Serology Test Evaluation Report for "SARS-CoV-2 Antibody Test (Lateral Flow Method)" from Wondfo

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1 Introduction

The SARS-CoV-2 Antibody Test (Lateral Flow Method) from Wondfo was tested on 2020-04-21 at the Frederick National Laboratory for Cancer Research (FNLCR), a Federally Funded Research and Development Center (FFRDC) sponsored by the National Cancer Institute (NCI). Tests were from lot number W19500328. The SARS-CoV-2 Antibody Test (Lateral Flow Method) is intended to qualitatively detect IgM and IgG together without differentiating between the two.

1.1 Panel composition

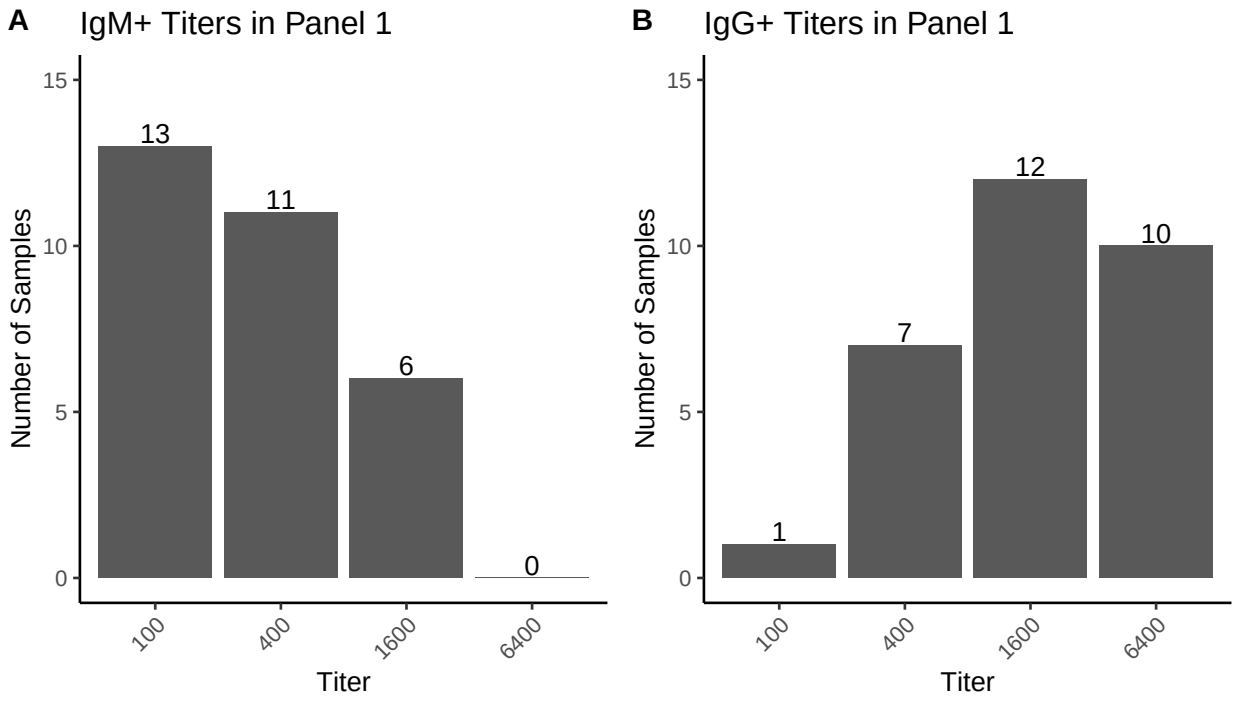

Figure 1: Titer levels for (A) IgM+ and (B) IgG+ samples according to the CDC SARS-CoV-2 Spike antigen assay

The test was evaluated against “Panel 1,” which includes frozen SARS-CoV-2 antibody-positive serum samples ($n = 30$) and frozen antibody-negative serum and plasma samples ($n = 80$). The panel size and composition were chosen to enable a laboratory-based evaluation and to provide reasonable estimates and confidence intervals for test performance in the context of limited sample availability. The sample size is comparable to that of a typical sample size used to support Emergency Use Authorization (EUA) by FDA for tests of this type.

1.1.1 Positive samples

Positive samples used in Panel 1 were from patients previously confirmed to have SARS-CoV-2 infection with a nucleic acid amplification test (NAAT). Time between symptom onset, NAAT testing, and sample collection is not known for all samples. Both SARS-CoV-2 IgM and IgG antibodies are present in all Panel 1 positive samples. The Centers for Disease Control and Prevention (CDC) detected the presence of IgG and IgM antibodies at their laboratory using their SARS-CoV-2 spike enzyme-linked immunosorbent assay (ELISA) tests.[1] The presence of antibodies was confirmed at FNLCR using CDC's developed ELISAs (Pan-Ig, IgG, and IgM) as well as an IgG Receptor Binding Domain (RBD) ELISA developed by the Krammer Laboratory at the Icahn School of Medicine at Mount Sinai.[2] The positive samples selected may not reflect the distribution of antibody levels in patient populations that would be evaluated by such a test. Because all samples are positive for both IgM and IgG, this evaluation cannot verify that tests intended to detect IgM and IgG antibodies separately detect these antibodies independently.

Positive samples were assessed at dilutions of 1:100, 1:400, 1:1600, and 1:6400 by CDC on their Pan-Ig assay, their IgM assay, and their IgG assay. Some samples were run at additional dilutions. Any samples that were positive at a dilution greater than 1:6400 were assigned a titer of 6400 because 1:6400 was the highest dilution at which all Panel 1 positive samples were assessed. Two of these samples, C0107 and C0176, were positive for IgG antibodies at a dilution of 1:25600.

1.1.2 Negative samples

All Panel 1 negative samples were collected prior to 2020, before the SARS-CoV-2 virus is known to have circulated in the United States. Panel 1 groups include:

- "Negatives" ($n = 70$): selected without regard for clinical status. This group includes a sample, C0063, that showed reactivity in the Pan-Ig CDC spike ELISA at FNLCR. It includes another sample, C0087, that showed reactivity in the IgG RBD ELISA at FNLCR.
- "HIV+" ($n = 10$): selected from banked serum from HIV+ patients.[3] This group includes 3 samples, C0018, C0155, and C0182, that showed reactivity in the IgG RBD ELISA at FNLCR.

All Panel 1 negative samples were assessed at dilutions of 1:100 and 1:400 by CDC on their Pan-Ig assay. A subset of samples was assessed in parallel at additional dilutions and on the CDC IgM

[1] See `https://www.cdc.gov/coronavirus/2019-ncov/lab/serology-testing.html`, which notes "CDC's serologic test is designed and validated for broad-based surveillance and research that will give us information needed to guide the response to the pandemic and protect the public's health. The test is not currently designed to test individuals who want to know if they have been previously infected with COVID-19."

[2] An implementation of this test, the COVID-19 ELISA IgG Antibody Test, has been granted an EUA authorization by FDA for use at the Mount Sinai Laboratory (MSL), Center for Clinical Laboratories, a division of the Department of Pathology, Molecular, and Cell-Based Medicine, New York, NY. See `https://www.fda.gov/media/137029/download`.

[3] HIV+ samples were deemed appropriate for inclusion in the panel: (1) to increase the sample size and reduce the confidence interval; and (2) to identify any possibility of cross-reactivity with HIV+ samples. It is anticipated that other types of samples, as they become available, may also be evaluated in any future analyses.

and IgG assays. All Panel 1 negative samples were negative at a dilution of 1:100 on the CDC Pan-Ig assay. These samples were assigned an undetectable titer (represented as zero (0) in the line data) for the Pan-Ig assay, the IgM assay, and the IgG assay.

1.2 Analysis

Samples used in this evaluation were not randomly selected, and sensitivity (PPA) and specificity (NPA) estimates in this report may not be indicative of the real-world performance of the Wondfo SARS-CoV-2 Antibody Test (Lateral Flow Method). Sensitivity and specificity were calculated for each antibody (e.g., IgM, IgG, IgA, and Pan-Ig, as applicable) separately. In addition, sensitivity and specificity were estimated in a combined manner, where a positive result for any antibody the Wondfo SARS-CoV-2 Antibody Test (Lateral Flow Method) is intended to detect was considered as a positive test result and a negative result meant that a sample tested negative for all antibodies the Wondfo SARS-CoV-2 Antibody Test (Lateral Flow Method) is intended to detect. Positive and negative predictive values were calculated for combined sensitivity and specificity assuming a prevalence of 5%. Cross-reactivity with HIV+ was evaluated, and results are presented separately. If cross-reactivity was detected, the samples with HIV+ were not included in calculations of specificity.

Confidence intervals for sensitivity and specificity were calculated per a score method described in CLSI EP12-A2 (2008).[4] Confidence intervals for PPV and NPV were calculated using the values from the 95% confidence intervals for sensitivity and specificity. For evaluation of cross-reactivity with HIV+, it was evaluated whether an increased false positive rate among antibody negative samples with HIV was statistically higher than the false positive rate among antibody negative samples without HIV (for this, a confidence interval for the difference in false positive rates was calculated per a score method described by Altman.[5])

1.3 Important caveats

Sensitivity and specificity estimates in this report may not be indicative of the real world performance of the Wondfo SARS-CoV-2 Antibody Test (Lateral Flow Method).

These results are based on serum and plasma samples only and may not be indicative of performance with other sample types, such as whole blood, including finger stick blood.

Information about anticoagulants used is not known.

The number of samples in the panel is a minimally viable sample size that still provides reasonable estimates and confidence intervals for test performance, and the samples used may not be representative of the antibody profile observed in patient populations.

[4] CLSI. *User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline—Second Edition*. CLSI document EP12-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2008. See `https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfStandards/detail.cfm?standard__identification_no=31791`.

[5] Statistics with Confidence: Confidence Intervals and Statistical Guidelines. (2013). Wiley.

1.4 Notes about the evaluation procedure

- The Wondfo SARS-CoV-2 Antibody Test (Lateral Flow Method) was used per the manufacturer's package insert.
- Devices were tested within any expiration dates provided.
- Devices were not obviously defective / compromised.
- Devices were stored at FNLCR within their labeled conditions.
- A single operator conducted and read the test.
- The personnel who performed the testing were blinded to the identity / code of the sample and the expected results.
- The testing was performed in a non-clinical laboratory environment.
- Negative and positive samples were ordered randomly and then tested serially.
- The operator trained on the SARS-CoV-2 Antibody Test (Lateral Flow Method) with positive and negative controls prior to testing.

2 Results

Table 1: Summary Results

	Comparator Method			Collected pre-2020		
	Antibody Positive			Antibody Negative		
SARS-CoV-2 Antibody Test (Lateral Flow Method)	IgM+, IgG+	IgM+, IgG-	IgM-, IgG+	Negative	HIV+	Total
(IgM / IgG)+	30					30
(IgM / IgG)-				70	10	80
Total	30			70	10	110

Table 2: Summary Statistics

Measure	Estimate	Confidence Interval
(IgM / IgG) Sensitivity	100% (30/30)	(88.7%; 100%)
(IgM / IgG) Specificity	100% (80/80)	(95.4%; 100%)
Combined Sensitivity	100% (30/30)	(88.7%; 100%)
Combined Specificity	100% (80/80)	(95.4%; 100%)
Combined PPV for prevalence = 5.0%	100%	(50.5%; 100%)
Combined NPV for prevalence = 5.0%	100%	(99.4%; 100%)
Cross-reactivity with HIV+	0.0% (0/10), not detected	

3 Line Data

In the table below, “Days” refers to “Days from symptom onset to blood collection.”

Table 3: Line Data

Sample Number	IgM Result	IgG Result	IgA Result	Pan Ig Result	(IgM / IgG) Result	Control	Sample ID	Type	CDC Spike Pan-Ig Titer	CDC Spike IgM Titer	CDC Spike IgG Titer	Days	Group
1					Negative	Pass	C0001	Serum	0	0	0		Negatives
2					Negative	Pass	C0002	Serum	0	0	0		Negatives
3					Negative	Pass	C0004	Plasma	0	0	0		Negatives
4					Negative	Pass	C0005	Plasma	0	0	0		Negatives
5					Negative	Pass	C0008	Plasma	0	0	0		Negatives
6					Positive	Pass	C0010	Serum	1600	400	1600	29	Positives
7					Negative	Pass	C0011	Serum	0	0	0		Negatives
8					Negative	Pass	C0012	Serum	0	0	0		Negatives
9					Negative	Pass	C0014	Serum	0	0	0		Negatives
10					Negative	Pass	C0016	Serum	0	0	0		Negatives
11					Negative	Pass	C0018	Plasma	0	0	0		HIV+
12					Negative	Pass	C0020	Serum	0	0	0		Negatives
13					Negative	Pass	C0024	Serum	0	0	0		Negatives
14					Negative	Pass	C0027	Serum	0	0	0		Negatives
15					Negative	Pass	C0029	Serum	0	0	0		Negatives
16					Negative	Pass	C0032	Plasma	0	0	0		Negatives
17					Negative	Pass	C0033	Serum	0	0	0		Negatives
18					Negative	Pass	C0034	Serum	0	0	0		Negatives
19					Positive	Pass	C0037	Serum	400	100	1600	19	Positives
20					Positive	Pass	C0038	Serum	100	100	100	20	Positives

Table 3: Line Data *(continued)*

Sample Number	IgM Result	IgG Result	IgA Result	Pan Ig Result	(IgM / IgG) Result	Control	Sample ID	Type	CDC Spike Pan-Ig Titer	CDC Spike IgM Titer	CDC Spike IgG Titer	Days	Group
21					Positive	Pass	C0040	Serum	1600	400	1600	24	Positives
22					Negative	Pass	C0041	Plasma	0	0	0		Negatives
23					Negative	Pass	C0043	Serum	0	0	0		Negatives
24					Negative	Pass	C0044	Serum	0	0	0		Negatives
25					Negative	Pass	C0048	Serum	0	0	0		Negatives
26					Positive	Pass	C0049	Serum	400	400	400	23	Positives
27					Negative	Pass	C0050	Serum	0	0	0		Negatives
28					Negative	Pass	C0051	Plasma	0	0	0		Negatives
29					Positive	Pass	C0053	Serum	1600	1600	1600	28	Positives
30					Negative	Pass	C0054	Plasma	0	0	0		HIV+
31					Negative	Pass	C0058	Serum	0	0	0		Negatives
32					Negative	Pass	C0059	Plasma	0	0	0		Negatives
33					Positive	Pass	C0061	Serum	1600	100	1600	26	Positives
34					Negative	Pass	C0062	Serum	0	0	0		Negatives
35					Negative	Pass	C0063	Plasma	0	0	0		Negatives
36					Positive	Pass	C0064	Serum	1600	1600	6400	20	Positives
37					Negative	Pass	C0065	Plasma	0	0	0		Negatives
38					Negative	Pass	C0066	Serum	0	0	0		Negatives
39					Negative	Pass	C0067	Serum	0	0	0		Negatives
40					Negative	Pass	C0069	Serum	0	0	0		Negatives
41					Negative	Pass	C0070	Serum	0	0	0		Negatives
42					Positive	Pass	C0071	Serum	6400	1600	6400	20	Positives
43					Negative	Pass	C0072	Serum	0	0	0		Negatives
44					Negative	Pass	C0073	Serum	0	0	0		Negatives

Table 3: Line Data *(continued)*

Sample Number	IgM Result	IgG Result	IgA Result	Pan Ig Result	(IgM / IgG) Result	Control	Sample ID	Type	CDC Spike Pan-Ig Titer	CDC Spike IgM Titer	CDC Spike IgG Titer	Days	Group
45					Positive	Pass	C0074	Serum	400	100	400	24	Positives
46					Negative	Pass	C0079	Plasma	0	0	0		Negatives
47					Positive	Pass	C0080	Serum	1600	400	1600	29	Positives
48					Negative	Pass	C0081	Serum	0	0	0		Negatives
49					Negative	Pass	C0083	Serum	0	0	0		Negatives
50					Positive	Pass	C0084	Serum	6400	100	6400	24	Positives
51					Negative	Pass	C0087	Serum	0	0	0		Negatives
52					Negative	Pass	C0089	Plasma	0	0	0		HIV+
53					Positive	Pass	C0090	Serum	1600	100	1600	22	Positives
54					Positive	Pass	C0092	Serum	1600	100	1600		Positives
55					Negative	Pass	C0093	Plasma	0	0	0		HIV+
56					Negative	Pass	C0094	Serum	0	0	0		Negatives
57					Negative	Pass	C0095	Plasma	0	0	0		Negatives
58					Negative	Pass	C0098	Plasma	0	0	0		Negatives
59					Negative	Pass	C0099	Plasma	0	0	0		HIV+
60					Negative	Pass	C0100	Serum	0	0	0		Negatives
61					Negative	Pass	C0101	Plasma	0	0	0		Negatives
62					Positive	Pass	C0102	Serum	400	400	400	25	Positives
63					Positive	Pass	C0107	Serum	6400	400	6400	36	Positives
64					Negative	Pass	C0109	Plasma	0	0	0		Negatives
65					Negative	Pass	C0110	Serum	0	0	0		Negatives
66					Negative	Pass	C0115	Serum	0	0	0		Negatives
67					Negative	Pass	C0116	Serum	0	0	0		Negatives
68					Negative	Pass	C0117	Plasma	0	0	0		Negatives

Table 3: Line Data *(continued)*

Sample Number	IgM Result	IgG Result	IgA Result	Pan Ig Result	(IgM / IgG) Result	Control	Sample ID	Type	CDC Spike Pan-Ig Titer	CDC Spike IgM Titer	CDC Spike IgG Titer	Days	Group
69					Negative	Pass	C0118	Serum	0	0	0		Negatives
70					Positive	Pass	C0119	Serum	1600	100	1600	20	Positives
71					Negative	Pass	C0120	Serum	0	0	0		Negatives
72					Negative	Pass	C0121	Plasma	0	0	0		Negatives
73					Positive	Pass	C0122	Serum	400	100	400	24	Positives
74					Negative	Pass	C0126	Serum	0	0	0		Negatives
75					Positive	Pass	C0127	Serum	400	400	1600	23	Positives
76					Negative	Pass	C0128	Serum	0	0	0		Negatives
77					Negative	Pass	C0131	Serum	0	0	0		Negatives
78					Positive	Pass	C0132	Serum	1600	1600	6400		Positives
79					Positive	Pass	C0136	Serum	6400	400	6400		Positives
80					Negative	Pass	C0138	Plasma	0	0	0		HIV+
81					Negative	Pass	C0139	Serum	0	0	0		Negatives
82					Negative	Pass	C0140	Plasma	0	0	0		Negatives
83					Positive	Pass	C0144	Serum	6400	1600	6400	21	Positives
84					Negative	Pass	C0146	Serum	0	0	0		Negatives
85					Negative	Pass	C0150	Plasma	0	0	0		HIV+
86					Positive	Pass	C0153	Serum	400	100	400	31	Positives
87					Negative	Pass	C0155	Plasma	0	0	0		HIV+
88					Negative	Pass	C0156	Plasma	0	0	0		Negatives
89					Negative	Pass	C0158	Serum	0	0	0		Negatives
90					Positive	Pass	C0160	Serum	1600	100	1600	17	Positives
91					Positive	Pass	C0161	Serum	400	100	400	25	Positives
92					Positive	Pass	C0164	Serum	1600	400	6400	23	Positives

Table 3: Line Data *(continued)*

Sample Number	IgM Result	IgG Result	IgA Result	Pan Ig Result	(IgM / IgG) Result	Control	Sample ID	Type	CDC Spike Pan-Ig Titer	CDC Spike IgM Titer	CDC Spike IgG Titer	Days	Group
93					Negative	Pass	C0165	Serum	0	0	0		Negatives
94					Negative	Pass	C0169	Serum	0	0	0		Negatives
95					Positive	Pass	C0172	Serum	1600	400	1600	19	Positives
96					Negative	Pass	C0173	Serum	0	0	0		Negatives
97					Negative	Pass	C0174	Serum	0	0	0		Negatives
98					Positive	Pass	C0176	Serum	6400	400	6400	19	Positives
99					Negative	Pass	C0179	Plasma	0	0	0		Negatives
100					Positive	Pass	C0180	Serum	6400	1600	6400	24	Positives
101					Negative	Pass	C0181	Serum	0	0	0		Negatives
102					Negative	Pass	C0182	Plasma	0	0	0		HIV+
103					Negative	Pass	C0185	Plasma	0	0	0		Negatives
104					Negative	Pass	C0186	Serum	0	0	0		Negatives
105					Positive	Pass	C0191	Serum	400	100	400	22	Positives
106					Negative	Pass	C0193	Plasma	0	0	0		Negatives
107					Negative	Pass	C0197	Plasma	0	0	0		HIV+
108					Negative	Pass	C0198	Plasma	0	0	0		Negatives
109					Negative	Pass	C0199	Plasma	0	0	0		Negatives
110					Negative	Pass	C0200	Serum	0	0	0		Negatives