Serology Test Evaluation Report for "SARS-COV-2 ELISA" from United Biomedical, Inc.

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1 Introduction

The SARS-COV-2 ELISA from United Biomedical, Inc. was tested on 2020-09-01 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 0153004. The SARS-COV-2 ELISA is intended to qualitatively detect IgG.

1.1 Panel composition

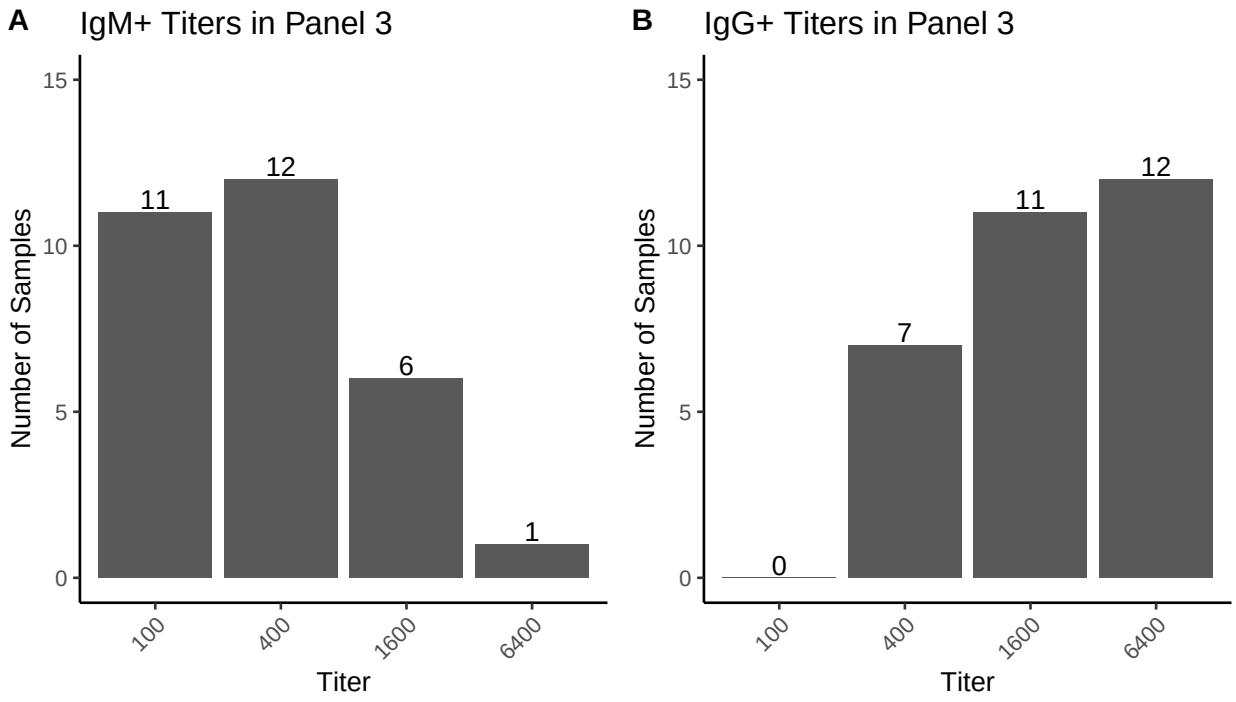

Figure 1: Titer levels for (A) IgM+ and (B) IgG+ samples according to the CDC SARS-CoV-2 Spike antigen assay

The test was evaluated against “Panel 3,” which includes frozen SARS-CoV-2 antibody-positive serum samples ($n = 30$) and frozen antibody-negative Anticoagulant Citrate Dextrose Solution Formula A (ACD-A) 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 3 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 3 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 positive samples used in these evaluations were assessed.

1.1.2 Negative samples

All Panel 3 negative samples were collected prior to 2020, before the SARS-CoV-2 virus is known to have circulated in the United States. Panel 3 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.
- "HIV+" ($n = 10$): selected from banked plasma 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 3 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 has been designed and validated for surveillance and research purposes. It is designed to estimate the percentage of the U.S. population previously infected with the virus – information needed to guide the response to the pandemic and protect the public's health. The CDC test is not currently designed to test individuals who want to know if they have been previously infected with SARS-CoV-2. Commercial tests are available to provide test results to individuals."

[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 3 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 United Biomedical, Inc. SARS-COV-2 ELISA. 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 United Biomedical, Inc. SARS-COV-2 ELISA 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 United Biomedical, Inc. SARS-COV-2 ELISA 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 United Biomedical, Inc. SARS-COV-2 ELISA.

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.

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 United Biomedical, Inc. SARS-COV-2 ELISA 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 the FNLCR within their labeled conditions.
- A single operator conducted 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 United Biomedical, Inc. SARS-COV-2 ELISA was run with positive and negative controls.

1.5 Additional notes, anomalies, and clarifications

The FNLCR provided the following additional information:

> The Instructions for Use describe the following testing strategy: the positives are marked as "Initially Positive". Follow up testing should be performed on the "Initially Positive" samples. These samples should be tested in duplicate to confirm the positive results. Follow up testing has not been performed at FNLCR as of 09SEP20.

2 Results

Table 1: Summary Results

	Comparator Method			Collected pre-2020		
	Antibody Positive			Antibody Negative		
SARS-COV-2 ELISA	IgM+, IgG+	IgM+, IgG-	IgM-, IgG+	Negative	HIV+	Total
IgG+	27					27
IgG-	3			70	10	83
Total	30			70	10	110

Table 2: Summary Statistics

Measure	Estimate	Confidence Interval
IgG Sensitivity	90.0% (27/30)	(74.4%; 96.5%)
IgG Specificity	100% (80/80)	(95.4%; 100%)
Combined Sensitivity	90.0% (27/30)	(74.4%; 96.5%)
Combined Specificity	100% (80/80)	(95.4%; 100%)
Combined PPV for prevalence = 5.0%	100%	(46.1%; 100%)
Combined NPV for prevalence = 5.0%	99.5%	(98.6%; 99.8%)
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	IgG Result	Control	Sample ID	Type	CDC Spike Pan-Ig Titer	CDC Spike IgM Titer	CDC Spike IgG Titer	Days	Group
1	Negative	Pass	C0105	Plasma	0	0	0		Negative
2	Positive	Pass	D0209	Serum	6400	1600	6400	42	Positive
3	Negative	Pass	C0137	Plasma	0	0	0		Negative
4	Negative	Pass	D0023	Plasma	0	0	0		Negative
5	Negative	Pass	D0086	Plasma	0	0	0		Negative
6	Positive	Pass	D0326	Serum	400	400	1600		Positive
7	Positive	Pass	D0168	Serum	400	100	400	24	Positive
8	Negative	Pass	D0049	Plasma	0	0	0		Negative
9	Negative	Pass	C0133	Plasma	0	0	0		Negative
10	Negative	Pass	D0113	Plasma	0	0	0		Negative
11	Negative	Pass	C0121	Plasma	0	0	0		Negative
12	Negative	Pass	D0007	Plasma	0	0	0		Negative
13	Positive	Pass	D0040	Serum	100	100	400	43	Positive
14	Negative	Pass	C0089	Plasma	0	0	0		HIV+
15	Negative	Pass	D0192	Plasma	0	0	0		Negative
16	Positive	Pass	D0386	Serum	6400	6400	6400	20	Positive
17	Positive	Pass	D0405	Serum	400	400	400	32	Positive
18	Negative	Pass	C0185	Plasma	0	0	0		Negative
19	Positive	Pass	D0307	Serum	6400	1600	6400	41	Positive
20	Negative	Pass	C0182	Plasma	0	0	0		HIV+
21	Negative	Pass	D0056	Plasma	0	0	0		Negative
22	Negative	Pass	D0166	Plasma	0	0	0		Negative

Table 3: Line Data *(continued)*

Sample Number	IgG Result	Control	Sample ID	Type	CDC Spike Pan-Ig Titer	CDC Spike IgM Titer	CDC Spike IgG Titer	Days	Group
23	Positive	Pass	D0451	Serum	1600	400	1600	35	Positive
24	Positive	Pass	D0231	Serum	6400	1600	6400	19	Positive
25	Negative	Pass	C0041	Plasma	0	0	0		Negative
26	Negative	Pass	C0054	Plasma	0	0	0		HIV+
27	Negative	Pass	D0123	Plasma	0	0	0		Negative
28	Negative	Pass	C0032	Plasma	0	0	0		Negative
29	Negative	Pass	D0135	Plasma	0	0	0		Negative
30	Negative	Pass	C0196	Plasma	0	0	0		Negative
31	Negative	Pass	D0187	Plasma	0	0	0		Negative
32	Positive	Pass	D0498	Serum	1600	400	1600	17	Positive
33	Positive	Pass	D0344	Serum	6400	1600	6400	27	Positive
34	Negative	Pass	C0095	Plasma	0	0	0		Negative
35	Negative	Pass	D0097	Plasma	0	0	0		Negative
36	Positive	Pass	D0349	Serum	1600	400	6400	19	Positive
37	Negative	Pass	C0018	Plasma	0	0	0		HIV+
38	Negative	Pass	D0031	Plasma	0	0	0		Negative
39	Negative	Pass	D0064	Plasma	0	0	0		Negative
40	Negative	Pass	C0156	Plasma	0	0	0		Negative
41	Negative	Pass	D0184	Plasma	0	0	0		Negative
42	Negative	Pass	C0103	Plasma	0	0	0		Negative
43	Negative	Pass	C0198	Plasma	0	0	0		Negative
44	Negative	Pass	C0098	Plasma	0	0	0		Negative
45	Negative	Pass	C0179	Plasma	0	0	0		Negative
46	Negative	Pass	C0059	Plasma	0	0	0		Negative
47	Negative	Pass	C0063	Plasma	0	0	0		Negative
48	Positive	Pass	D0102	Serum	400	100	400	24	Positive

Table 3: Line Data *(continued)*

Sample Number	IgG Result	Control	Sample ID	Type	CDC Spike Pan-Ig Titer	CDC Spike IgM Titer	CDC Spike IgG Titer	Days	Group
49	Negative	Pass	C0117	Plasma	0	0	0		Negative
50	Negative	Pass	C0193	Plasma	0	0	0		Negative
51	Negative	Pass	C0101	Plasma	0	0	0		Negative
52	Negative	Pass	C0093	Plasma	0	0	0		HIV+
53	Negative	Pass	D0015	Plasma	0	0	0		Negative
54	Negative	Pass	D0025	Plasma	0	0	0		Negative
55	Positive	Pass	D0270	Serum	1600	100	1600	20	Positive
56	Negative	Pass	D0112	Plasma	0	0	0		Negative
57	Negative	Pass	D0183	Plasma	0	0	0		Negative
58	Negative	Pass	C0051	Plasma	0	0	0		Negative
59	Negative	Pass	C0079	Plasma	0	0	0		Negative
60	Negative	Pass	D0172	Plasma	0	0	0		Negative
61	Negative	Pass	C0005	Plasma	0	0	0		Negative
62	Negative	Pass	C0138	Plasma	0	0	0		HIV+
63	Negative	Pass	D0142	Plasma	0	0	0		Negative
64	Positive	Pass	D0493	Serum	1600	400	1600	43	Positive
65	Negative	Pass	D0141	Plasma	0	0	0		Negative
66	Negative	Pass	D0148	Plasma	0	0	0		Negative
67	Positive	Pass	D0038	Serum	400	100	1600	44	Positive
68	Negative	Pass	D0060	Plasma	0	0	0		Negative
69	Negative	Pass	D0053	Plasma	0	0	0		Negative
70	Negative	Pass	C0075	Plasma	0	0	0		Negative
71	Negative	Pass	C0140	Plasma	0	0	0		Negative
72	Negative	Pass	C0150	Plasma	0	0	0		HIV+
73	Negative	Pass	D0159	Serum	100	100	400	24	Positive
74	Positive	Pass	D0455	Serum	1600	1600	6400	23	Positive

Table 3: Line Data *(continued)*

Sample Number	IgG Result	Control	Sample ID	Type	CDC Spike Pan-Ig Titer	CDC Spike IgM Titer	CDC Spike IgG Titer	Days	Group
75	Negative	Pass	D0003	Plasma	0	0	0		Negative
76	Negative	Pass	C0008	Plasma	0	0	0		Negative
77	Positive	Pass	D0497	Serum	400	400	1600	25	Positive
78	Negative	Pass	D0178	Plasma	0	0	0		Negative
79	Negative	Pass	C0197	Plasma	0	0	0		HIV+
80	Positive	Pass	D0182	Serum	6400	1600	6400	17	Positive
81	Negative	Pass	C0109	Plasma	0	0	0		Negative
82	Negative	Pass	D0085	Plasma	0	0	0		Negative
83	Positive	Pass	D0090	Serum	1600	400	6400	45	Positive
84	Positive	Pass	D0091	Serum	400	100	1600	40	Positive
85	Negative	Pass	D0170	Plasma	0	0	0		Negative
86	Positive	Pass	D0020	Serum	1600	400	6400	42	Positive
87	Negative	Pass	D0132	Plasma	0	0	0		Negative
88	Negative	Pass	C0026	Plasma	0	0	0		Negative
89	Negative	Pass	C0065	Plasma	0	0	0		Negative
90	Negative	Pass	D0080	Plasma	0	0	0		Negative
91	Positive	Pass	D0466	Serum	400	100	1600	19	Positive
92	Negative	Pass	D0154	Plasma	0	0	0		Negative
93	Negative	Pass	D0129	Plasma	0	0	0		Negative
94	Negative	Pass	D0006	Plasma	0	0	0		Negative
95	Negative	Pass	C0134	Plasma	0	0	0		Negative
96	Negative	Pass	D0077	Plasma	0	0	0		Negative
97	Negative	Pass	C0155	Plasma	0	0	0		HIV+
98	Negative	Pass	C0099	Plasma	0	0	0		HIV+
99	Positive	Pass	D0268	Serum	400	100	1600		Positive
100	Negative	Pass	D0367	Serum	400	400	400	37	Positive

Table 3: Line Data *(continued)*

Sample Number	IgG Result	Control	Sample ID	Type	CDC Spike Pan-Ig Titer	CDC Spike IgM Titer	CDC Spike IgG Titer	Days	Group
101	Negative	Pass	C0004	Plasma	0	0	0		Negative
102	Negative	Pass	C0199	Plasma	0	0	0		Negative
103	Negative	Pass	C0162	Plasma	0	0	0		Negative
104	Negative	Pass	C0019	Plasma	0	0	0		Negative
105	Positive	Pass	D0254	Serum	400	400	1600	34	Positive
106	Negative	Pass	D0396	Serum	100	100	400	26	Positive
107	Positive	Pass	D0297	Serum	1600	400	6400	20	Positive
108	Positive	Pass	D0465	Serum	1600	100	6400	31	Positive
109	Negative	Pass	D0153	Plasma	0	0	0		Negative
110	Negative	Pass	D0145	Plasma	0	0	0		Negative