Serology Test Evaluation Report for "SARS-CoV-2 IgM/IgG Antibody Test Kit" from Biohit Healthcare (Hefei) Co., Ltd.

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1 Introduction

The SARS-CoV-2 IgM/IgG Antibody Test Kit from Biohit Healthcare (Hefei) Co., Ltd. was tested on 2020-05-28 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 SA200301. The SARS-CoV-2 IgM/IgG Antibody Test Kit is intended to qualitatively detect IgM and IgG separately.

1.1 Panel composition

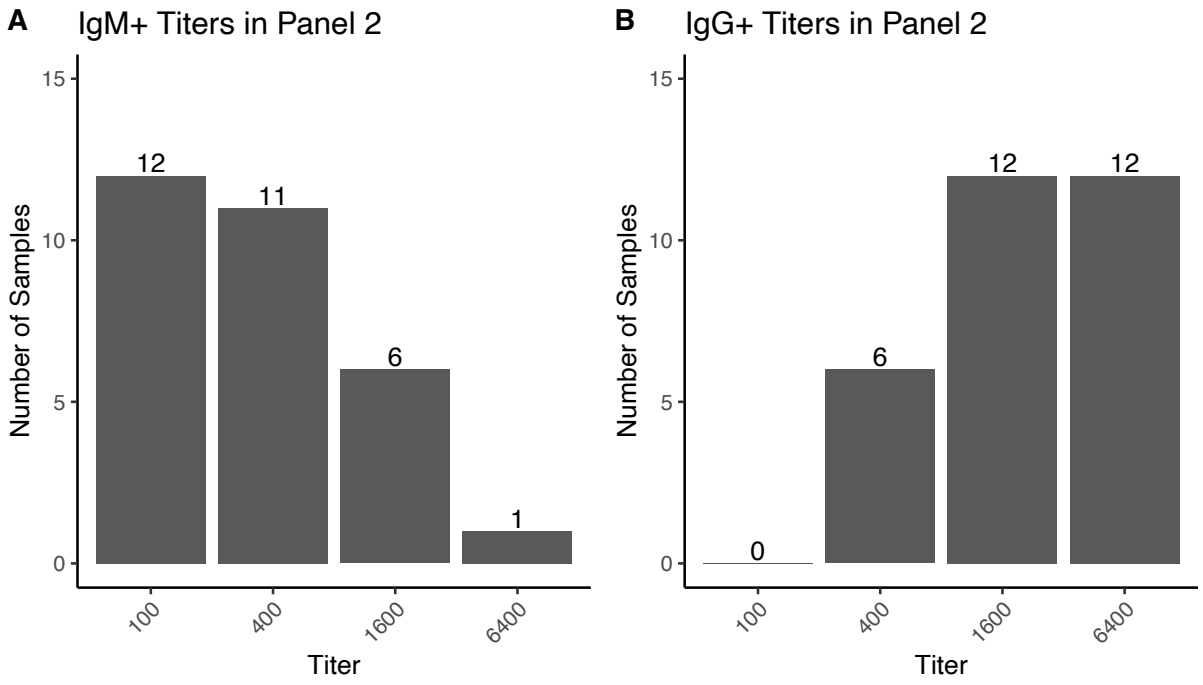

Figure 1: Titer levels for (A) IgM+ and (B) IgG+ samples according to the CDC SARS-CoV-2 Spike antigen assay

The test was evaluated against "Panel 2," 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 2 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 2 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 2 positive samples were assessed.

1.1.2 Negative samples

All Panel 2 negative samples were collected prior to 2020, before the SARS-CoV-2 virus is known to have circulated in the United States. Panel 2 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 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 2 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 and IgG assays. All Panel 2 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] 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.

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 Biohit Healthcare (Hefei) Co., Ltd. SARS-CoV-2 IgM/IgG Antibody Test Kit. 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 Biohit Healthcare (Hefei) Co., Ltd. SARS-CoV-2 IgM/IgG Antibody Test Kit 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 Biohit Healthcare (Hefei) Co., Ltd. SARS-CoV-2 IgM/IgG Antibody Test Kit 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 Biohit Healthcare (Hefei) Co., Ltd. SARS-CoV-2 IgM/IgG Antibody Test Kit.

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 Biohit Healthcare (Hefei) Co., Ltd. SARS-CoV-2 IgM/IgG Antibody Test Kit 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 IgM/IgG Antibody Test Kit 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 IgM/IgG Antibody Test Kit	IgM+, IgG+	IgM+, IgG-	IgM-, IgG+	Negative	HIV+	Total
IgM+, IgG+	29			4		33
IgM+, IgG-						
IgM-, IgG+						
IgM-, IgG-	1			66	10	77
Total	30			70	10	110

Table 2: Summary Statistics

Measure	Estimate	Confidence Interval
IgM Sensitivity	96.7% (29/30)	(83.3%; 99.4%)
IgM Specificity	95.0% (76/80)	(87.8%; 98%)
IgG Sensitivity	96.7% (29/30)	(83.3%; 99.4%)
IgG Specificity	95.0% (76/80)	(87.8%; 98%)
Combined Sensitivity	96.7% (29/30)	(83.3%; 99.4%)
Combined Specificity	95.0% (76/80)	(87.8%; 98%)
Combined PPV for prevalence = 5.0%	50.4%	(26.5%; 72.7%)
Combined NPV for prevalence = 5.0%	99.8%	(99%; 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	Negative				Pass	C0004	Plasma	0	0	0		Negative
2	Negative	Negative				Pass	C0005	Plasma	0	0	0		Negative
3	Negative	Negative				Pass	D0007	Plasma	0	0	0		Negative
4	Negative	Negative				Pass	D0011	Serum	0	0	0		Negative
5	Positive	Positive				Pass	D0012	Serum	6400	100	6400	28	Positive
6	Negative	Negative				Pass	C0155	Plasma	0	0	0		HIV+
7	Negative	Negative				Pass	D0015	Plasma	0	0	0		Negative
8	Positive	Positive				Pass	C0031	Serum	1600	400	6400	21	Positive
9	Negative	Negative				Pass	C0019	Plasma	0	0	0		Negative
10	Positive	Positive				Pass	D0020	Serum	1600	400	6400	42	Positive
11	Negative	Negative				Pass	C0089	Plasma	0	0	0		HIV+
12	Positive	Positive				Pass	D0022	Serum	400	100	1600	46	Positive
13	Positive	Positive				Pass	C0049	Serum	400	400	400	23	Positive
14	Negative	Negative				Pass	D0027	Serum	0	0	0		Negative
15	Positive	Positive				Pass	D0028	Serum	6400	400	6400	32	Positive
16	Negative	Negative				Pass	C0032	Plasma	0	0	0		Negative
17	Negative	Negative				Pass	D0034	Serum	0	0	0		Negative
18	Negative	Negative				Pass	C0099	Plasma	0	0	0		HIV+
19	Negative	Negative				Pass	C0041	Plasma	0	0	0		Negative
20	Negative	Negative				Pass	C0008	Plasma	0	0	0		Negative

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	Negative	Negative				Pass	C0051	Plasma	0	0	0		Negative
22	Negative	Negative				Pass	C0018	Plasma	0	0	0		HIV+
23	Negative	Negative				Pass	D0047	Serum	0	0	0		Negative
24	Negative	Negative				Pass	D0049	Plasma	0	0	0		Negative
25	Negative	Negative				Pass	C0185	Plasma	0	0	0		Negative
26	Positive	Positive				Pass	C0172	Serum	1600	400	1600	19	Positive
27	Negative	Negative				Pass	C0093	Plasma	0	0	0		HIV+
28	Positive	Positive				Pass	C0053	Serum	1600	1600	1600	28	Positive
29	Negative	Negative				Pass	D0056	Plasma	0	0	0		Negative
30	Negative	Negative				Pass	D0058	Serum	0	0	0		Negative
31	Negative	Negative				Pass	C0059	Plasma	0	0	0		Negative
32	Positive	Positive				Pass	C0145	Serum	6400	1600	6400	17	Positive
33	Negative	Negative				Pass	D0064	Plasma	0	0	0		Negative
34	Negative	Negative				Pass	C0065	Plasma	0	0	0		Negative
35	Negative	Negative				Pass	C0075	Plasma	0	0	0		Negative
36	Negative	Negative				Pass	D0073	Serum	0	0	0		Negative
37	Negative	Negative				Pass	C0153	Serum	400	100	400	31	Positive
38	Negative	Negative				Pass	C0197	Plasma	0	0	0		HIV+
39	Positive	Positive				Pass	C0144	Serum	6400	1600	6400	21	Positive
40	Negative	Negative				Pass	C0179	Plasma	0	0	0		Negative
41	Negative	Negative				Pass	D0083	Serum	0	0	0		Negative
42	Positive	Positive				Pass	D0084	Serum	1600	100	1600	41	Positive
43	Negative	Negative				Pass	D0086	Plasma	0	0	0		Negative
44	Positive	Positive				Pass	C0132	Serum	1600	1600	6400		Positive

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	Positive				Pass	C0064	Serum	1600	1600	6400	20	Positive
46	Negative	Negative				Pass	D0094	Serum	0	0	0		Negative
47	Negative	Negative				Pass	C0095	Plasma	0	0	0		Negative
48	Negative	Negative				Pass	D0097	Plasma	0	0	0		Negative
49	Negative	Negative				Pass	C0098	Plasma	0	0	0		Negative
50	Negative	Negative				Pass	C0063	Plasma	0	0	0		Negative
51	Negative	Negative				Pass	C0101	Plasma	0	0	0		Negative
52	Negative	Negative				Pass	C0103	Plasma	0	0	0		Negative
53	Positive	Positive				Pass	D0104	Serum	400	100	400	17	Positive
54	Negative	Negative				Pass	C0105	Plasma	0	0	0		Negative
55	Negative	Negative				Pass	C0109	Plasma	0	0	0		Negative
56	Negative	Negative				Pass	C0198	Plasma	0	0	0		Negative
57	Negative	Negative				Pass	D0115	Serum	0	0	0		Negative
58	Negative	Negative				Pass	C0117	Plasma	0	0	0		Negative
59	Negative	Negative				Pass	D0120	Serum	0	0	0		Negative
60	Negative	Negative				Pass	C0121	Plasma	0	0	0		Negative
61	Negative	Negative				Pass	D0123	Plasma	0	0	0		Negative
62	Positive	Positive				Pass	D0126	Serum	0	0	0		Negative
63	Negative	Negative				Pass	D0128	Serum	0	0	0		Negative
64	Negative	Negative				Pass	D0130	Serum	0	0	0		Negative
65	Negative	Negative				Pass	D0131	Serum	0	0	0		Negative
66	Negative	Negative				Pass	D0132	Plasma	0	0	0		Negative
67	Negative	Negative				Pass	C0133	Plasma	0	0	0		Negative
68	Negative	Negative				Pass	C0134	Plasma	0	0	0		Negative

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	Positive	Positive				Pass	D0135	Plasma	0	0	0		Negative
70	Negative	Negative				Pass	C0137	Plasma	0	0	0		Negative
71	Positive	Positive				Pass	C0127	Serum	400	400	1600	23	Positive
72	Negative	Negative				Pass	C0199	Plasma	0	0	0		Negative
73	Negative	Negative				Pass	C0140	Plasma	0	0	0		Negative
74	Negative	Negative				Pass	D0141	Plasma	0	0	0		Negative
75	Negative	Negative				Pass	D0142	Plasma	0	0	0		Negative
76	Negative	Negative				Pass	D0145	Plasma	0	0	0		Negative
77	Negative	Negative				Pass	C0054	Plasma	0	0	0		HIV+
78	Negative	Negative				Pass	D0148	Plasma	0	0	0		Negative
79	Positive	Positive				Pass	D0149	Serum	400	100	400	43	Positive
80	Positive	Positive				Pass	C0152	Serum	400	400	6400	24	Positive
81	Negative	Negative				Pass	D0153	Plasma	0	0	0		Negative
82	Negative	Negative				Pass	D0154	Plasma	0	0	0		Negative
83	Negative	Negative				Pass	C0156	Plasma	0	0	0		Negative
84	Negative	Negative				Pass	D0158	Serum	0	0	0		Negative
85	Positive	Positive				Pass	D0159	Serum	100	100	400	24	Positive
86	Positive	Positive				Pass	D0161	Serum	1600	100	1600	44	Positive
87	Negative	Negative				Pass	C0162	Plasma	0	0	0		Negative
88	Positive	Positive				Pass	C0026	Plasma	0	0	0		Negative
89	Negative	Negative				Pass	D0166	Plasma	0	0	0		Negative
90	Negative	Negative				Pass	C0138	Plasma	0	0	0		HIV+
91	Negative	Negative				Pass	D0169	Serum	0	0	0		Negative
92	Negative	Negative				Pass	D0170	Plasma	0	0	0		Negative

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	Negative				Pass	D0173	Serum	0	0	0		Negative
94	Positive	Positive				Pass	D0180	Serum	6400	400	6400	39	Positive
95	Negative	Negative				Pass	C0079	Plasma	0	0	0		Negative
96	Positive	Positive				Pass	D0184	Plasma	0	0	0		Negative
97	Positive	Positive				Pass	C0071	Serum	6400	1600	6400	20	Positive
98	Negative	Negative				Pass	D0187	Plasma	0	0	0		Negative
99	Positive	Positive				Pass	D0188	Serum	400	400	1600	24	Positive
100	Negative	Negative				Pass	C0193	Plasma	0	0	0		Negative
101	Negative	Negative				Pass	C0150	Plasma	0	0	0		HIV+
102	Negative	Negative				Pass	C0182	Plasma	0	0	0		HIV+
103	Negative	Negative				Pass	C0196	Plasma	0	0	0		Negative
104	Positive	Positive				Pass	C0187	Serum	6400	6400	6400	29	Positive
105	Positive	Positive				Pass	C0080	Serum	1600	400	1600	29	Positive
106	Positive	Positive				Pass	D0201	Serum	100	100	400	33	Positive
107	Positive	Positive				Pass	D0205	Serum	400	400	1600	26	Positive
108	Positive	Positive				Pass	D0206	Serum	1600	100	1600	25	Positive
109	Positive	Positive				Pass	D0208	Serum	400	100	1600	22	Positive
110	Positive	Positive				Pass	D0210	Serum	1600	100	1600	26	Positive