Validation of FlexImmArray™ SARS-CoV-2 Human IgG Antibody Test, a High-Performance & Multiplex Serology Assay

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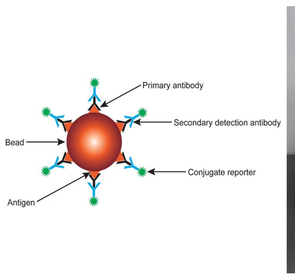
COVID-19 is a disease caused by a respiratory virus first identified in Wuhan, Hubei Province, China in December 2019. Severe Acute Respiratory Syndrome (SARS) corona virus 2 (CoV-2) is a new virus that hasn't caused illness in humans before. Worldwide, COVID-19 has resulted in millions of human infections, causing illness and in many cases death. COVID-19 has spread throughout the world, with more 7.5 million cases reported. A high performance multiplex serology assay is developed to serve as a valuable tool in the fight against COVID-19 for detection of human IgG antibody. The Tetracore® FlexImmArray™ SARS-CoV-2 Human IgG Antibody Test is a multiplex assay based on Luminex® x-MAP® technology using magnetic microspheres. It is intended for qualitative detection of IgG antibodies to SARS-CoV-2 in human serum and plasma. FlexImmArray SARS-CoV-2 Human IgG Antibody Test is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. United States Food and Drug Administration (FDA) has been notified about the validation of this test. Details about the validation of the test is given in this presentation. Characteristics of this test make it a high-performance multiplex assay by simultaneously measuring immunoreactivity to three different SARS-CoV-2 antigens. Incorporation of four quality assurance features in every single well of the test enhances the confidence in the assay results. This test is amenable to high-throughput automation and can be used for large-scale COVID-19 serology testing.

BACKGROUND

As stated in Section IV.D of the FDA's *Policy for Coronavirus Disease-2019 Tests*, the FDA does not intend to object to a commercial manufacturer's development and distribution of serology tests to identify antibodies to SARS-CoV-2 for a reasonable period of time, where the test has been validated and while the manufacturer is preparing its EUA request, where the manufacturer gives notification to the FDA and information that helps users and patients understand the test results, and clearly indicates the limitations of the tests in the instructions for use. These tests are to be used in qualified and certified laboratories capable of performing high-complexity laboratory testing. Simple depiction of the multiplex serology test, a indirect immunoassay where the specific IgG antibody in the human sample bound to the microsphere-coupled antigen measured using a secondary fluorescent labeled detection antibody, is shown in Figure 1.



#	Microsphere Functionality
1	SARS-CoV-2 Protein 1
2	SARS-CoV-2 Protein 2
3	SARS-CoV-2 Protein 3
4	IC—Instrument Control
5	NC—Non-specific binding control
6	FC—Fluorescent Control
7	ScG—Human IgG Sample Control

Figure 1. FlexImmArray SARS-Cov-2 Human IgG antibody test depiction and the MAGPIX® instrument used for multiplex assay reading

MATERIALS AND METHODS

Tetracore FlexImmArray SARS-CoV-2 human IgG antibody test kits were used for this validation. We used de-identified human serum / plasma samples, and MAGPIX instrument for reading. Testing of the samples were done as per the manufacturer's instructions for use given in the test kit (https://www.tetracore.com/). For this kit, Emergency Use Authorization application was submitted to FDA, and the FDA acknowledgement number is PEUA201215. For this validation, the analytical sensitivity of the test was determined followed by reproducibility testing. Cross-reactivity testing was done with samples from normal healthy individuals and non-COVID-19 disease state samples. Then clinical agreement studies were performed using SARS-CoV-2 RT-PCR tested, clinical samples from individuals with COVID-19 disease symptoms. Statistical analyses were performed to find out the clinical truth by determining the positive and negative percent agreement of the test. Further assessment was also done to calculate the positive and negative predictive values of the test at 5% prevalence rate.

RESULTS

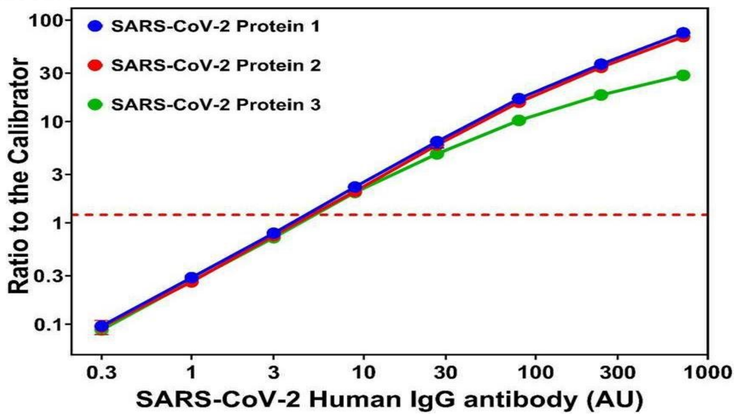


Figure 2. SARS-Cov-2 Human IgG antibody titration curve. X-axis shows the antibodies in antibody units (AU). The ratio of the fluorescent intensity of sample to that of the calibrator is plotted in the Y-axis. Both the axes are in Log scale.

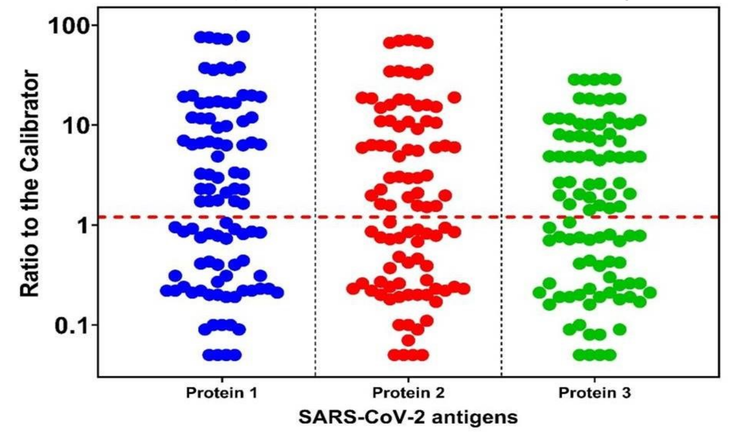


Figure 3. Scatter plot showing the individual data points during analytical sensitivity testing. X-axis shows the reactivity to each of the SARS-CoV-2 antigen.

Table 1. Intra-assay reproducibility testing done with seven replicates of four samples that include 3 positives and 1 negative sample

Sample N=7	SARS-CoV-2 Protein 1			SARS-CoV-2 Protein 2			SARS-CoV-2 Protein 3		
	Mean	SD	% CV	Mean	SD	% CV	Mean	SD	% CV
Sample 1	4.2	0.2	5.3	3.7	0.2	6.5	3.7	0.2	4.5
Sample 2	1.8	0.1	6.4	2.0	0.1	6.0	1.8	0.1	6.4
Sample 3	1.6	0.1	8.2	1.4	0.1	8.1	1.6	0.1	7.7
Sample 4	0.1	0.0	2.9	0.2	0.0	2.9	0.1	0.0	7.7

Table 2. Inter-assay reproducibility determined by test performed by three different performers using four samples (3 positives and 1 negative) and seven replicates of each sample

Sample N=21	SARS-CoV-2 Protein 1			SARS-CoV-2 Protein 2			SARS-CoV-2 Protein 3		
	Mean	SD	% CV	Mean	SD	% CV	Mean	SD	% CV
Sample 1	4.2	0.5	11.7	3.7	0.5	12.2	3.8	0.5	12.0
Sample 2	2.0	0.2	8.9	1.8	0.2	10.0	2.0	0.1	6.3
Sample 3	1.6	0.2	14.2	1.5	0.2	14.3	1.7	0.2	12.9
Sample 4	0.2	0.1	33.8	0.2	0.1	26.4	0.1	0.1	32.9

Cross-reactivity Testing

Table 3. Testing of human serum / plasma samples from normal healthy individuals and presumed SARS-CoV-2 antibody negative samples

Presumed negative human serum/plasma samples	Total	Percent Cross-Reactivity
Samples collected before December 2019	299	0 %
Samples collected during 2020	197	0 %
Total	**496**	0 %

Table 4. Distribution of the testing of human serum / plasma samples from different disease-state individuals and presumed SARS-CoV-2 antibody negative

Other disease states	Before 2019	Percent Cross-Reactivity
Zika IgG Positive	10	0 %
Dengue IgG Positive	64	0 %
Chikungunya IgG Positive	6	0 %
HIV positive	18	0 %
HBV positive	86	0 %
HCV positive	52	0 %
EBV positive	6	0 %
CMV positive	6	0 %
HCoV 229 E	2	0 %
HCoV NL 63	2	0 %
HAMA	1	0 %
Rheumatoid Factor	1	0 %
Total	**254**	0 %

Clinical Agreement Testing:

Table 5. Testing of human clinical serum / plasma from SARS-CoV-2 RT-PCR tested, and convalescent samples

FlexImmArray SARS-CoV-2 Human IgG antibody test	SARS-CoV-2 RT- PCR Test		
	Positive	Negative	Total
Positive	28	0	28
Negative	0	31	31
Summary Statistics	**Percent**	**Low Limit**	**High Limit**
Positive Agreement (PPA)	100%	87.9%	100%
Negative Agreement (NPA)	100%	89.0%	100%
Overall Agreement (OAA)	100%	93.9%	100%

Table 6. Testing of human serum / plasma from individuals with presumed COVID-19 disease and presumed SARS-CoV-2 antibody negative samples

FlexImmArray SARS-CoV-2 Human IgG antibody test	Presumed COVID-19		
	Positive	Negative	Total
Positive	28	0	**28**
Negative	0	750	**750**
Summary Statistics	**Percent**	**Low Limit**	**High Limit**
Positive Agreement (PPA)	100%	87.9%	100%
Negative Agreement (NPA)	100%	99.5%	100%
Overall Agreement (OAA)	100%	99.5%	100%
Assumed Prevalence	5.0%		
Positive Predictive value (PPV)	100%	90.2%	100%
Negative Predictive Value (PPV)	100%	99.4%	100%

Acknowledgments:

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