ProterixBio

SARS-CoV-2 (RBD) IgG Antibody Test

- Measures antibodies that bind the RBD of the spike protein of the SARS-CoV-2 virus
- Output is a numerical index to better understand the course of an individual's immune response
- Track levels of IgG antibodies to the RBD which are associated with neutralizing activity
- Excellent sensitivity, specificity and quantitative performance
- Multiple blood sample types supported: serum, plasma and dried blood spot (DBS)

Clinical Significance

SARS-CoV-2 is a beta-coronavirus that causes COVID-19. Antibodies to SARS-CoV-2 appear during the first week after symptoms onset. Typically, levels of IgG are reliably detected after 15 days and persist for months.

The Receptor Binding Domain (RBD) is the region of the spike protein that binds to the ACE-2 receptor of host cells and facilitates virus entry. Studies have shown that antibodies to the RBD are associated with neutralizing activity, which plays a critical role in the adaptive immune response and the potential development of immunity. Several of the leading candidate SARS-CoV-2 vaccine designs incorporate sequences of the RBD.

Measurement of antibodies that bind the RBD is the most specific indicator of prior SARS-CoV-2 infection. Aside from SARS-CoV, the RBD of other common coronaviruses (e.g., NL63, HKU1) exhibit much less sequence similarity and, correspondingly, reduced potential for cross-reactivity.

Sensitivity and Specificity

The sensitivity of the SARS-COV-2 (RBD) IgG Antibody test was assessed by analyzing blood samples from individuals with prior SARS-CoV-2 infections confirmed by PCR. The timing of the blood sampling relative to the onset of symptoms was also recorded. As shown in the table below, the Positive Percent Agreement (PPA) was 100% for all samples acquired ≥15 days post onset of symptoms. For samples acquired earlier in the disease course (0-14 days), the PPA decreased to 50% reflecting variable seroconversion rates among individuals. Typically, it takes at least 15 days after the onset of symptoms for IgG levels to be reliably detected.

Positive Percent Agreement (PPA)

Days after Symptoms Onset	ProterixBio SARS-CoV-2 (RBD) IgG Antibody Test			
	Total	Positive	Negative	PPA
0 - 14 days	6	3	3	50%
15 - 20 days	4	4	0	100%
≥ 21 days	27	27	0	100%

The specificity of the SARS-COV-2 (RBD) IgG Antibody test was assessed by analyzing blood samples acquired pre-pandemic. This U.S.-based population covers a range of demographic categories (age, race, ethnicity), chronic conditions, exposure to common infectious diseases (influenza, common cold, hepatitis, etc) and vaccination history. As shown in the table below, the Negative Percent Agreement (NPA) was 100%.

Negative Percent Agreement (NPA)

Group	ProterixBio SARS-CoV-2 (RBD) IgG Antibody Test			
	Total	Positive	Negative	NPA
Pre-2019 adults, U.S. population	127	0	127	100%

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Additional Cross-Reactivity Testing

The specificity of the assay was further tested with samples with confirmed levels of antibodies to other infectious agents, including: Human Immunodeficiency Virus (HIV-1), Hepatitis A (HAV), Hepatitis B (HBc and HBs), Hepatitis C (HCV), Cytomegalovirus (CVM) and Human T-Cell Leukemia Virus (HTLV-1). All samples produced negative results. Additionally, spiking these materials into COVID-19 seropositive samples did not change the readout.

Quantitative Performance

The assay output is a numerical index that is the ratio of the antibody concentration of the sample to that of a calibrator level set at the positive/negative cut-off. The table below illustrates excellent precision across the measurement range. Additionally, the assay recovers to within 10% of expected for a range of dilutions. Samples with very high levels above the assay range can be serially diluted to achieve a reportable result.

Precision

Sample I.D.	N	Mean Index	Within Run		Day to Day	
			SD	CV%	SD	CV%
Sample A	80	16.65	1.23	7.1	0.60	3.6
Sample B	80	6.80	0.52	7.3	0.50	7.4
Sample C	80	3.39	0.17	4.7	0.28	8.2
Sample D	80	1.66	0.11	7.1	0.28	16.7
Sample E	80	0.54*	0.05	11.3	0.10	17.8

*Measured level is below the pos/neg cut-off (Index = 1) and just above LoQ for the assay

Linearity

Sample I.D.	Dilution	Observed Index	Expected Index	Recovery (%)
Sample F (representative of multiple individual samples tested)		11.5		
	1:2	5.8	5.8	93.0
	1:4	2.9	2.9	99.7
	1:8	1.4	1.4	100.5

References

- CDC Overview of Testing for SARS-CoV-2 (COVID-19), https://www.cdc.gov/coronavirus/2019-ncov/hcp/testing-overview.html, Aug 2020.
- FDA Policy for COVID-19 Tests During the Public Health Emergency (Revised), https://www.fda.gov/regulatory-information/search-fda-guidance-documents/policy-coronavirus-disease-2019-tests-during-public-health-emergency-revised, Aug 2020
- Krammer, F. and Simon, V., Serology assays to manage COVID-19, Science, 15May, 2020.
- Long et al, Antibody responses to SARS-CoV-2 in COVID-19 patients: the perspective application of serological tests in clinical practice, medRxiv, 20March, 2020.
- Okba et al, Severe Acute Respiratory Syndrome Coronavirus 2–Specific Antibody Responses in Coronavirus Disease Patients Emerg Infect Dis, 2020; 26(7).
- Atjeo et al, Distinct early serological signatures track with SARS-CoV-2 survival, Immunity 53, 1-9, 2020.
- Premkumar et al, The receptor binding domain of the viral spike protein is an immunodominant and highly specific target of the antibodies in the SARS-CoV-2 patients, Science Immunology, 11June 2020.
- Seow et al, Longitudinal evaluation and decline of antibody responses in SARS-CoV-2 infection, medRxiv, 11July, 2020
- Gudbjartsson et al., Humoral Immune Response to SARS-CoV-2 in Iceland, NEJM, 1Sept 2020.