

**ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
SARS-COV-2 RBD IGG FOR ANTIBODY DETECTION
(EMORY MEDICAL LABORATORIES)**

For *In vitro* Diagnostic Use
Rx Only
For use under Emergency Use Authorization (EUA) only

(The SARS-CoV-2 RBD IgG test for Antibody Detection will be performed at the Emory Medical Laboratories, Atlanta, GA, a laboratory certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a that meets the requirements to perform high complexity tests as per Emory Medical Laboratories SARS-CoV-2 RBD IgG Test Standard Operating Procedure (SOP) that was reviewed by the FDA under this EUA.)

INTENDED USE

The SARS-CoV-2 RBD IgG test is an Enzyme-Linked Immunosorbent Assay (ELISA) intended for the qualitative detection of IgG antibodies to SARS-CoV-2 in human serum. The SARS-CoV-2 RBD IgG 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. At this time, it is unknown for how long antibodies persist following infection and if the presence of antibodies confers protective immunity. The SARS-CoV-2 RBD IgG test should not be used to diagnose acute SARS-CoV-2 infection.

Testing is limited to Emory Medical Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meets the requirements to perform high-complexity tests. Results are for the detection of SARS CoV-2 antibodies. IgG antibodies to SARS-CoV-2 are generally detectable in blood several days after initial infection, although the duration of time antibodies are present post-infection is not well characterized. Individuals may have detectable virus present for several weeks following seroconversion.

Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

The sensitivity of the SARS-CoV-2 RBD IgG test early after infection is unknown. Negative results do not preclude acute SARS-CoV-2 infection. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary.

False positive results for the SARS-CoV-2 RBD IgG test may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

The SARS-CoV-2 RBD IgG test is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The SARS-CoV-2 RBD IgG test uses a recombinant form of the RBD region of the spike protein from SARS-CoV-2 attached to a solid support (ELISA plate) to capture IgG antibodies that may be present in a serum sample. Serum samples from subjects exposed to SARS-CoV-2 may contain antibodies against viral targets, including RBD. If IgG antibodies against RBD are present in the sample, they are detected using a secondary antibody (goat-anti-human IgG) conjugated to horseradish peroxidase, the reaction product of which is quantified using a BioTek 800 TS Absorbance Reader.

COMPONENTS SPECIFIC TO THE TEST

Components specific to the SARS-CoV-2 RBD IgG test for Antibody Detection include Receptor Binding Domain (RBD) fusion protein and high positive, low positive, and negative controls.

COMPONENTS REQUIRED BUT NOT SPECIFIC TO THE TEST

- Flat-Bottom Immuno Nonsterile 96-Well Plates: Catalog #: 3855 Fisher Healthcare
- Microtiter Plate Sealer: Catalog #: 14-245-18 Fisher Healthcare
- Bovine Serum Albumin (BSA) Powder: Catalog #: BP1600-100 Fisher Healthcare
- Wash Solution: 1 X PBS: Catalog #: MT20031CV Fisher Healthcare
- Tween 20: Catalog #: BP337-100 Fisher Healthcare
- Sterile Reservoirs: Catalog #: 07-200-127 Fisher Healthcare
- Elisa Buffer: 1%BSA, 0.2%Tween 20, 1 X PBS
- Conjugate: Goat Anti-Human IgG HRP in Elisa Buffer: Catalogs #: 62-8420 Fisher Healthcare
- Development Solution: Sigmafast OPD tablets in DI H₂O: Catalog #: P9187 Sigma Aldrich
- Stop Solution: 1M Hydrochloric Acid: Catalog #: S25856 Fisher Healthcare
- Paper Towels or Wypall Wipes: Catalog #: 1523560 Fisher Healthcare
- BioTek 50TS12 MicroPlate Washer 12 well: Serial #; 2004076
- BioTek 800TS Absorbance Reader: Serial #: 2003191F

CONTROLS TO BE USED WITH THE SARS-CoV-2 RBD IgG

Each 96-well plate contains 7 control samples: high-positive control (1), low-positive control (in triplicate), and negative control (in triplicate).

The high-positive control is produced by combining remaining serum from at least five prior samples with high positive results (OD value > 0.7); after verifying that the pool produces OD value > 0.7 when tested, it is aliquoted and frozen in single-use volumes.

The negative control is produced by pooling several previously-confirmed negative serum samples, and then testing and aliquoting them in the same way.

The low-positive control is produced by diluting the CR3022 monoclonal antibody into aliquots of negative control sera. When diluted 1:200 like patient samples, the resulting sample should produce OD = 0.175 - 0.400 (the positive cut-off value is set at 0.175).

INTERPRETATION OF RESULTS

Assessment of the SARS-CoV-2 RBD IgG test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted.

The cut-off for negative samples is set at 0.1 OD value. All 3 negative control samples must have OD values < 0.1 for the run to be valid. If the run is valid, all patient samples with OD values < 0.1 are interpreted as NEGATIVE.

The positive threshold is 0.175 OD value. All 3 low-positive control samples must have OD values > 0.175 for the run to be valid. If the run is valid, all patient samples with OD values > 0.175 are interpreted as POSITIVE.

The indeterminate range is set at 0.1 – 0.175 OD values. If the run is determined to be valid based on the results with the negative and positive controls, then samples that have OD in the indeterminate range are repeated in duplicate. If both duplicates are negative or positive, then the final interpretation is rendered as NEGATIVE or POSITIVE, respectively. If the OD readings for either 2 or 3 of the replicates place them in the indeterminate range (or if the three samples collectively tested negative, indeterminate, and positive), then the final interpretation is INDETERMINATE.

Table 1 summarizes the final interpretation of results obtained with the SARS-CoV-2 RBD IgG.

Table 1. Final interpretation of results.

SARS-CoV-2 RBD IgG test initial result (OD)	Retest result (OD)	Final result (OD)	Test result interpretation
< 0.1	N/A	Negative	
0.1 < x ≤ 0.175	2 results out of 3 are < 0.1	Negative	IgG antibodies to SARS-CoV-2 were not detected*. No retest is required.
	2 results out of 3 are between 0.1 and 0.175 OR 1 result is <0.1, 1 result is between 0.1 and 0.175, and 1 result is above 0.175	Indeterminate	Your specimen produced a borderline result. Repeat testing is indicated in 1-2 weeks.
	2 results out of 3 are ≥ 0.175	Positive	IgG antibodies to SARS-CoV-2 (the COVID-19 virus) were detected**. No retest is required.
> 0.175	N/A	Positive	

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* Negative results do not rule out SARS-CoV-2 infection, particularly in those who have been in contact with the virus. If clinically indicated, repeat testing can be considered at a later time. If an individual is symptomatic, direct viral testing is indicated.

** Consistent with a previous or recent infection with this virus. Positive results may also be due to past or present infection with non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E. A positive result may not indicate previous SARS-CoV-2 infection. Consider other information including clinical history and local disease prevalence, in assessing the need for a second but different serology test to confirm an immune response.

PERFORMANCE EVALUATION

1) Analytical Sensitivity:

There is no standard reference SARS-CoV-2 antigen material available; accordingly, absolute analytical sensitivity cannot be calculated.

2) Analytical Specificity

Reactivity/Inclusivity

Although mutations in the SARS-CoV-2 genome have been identified as the virus has spread, no serologically unique strains have been described relative to the originally isolated virus (this research is limited at present).

Cross reactivity

Cross-reactivity of the SARS-CoV-2 RBD IgG test was evaluated using 45 serum samples seropositive for various pathogens (characterized by ELISA) that were tested with the SARS-CoV-2 RBD IgG test. All of these samples produced negative results. No false positive results were observed. (see **Table 1**).

Table 1. Summary of the results of testing of samples antibody-positive for other pathogens/conditions.

Antibody Positive Serum	Source	Number of samples	SARS-CoV-2 RBD IgG test Results		
			Negative results	OD	Positive Results
Anti-Influenza A (IgG)	ELISA kit positive controls	3	3	<0.010	0
Anti-Influenza B (IgG)	Archived patient sample	1	1	<0.09	0
Anti-HCV (IgG and IgM)	Proficiency testing samples	5	5	<0.049	0
Anti-HBV (IgG and IgM)	Proficiency testing samples	5	5	<0.029	0
Anti-Haemophilus influenzae (IgG)	Archived patient samples	5	5	<0.073	0
Anti-229E (IgG)	Archived patient samples	4	4	<0.046	0
Anti-NL63	None identified to date				
Anti-OC43 (IgG)	Archived patient sample	1	1	<0.049	0

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Anti-HKU1	None identified to date				
ANA (IgG and IgM)	Proficiency testing samples	5	5	<0.010	0
Anti-Rhinovirus (IgG)	Archived patient samples	5	5	<0.091	0
Anti-Respiratory Syncytial Virus (IgG)	Archived patient samples and ELISA kit positive controls	5	5	<0.012	0
Anti-HIV (IgG)	Proficiency testing samples	6	6	<0.086	0

Class Specificity

The SARS-CoV-2 RBD IgG test uses the secondary antibody that is marketed for specific detection of human IgG.

In addition, the class specificity of the secondary antibody was confirmed by performing the SARS-CoV-2 RBD IgG test with IgG, IgM, and IgA polyclonal human antibody preparations (Sigma) coated separately onto several wells of ELISA plates at concentrations ranging from 1000-0.1 ng/mL. After blocking and washing the plate, either IgG, IgM, or IgA secondary antibodies conjugated with horse radish peroxidase were added. The plates were incubated, washed, and then the reactions were developed using the same protocol as for the SARS-CoV-2 RBD IgG test. The IgG secondary antibody is the exact lot, and was used at the same dilution, as for the clinical SARS-CoV-2 RBD IgG test. It was shown that the secondary antibody is highly specific for human IgG, with no cross-reactivity against either human IgM or IgG.

3) Clinical Performance

Clinical Sensitivity

The clinical performance of the SARS-CoV-2 RBD IgG test was evaluated by prospectively collecting 231 serum samples from inpatients who had prior (or coincident) positive nasopharyngeal RT-PCR results. The clinical comparator was one of three FDA authorized SARS-CoV-2 RT-PCR tests used on nasopharyngeal specimens: the Roche SARS-CoV-2 EUA, the Cepheid Xpert Xpress SARS-CoV-2 EUA, or the Emory-modified CDC EUA.

The samples were collected at Emory University Hospital (EUH; n=132) or Emory University Hospital-Midtown (EUHM; n=99). All testing was performed by a group of eight medical technologists in the Clinical Immunology Laboratory of EML.

Positive Percent Agreement (PPA) was determined by testing 231 samples collected over the course of time from unique subjects with a clinical diagnosis of COVID-19 based on positive results obtained with the FDA authorized PCR methods listed above. The following table describes positive percent agreement by time of sampling following a positive SARS-CoV-2 PCR result (see **Table**).

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Table 3. Results of the clinical study by days from positive PCR to serum collection.

Days from positive PCR	No. of specimens from PCR-confirmed positive subjects	No. of specimens positive with the SARS-CoV-2 RBD IgG test	PPA	95%CI
0 - 7	163	119	73.01%	65.72% - 79.23%
8 - 14	38	38	100.00%	90.82% - 100.00%
≥15	30	30	100.00%	95.58% - 100.00%

Clinical Specificity

For evaluation of the negative percent agreement (NPA), 388 serum specimens that were archived prior to December 2019 were tested with the SARS-CoV-2 RBD IgG test. Among the 388 archived pre-COVID-19 specimens, 379 had negative results (see **Table 2**). The NPA in this study is therefore 97.68% (95%CI: 95.65% to 98.78%).

Additionally, EML performed a large sero-surveillance study of health care workers (HCW) in the Emory Healthcare system. Out of 250 HCW whose RT-PCR results were negative, 236 tested negative, 5 indeterminate, and 9 positive by the SARS-CoV-2 RBD IgG test. Considering 236 out of 250 samples had SARS-CoV-2 RBD IgG test negative results, the NPA is 94.4% (95% CI: 90.82% to 96.64%) (see **Table 2**).

Table 2. NPA per clinical study.

Clinical Study	Sample Set	NPA and 95% CI
1	Prior to COVID-19	97.68% (379/388) 95% CI: 95.65% to 98.78%
2	Health Care workers (during COVID-19)	94.4% (236/250) 95% CI: 90.82% to 96.64%

Warnings

- This test has not been FDA cleared or approved.
- This test has been authorized by FDA under an emergency use authorization only for use by the authorized laboratory, Emory Medical Laboratories.
- This test has been authorized only for the detection of IgG antibodies to SARS-CoV-2, not for any other viruses or pathogens.
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
- For In Vitro Diagnostic Use Only
- Rx Only
- Not for the screening of donated blood

Limitations

- It is not known at this time if the presence of antibodies to SARS-CoV-2 confers immunity to infection. Results should only be interpreted in conjunction with the patient's medical history, clinical presentation, and other findings.
- The assay performance characteristics have not been established for matrices other than serum.
- This assay has not been evaluated with fingerstick specimens. This test is not authorized for use with fingerstick whole blood.
- The immune response may be depressed in elderly, immunocompromised, or immunosuppressed patients. Immunocompromised patients who have COVID-19 may have a delayed antibody response and produce levels of antibody which may not be detected as reactive by the assay.
- Results from antibody testing should not be used to diagnose or exclude active SARS-CoV-2 infection.
- A negative result for an individual subject indicates absence of detectable anti-SARS-CoV-2 antibodies. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions.
- A negative result can occur if the quantity of the anti-SARS-CoV-2 antibodies present in the specimen is below the detection limits of the assay, or the antibodies that are detected are not present during the stage of disease in which a sample is collected.
- Positive results may be due to past or present infection with non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E.
- A positive result may not indicate previous SARS-CoV-2 infection. Consider other information including clinical history and local disease prevalence, in assessing the need for a second but different serology test to confirm an immune response.
- The performance of this device has not been established in individuals that have received a COVID-19 vaccine. The clinical significance of a positive or negative antibody result following COVID-19 vaccination has not been established, and the result from this assay should not be interpreted as an indication or degree of protection from infection after vaccination.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.