

**ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
COVID-19 ELISA IGG ANTIBODY TEST
(MOUNT SINAI LABORATORY)**

For *In vitro* Diagnostic Use
Rx Only

For use under Emergency Use Authorization (EUA) only

(The COVID-19 ELISA IgG Antibody Test will be performed at Mount Sinai Laboratory (MSL), Center for Clinical Laboratories, New York, NY, a laboratory certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a to perform high complexity tests as per Laboratory Instructions for Use that was reviewed by the FDA under this EUA.)

INTENDED USE

The COVID-19 ELISA IgG Antibody Test consists of two serial direct Enzyme-Linked Immunosorbent Assays (ELISA) for the qualitative detection of human IgG antibodies in serum and plasma specimens collected from individuals suspected of prior infection with the virus that causes COVID-19 by their healthcare provider. An initial ELISA is performed against recombinant Receptor Binding Domain of SARS-CoV-2 in serum and plasma, followed for positive specimen by a confirmatory ELISA against full length SARS-CoV-2 Spike protein in serum and plasma. The COVID-19 ELISA IgG Antibody Test detects IgG antibodies as indicative of an immune response to SARS-CoV-2 in patients suspected of previous SARS-CoV-2 infection, or for the detection of IgG seroconversion in patients following known recent SARS-CoV-2 infection. The test is an aid in the diagnosis of patients with suspected of prior COVID-19 in conjunction with clinical presentation and the results of other laboratory tests. Results from the COVID-19 ELISA IgG Antibody Test should not be used as the sole basis for diagnosis and should not be used for the diagnosis of patients with acute COVID-19 infection. Testing is limited to the Mount Sinai Laboratory (MSL), Center for Clinical Laboratories), Department of Pathology, Molecular, and Cell-Based Medicine, New York, NY-10029, that is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests.

Results are for the detection of IgG SARS-CoV-2 antibodies. IgG antibodies to SARS-CoV-2 generally become detectable beginning 10 – 14 days following infection but may occur later. The presence of IgG antibodies, following previously negative testing, defines IgG antibody seroconversion following SARS-CoV-2 infection. The COVID-19 ELISA IgG Antibody Test may also be used to identify positive specimens with an antibody titer up to a dilution of 1:2880 for the identification of individuals with higher antibody titers.

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

Negative results do not preclude acute SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. IgG antibodies may not be present for

more than two weeks following infection, and patients may remain infectious during acute infection even if IgG antibody is present. Results must be combined with clinical observations, patient history, and epidemiological information. The sensitivity of the COVID-19 ELISA Antibody IgG Test early after infection is unknown.

False positive results for IgG antibodies may occur due to cross-reactivity from pre-existing antibodies or other possible causes. Prevalence of SARS-CoV-2 infection in the area where testing has occurred should be considered when interpreting positive test results.

At this time, it is unknown for how IgG antibodies may persist following infection.

The COVID-19 ELISA IgG Antibody Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The COVID-19 ELISA IgG Antibody Test has been developed for the qualitative detection of human SARS-CoV-2 IgG antibody and measurement of circulating antibody titer in serum via direct Enzyme-Linked ImmunoSorbent Assay (ELISA). Assay controls and patient serum samples are diluted 1:50 and added to Thermo Scientific Immulon 96-wells microtiter plate that was coated with SARS-CoV-2 recombinant Receptor Binding Domain protein (RBD). The coated RBD protein combines with patient's SARS-CoV-2 IgG antibodies. The subsequent addition of a secondary anti-human IgG (Fab specific) HRP labeled antibody creates a specific complex of antigen-antibody bound to the plate surface. The binding reaction is then enhanced visually with Sigma-Aldrich *SIGMAFAST OPD* (o-Phenylenediamine dihydrochloride) substrate generating a yellow color for positive specimens. After application of the stop solution (3M Hydrochloric acid), the color changes to orange and optical density is monitored at 490 nm. The depth of color is relative with the content of the SARS-CoV-2 IgG antibodies. When the value of color is greater than the cut-off value (OD₄₉₀ = 0.15), the specimens are reported as a presumptive positive screen result. Positive screen specimens are then serially diluted for assessment of total circulating antibody titer (80x, 160x, 320x, 960x, 2800x) using SARS-CoV-2 Spike protein coated Thermo Scientific Immulon 96-wells microtiter plate. There is a stronger reaction against the full-length Spike protein than against the RBD, likely reflecting the higher numbers of epitopes found on the much larger Spike protein. A positive spike protein response is detection of antibody at a titer of $\geq 1:80$. Positive results from both the RBD antibody ELISA and the spike protein ELISA are necessary for an overall positive test result.

COMPONENTS SPECIFIC TO THE TEST

Components specific to the COVID-19 ELISA IgG Antibody Test include the recombinant RBD protein and the recombinant full-length Spike protein are both developed at the Mount Sinai Laboratory (MSL).

COMPONENTS REQUIRED BUT NOT SPECIFIC TO THE TEST

- Flat-Bottom Immuno Nonsterile 96-Well Plates 4 HBX (Thermo Scientific #3855, or equivalent)
- Thermo Scientific (Waltham, MA) Nunc MicroWell 96-Well Microplates, Non-Treated, Cat# 260895, or equivalent
- Milk Powder, Fisher Scientific, Cat# NC0115668, or equivalent. Store at room temperature, stable until expiration date
- PBS (1X) (Gibco), ThermoFisher, Cat# 100010023, or equivalent. Store at room temperature, stable 24 months from date of manufacture
- Water for Injection (WFI) for Cell Culture (Gibco #A1287301 or equivalent)
- Tween 20 (C58H114O26; MW: 1227.5), Fisher BioReagents, Fisher Scientific, Cat# BP337-500, CAS# 9005-64-5, or equivalent, store at room temperature, stable until expiration date.
- Phosphate Buffered Saline (10X), Fisher Scientific, Cat# MT-46013CM, or equivalent, store at room temperature, stable until manufacturers expiration date.
- Eppendorf (Hamburg, Germany) Repeater M4 pipettor; 10 mL Eppendorf tips (Cat# 30089820), 5 mL Eppendorf tips (Cat# 30089812) and 1 mL Eppendorf tips (Cat# 30089790)
- Micropipette tips
 - Fisher Scientific (Hampton, NH) Fisherbrand SureOne Micropoint Pipette Tips, Universal Fit, Non-Filtered 0.1-10 μ L pipette tips (Cat. # 02-707- 420), or equivalent
 - Fisher Scientific (Hampton, NH) Fisher Brand 1-200 μ L pipette tips (Cat. # 02-707- 420), or equivalent
 - USA Scientific, 200 μ L TIPONE PIPETTE TIP STACKS, Cat# 1111-0240, or equivalent
 - Fisher Scientific (Hampton, NH) Fisher Brand 100-1250 μ L pipette tips (Cat. #02-707- 403), or equivalent
- Sterile, serological pipettes
 - 5mL (Falcon #356543 or equivalent)
 - 10mL (Falcon #357551 or equivalent)
 - 25 mL (Falcon #357535 or equivalent)
 - 50 mL (Falcon #356550 or equivalent)
- Polypropylene sterile conical tubes
 - Fisher Scientific Centrifuge Tubes, 15 mL (17 X 120mm), Molded with Graduations, Polypropylene with screw cap, Fisher Scientific, Cat# 50-145-8829, or equivalent.
 - Fisher Scientific Tubes, 50 mL (30 X 115mm), Molded with Graduations, Polypropylene with screw cap, Fisher Scientific, Cat# 50-145-8841, or equivalent.
- Anti-Human IgG (Fab specific, in 0.01 M phosphate buffered saline, pH 7.4, containing 0.01% thimerosal)-Peroxidase antibody produced in goat, Sigma Aldrich (St. Louis, MO), Cat# A-0293. Store at - 20°C freezer, stable until manufacturer's expiration date.
- Hydrochloric Acid 3.0M (HCl; MW:36.4), Fisher Scientific, Cat# S25856, CAS# 7647-01-0, or equivalent. Store at room temperature in flammable safety cabinet.

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- SIGMAFAST OPD (*o*-Phenylenediamine dihydrochloride), Sigma-Aldrich (St. Louis, MO), #P9187 or equivalent, storage temperature 2-8 C, stable until manufacturer's expiration date.
- Precision pipette capable of delivering 0-20 µL, Eppendorf Research Plus or equivalent, (San Diego, CA) Cat #3120000810)
- Precision pipette capable of delivering 0-2.5 µL, Eppendorf Research Plus or equivalent, (San Diego, CA) Cat#3120000011)
- Precision pipette capable of delivering 0-200 µL, Eppendorf Research Plus or equivalent, (San Diego, CA) Cat#3123000055)

CONTROLS TO BE USED WITH THE COVID-19 ELISA IgG Antibody Test

Quality control serum is human serum with known acceptable results for RBD screen and titer determination. Each assay must include both negative and positive controls. The acceptable average value of the absorbance of the negative control is less than 0.1 at 490 nm, and the acceptable average value for the absorbance of the positive control set to greater than 0.15.

INTERPRETATION OF RESULTS

The cut-off for screening by receptor binding domain protein (RBD) and titer analysis by spike protein is ≥ 0.15 at an absorbance of 490 nm.

Negative result: A negative antibody screen result indicates that a serum dilution of 1/50 showed no specific antibodies to SARS-CoV-2 virus in RBD ELISA.

Positive result: The specimen is classified (reported) as POSITIVE for SARS-CoV-2 IgG antibodies when both the RBD screen and Spike protein testing are positive. A positive antibody screen result indicates IgG antibodies to SARS-CoV-2 virus at a dilution greater than 1/50. Subsequent testing by the spike protein is considered positive if antibody is detected at a titer of 1:80 or greater. The test result is considered overall positive only if positive results are seen for the RBD screen ELISA and subsequent spike protein ELISA.

Interpretation of Results

Test	Result	FINAL RESULT	
RBD screen (Part A)	NEGATIVE	NEGATIVE	No further testing
	POSITIVE*		Presumptive positive; test by spike protein
Spike protein (Part B)	NEGATIVE	NEGATIVE	
	POSITIVE* (titer > 1:80)	POSITIVE	Confirmed positive for IgG antibody

* > 0.15 at an absorbance of 490 nm

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).

Analytical Specificity and Microbial Interference:

Cross-reactivity of non SARS-CoV-2 specific Ab against RBD protein was examined using sera with known antibodies against confirmed past infections or elevated gamma globulins described below. No interference was observed. Negative screen results using RBD protein have been shown to be negative when tested at 1:80 dilution using Spike protein as antigen.

Antibody positive sera	N	COVID-19 ELISA IgG Antibody Test Result
Varicella	5	NEGATIVE
Influenza	4	NEGATIVE
HSV	7	NEGATIVE
Rubella	5	NEGATIVE
Hepatitis	4	NEGATIVE
HIV	4	NEGATIVE
Elevated IgG	5	NEGATIVE
Elevated IgM	4	NEGATIVE
CMV	2	NEGATIVE

3) Precision

Assay within day and between-day precision was evaluated using negative and positive controls for both the RBD and spike protein confirmation ELISAs. Precision was reported as the coefficient of variation (CV). Within day precision was calculated by analyzing 10 replicates of negative and positive controls on one day. Between day precision was calculated by analyzing negative and positive controls for 21 different days. The acceptance criterion for within day and between day precision (%CV) was ≤ 20% for each control tested. The cut-off for positive control was > 0.15 at OD490 nm and for the negative control was < 0.15

For the RBD and spike proteins assays, precision was measured over 22 days. For the spike protein, 5 dilutions of the positive and negative controls were tested. Within day and between day precision measurements for both the RBD screen and spike protein confirmation met the specified acceptance criteria.

4) **Clinical Evaluation:**

Test performance was assessed for samples obtained from patients with clinical findings suggestive of COVID-19 infection; patients were subsequently identified to include both PCR+ or PCR- negative patients. Initially 58 serum samples were collected between 7 and 14 days after onset of symptoms. Fifty five presumed true negative samples that had been collected prior to the onset of the emergence of COVID-19 pandemic were also randomized before sending to the clinical laboratory for testing. The COVID-19 ELISA IgG Antibody Test on samples at or before 14 days were positive in 67% of cases. PCR status of patients was subsequently confirmed, and all PCR+ patients retested at 21 days and results reanalyzed for positive and negative agreement. PCR+ samples were 92.5 % positive and all negative samples returned a negative result for 100% negative agreement. Results are shown in the table below:

	SARS-CoV-2 PCR Status			Total
		Positive	Negative	
COVID-19 Elisa IgG Antibody Test	Positive	37	0	37
	Negative	3	74	77
	Total	40	74	114

Positive agreement: 92% (95% CI: 79%, 98%)

Negative agreement: 100% (95% CI: 94%, 100%)

For positive samples, titers to 1:2880 were determined by dilution. Samples with titers to 1:2880 represent approximately 44% of all samples positive above the threshold titer of 1:80 for the spike protein confirmatory assay.