COVID-19 IgG/IgM
Antibody Test

Novel Coronavirus COVID-19 IgG and IgM ELISA Tests Performed by Diagnostic Solutions Laboratory

Testing has proven to be paramount in the detection and containment of SARS-CoV-2, the virus that causes COVID-19. Nasopharyngeal swab using RT-PCR to detect viral RNA is the diagnostic test of choice during acute illness. However, serological studies measuring IgG and IgM antibodies can aid in COVID-19 diagnosis and give further information about current and late immune response to the virus. The unprecedented urgency and poor regulation of testing during this global crisis has led to a flood of tests into the market. Clinicians and consumers alike deserve details about the performance of SARS-CoV-2 assays in order to select the most accurate and precise test for medical care.

This document provides technical information about the COVID-19 IgG/IgM Antibody Test used by Diagnostic Solutions Laboratory. Validation data (below) shows that the assays are repeatable and reproducible with low variability < 10%. They accurately measure the correct immunoglobulin class and don't cross-react with other common viruses or antibodies.

Diagnostic Solutions laboratory

In studies of clinical relevance, the EDI IgG assay had a clinical sensitivity of 100% when blood was collected 15–22 days after symptom onset.[1] Ambroise Pare Hospital showed the IgG assay was 96% sensitive and 99% specific when 80 known positives were tested 14 days after symptoms.[2] Clinical sensitivity was 100% and clinical specificity was 88.7% for the IgG assay in a study of 50 patients with COVID-19.[3] For the IgG assay, EDI (the kit manufacturer) found that the positive predictive value (PPV) was 98.4% and the negative predictive value (NPA) was 99.8%.

The IgM assay showed clinical sensitivity of 81% and clinical specificity of 97%, when blood samples were collected 10–14 days after symptoms.[2] IgM positive predictive value was 94% at 15–22 days after initial symptoms.[1] EDI found that the IgM assay had positive predictive value of 73.8% and negative predictive value of 100%.

The false positivity rate was < 3% for both EDI IgG and IgM kits.[1] Study details can be found below.

Clinical **sensitivity** is the percentage of true positives detected by a test *(e.g. 90% sensitivity = 90% of positives will be true positives).*

Clinical **specificity** is the percentage of true negatives *(e.g. 99% specificity = 99% of negatives will be true negatives).*

Positive Predictive Value (PPV) is the probability that subjects with a positive screening test truly have the disease. Unlike clinical sensitivity, it also depends on the *prevalence* of the disease.

Negative Predictive Value (NPV) is the probability that subjects with a negative screening test truly don't have the disease. Unlike clinical specificity, it depends also on the *prevalence* of the disease.

Assay Manufacturer

The assay developers, Epitope Diagnostics (EDI), have a 16-year history of developing and producing high-quality in vitro diagnostic tests for the research, pharmaceutical, and healthcare community. Located in San Diego, they are a certified and licensed medical device manufacturer by the State of California Department of Health.

Method

The EDI IgM and IgG SARS-CoV-2 tests are performed using a sandwich ELISA (enzyme-linked immunosorbent assay) method. This is a very well respected and commonly used methodology in scientific research and clinical laboratory medicine. It starts with a plate that is coated with SARS-CoV-2 recombinant full length nucleocapsid protein. The patient's serum or plasma is added to the plate. Using the IgG kit as an example, any SARS-CoV-2 IgG antibodies present in the patient's blood will bind to the nucleocapsid protein on the plate. The last part of the "sandwich" is to add anti-human IgG tracer antibody to the plate. It will bind any complexes on the plate consisting of SARS-CoV-2 and human IgG antibodies. The tracer is a fluorescent molecule that can be measured on a spectrophotometric plate reader. The amount of tracer bound to the anti-SARS-CoV-2 IgG antibodies is proportional to the amount of IgG antibodies in the patient specimen. On the plate reader, if the patient's sample optical density (OD) was below the positive cutoff the result was reported negative; If the patient's sample OD was equal or above the positive cutoff the patient was reported as positive.

Analytical Performance

The EDI IgG and IgM assays were repeatable, meaning that many replicates in the same batch gave the same result, with variation < 10%. The assays were reproducible, meaning that when the same samples were tested across twelve different batches, the results were the same, with variation less than 3.92%. The manufacturer found that the limit of detection was 0.669 and even at the top of that range, the assay had acceptable variation of < 6.46%. Class specificity

tests showed that the assays detected the correct immunoglobulin class *(for example, the IgG kit detected IgG only, not IgM)*. EDI evaluated 624 coronavirus-negative patient samples with the IgM and IgG kits and found no cross-reactivity with other common viruses or antibodies. Validation details can be found below.

Limit of Detection

Three lots of material were tested with one assay using a blank control in sixteen replicates. LoD was calculated as the mean of the OD for the blank control plus three times the standard deviation. The highest of the three runs was established for the LoD at 0.0669 for IgM and 0.0666 for IgG.

The IgM Results Were as Follows:

	Average OD (450 nm)	CV (%)	LOD (x? + 3 SD)
Run 1	0.0560	5.32%	0.0649
Run 2	0.0568	5.63%	0.0663
Run 3	0.0561	6.46%	0.0669

The IgG Results Were as Follows:

	Average OD (450 nm)	CV (%)	LOD (x? + 3 SD)
Run 1	0.0481	4.83%	0.0550
Run 2	0.0518	5.71%	0.0606
Run 3	0.0531	8.44%	0.0666

Repeatability

One lot of material was tested with one assay using three samples *(strong positive, light positive, and negative)* in sixteen replicates. For all sixteen replicates, sample 1 and 2 were positive and in sample 3 all were negative. The repeatability results were very satisfactory with acceptable CVs less than 10%.

The IgM Results Were as Follows:

	Average OD (450 nm)	Results	CV (%)
Run 1	1.023	16/16 are Positive	4.48%
Run 2	0.443	16/16 are Positive	4.83%
Run 3	0.125	16/16 are Negative	9.17%

The IgG Results Were as Follows:

	Average OD (450 nm)	Results	CV (%)
Run 1	1.071	16/16 are Positive	6.35%
Run 2	0.631	16/16 are Positive	3.11%
Run 3	0.199	16/16 are Negative	4.99%

Reproducibility

One lot of material was tested over twelve assays using three samples *(strong positive, light positive, and negative)* in two replicates and a set of positive and negative controls in three replicates. For all twelve assays, samples 1 and 2 were positive and sample 3 was negative. The results for reproducibility were very satisfactory with an acceptable CV less than 4.66%.

The IgM Results Were as Follows:

ID	Average OD (450 nm)	Results	CV (%)
Sample 1	1.18	12/12 are Positive	1.93%
Sample 2	0.53	12/12 are Positive	2.37%
Sample 3	0.13	12/12 are Negative	3.32%
Negative Control	0.09	12/12 are Negative	3.92%
Positive Control	0.89	12/12 are Positive	3.49%

The IgG Results Were as Follows:

ID	Average OD (450 nm)	Results	CV (%)
Sample 1	1.11	12/12 are Positive	1.96%
Sample 2	0.65	12/12 are Positive	3.47%
Sample 3	0.19	12/12 are Negative	4.66%
Negative Control	0.17	12/12 are Negative	3.15%
Positive Control	0.65	12/12 are Positive	4.14%

Class Specificity

To evaluate class specificity, ten RT-PCR confirmed COVID-19 patient serum samples were tested in duplicate in the Epitope Diagnostics, Inc. IgG and IgM ELISA Kits. Samples 1–5 were IgM positive and IgG negative. Sample 1 is a natural and untreated IgM positive, IgG negative. Sample 2–5 were originally positive for IgG and IgM but used protein A/ProSep A to remove the IgG. Samples 6–10 were IgG positive and IgM negative. All samples 6–10 were natural and untreated IgG positive, IgM negative. There was 100% agreement between the results of this

test. This demonstrates that the assay is specific to the detection of IgM class without cross reaction to COVID-19 IgG class and vice versa.

The IgM Results Were as Follows:

Sample ID	IgM Result	IgG Result
Sample 1	+	—
Sample 2	+	—
Sample 3	+	—
Sample 4	+	—
Sample 5	+	—
Sample 6	—	+
Sample 7	—	+
Sample 8	—	+
Sample 9	—	+
Sample 10	—	+

Assay Cross-Reactivity

Six hundred twenty-four (N=624) known negative samples collected in the US prior to December 2019 were tested from a population with a high prevalence of vaccination against, and/or infection with, the following viruses. Specificity of 99.8% was observed. Therefore, cross-reactivity for the following viruses would be extremely unlikely at this time.

- Anti-influenza A (IgG and IgM)
- Anti-influenza B (IgG and IgM)
- Anti-HCV (IgG and IgM)
- Anti-HBV (IgG and IgM)
- Anti-Haemophilus influenzae (IgG and IgM)
- Anti-229E (alpha coronavirus)
- Anti-NL63 (alpha coronavirus)
- Anti-OC43 (beta coronavirus)
- Anti-HKU1 (beta coronavirus)
- ANA
- Anti-respiratory syncytial virus (IgG and IgM)
- Anti-HIV

To demonstrate cross-reactivity of the test, Epitope Diagnostics, Inc. used the FDA required minimum of 5 individual samples tested in duplicate for each disease/infectious agent using human specimens confirmed by outside commercially available diagnostic tests. All samples were sourced from human specimens using sera from patients with the underlying diseases in the acute or convalescent stages of infection. Anti-haemophilus influenzae and rhinovirus could not be tested due to lack of availability. Cross-reactivity with these viruses and antibodies was not seen.

Agent	Disease State Confirmation Test	Results
Influenza A	Viron/Serion	5/5, Negative
Influenza B	Viron/Serion	5/5, Negative
Respiratory syncytial virus	EIA, Virion/Serion	5/5, Negative
Hepatitis C Virus	Roche Ampliprep/Taqman	5/5, Negative
Antinuclear Antibodies	Bio-Rad Hep 2	5/5, Negative
Hepatitis B Virus	Siemens	5/5, Negative

Clinical Sensitivity and Specificity
(Positive Predictive Value and Negative Predictive Value)

Clinical Relevance Studies by the Kit Manufacturer

Epitope Diagnostics measured IgG antibodies and IgM antibodies in known positive and negative patient sera. For IgM, the positive predictive value (PPV) was 73.8% and negative predictive value (NPA) was 100% using a total of 195 human specimens. For the IgG assay, using 811 human specimens, PPV was 98.4% and NPA was 99.8%. The assays demonstrate acceptable PPV and NPA, when used together. Assay details follow.

Patient samples were tested using the IgM ELISA kit at three sites: Center for Disease Control and Prevention in China, a University Hospital in China, and a laboratory in the United States. The combined cohort consisted of normal healthy patients with samples collected prior to the COVID-19 outbreak (N=153) and RT-PCR confirmed positive patients (N = 42).

The Results Were as Follows:

		Confirmed Positive	Confirmed Negative
EDI™ Novel Coronavirus COVID-19 IgM ELISA Kit	**Positive**	30	0
	Negative	11	233
	Total	41	233
PPA	73.8%	95% CI (Wilson's Score):	0.581–0.843
NPA	100%	95% CI (Wilson's Score):	0.976–1.000

Patient samples were tested using the IgG ELISA kit at four sites: Center for Disease Control and Prevention in China, a University Hospital in China, a laboratory in the United States, and a University Hospital Laboratory in the United States. The combined cohort consisted of normal healthy patients with samples collected prior to the COVID-19 outbreak (N=624) and RT-PCR confirmed positive patients (N = 187).

The Results Were as Follows:

		Confirmed Positive	Confirmed Negative
EDI™ Novel Coronavirus COVID-19 IgM ELISA Kit	**Positive**	184	1
	Negative	3	623
	Total	187	624
PPA	98.4%	95% CI (Wilson's Score):	0.954–0.995
NPA	99.8%	95% CI (Wilson's Score):	0.991–0.9997

Clinical Relevance Studies by Independent Third Parties

The EDI IgM showed clinical sensitivity of 94%, while IgG had a clinical sensitivity of 100%, in 18 confirmed COVID-19 blood samples drawn 15–22 days after symptom onset. The false positivity rate for the EDI kits was < 3% when testing non-coronavirus specimens collected from healthy blood donors and intensive care patients prior to December 2019 (n=456).[1]

Ambroise Pare Hospital evaluated the IgG and IgM kits from Epitope Diagnostics. Serum from 80 patients with SARS-CoV-2 infection (confirmed by RT-PCR) and 92 serum specimens collected prior to December 2019 (negatives) were tested for IgM and IgG. For IgM, sensitivity was 81% days 10–14 after symptoms and specificity was 97%. For IgG, sensitivity was 96% after 14 days of symptoms and specificity was 99%. The authors stated, *"the performances are very satisfactory with IgG: specificity of 99% and sensitivity of 96% at more than 14 days from the onset of symptoms."*

Sensitivity Results as a Function of Time Between Symptoms or PCR and Specimen Collection

IgM	Days Between Onset of Symptoms and Specimen Collection			
	< 10	**10–14**	**> 14**	**Total**
Ind			2	2
Neg	8	3	14	25
Pos	4	13	33	50
% Pos/Tot	33%	81%	67%	
% Pos/Neg	**33%**	**81%**	**70%**	
Total Tested	*12*	*16*	*49*	*77*

IgM	Days Between RT-PCR and Specimen Collection			
	< 10	**10–14**	**> 14**	**Total**
Ind	2	1	1	4
Neg	12		12	24
Pos	21	14	16	51
% Pos/Tot	60%	93%	55%	
% Pos/Neg	**64%**	**100%**	**57%**	
Total Tested	*35*	*15*	*29*	*79*

Pos: *Positive;* ***Neg:*** *Negative;* ***Ind:*** *Undetermined*

IgG	Days Between Onset of Symptoms and Specimen Collection			
	< 10	10–14	> 14	Total
Ind	3	2	2	7
Neg	7	4	2	13
Pos	2	10	46	58
% Pos/Tot	17%	63%	92%	
% Pos/Neg	**22%**	**71%**	**96%**	
Total Tested	*12*	*16*	*50*	*78*

IgG	Days Between RT-PCR and Specimen Collection			
	< 10	10–14	> 14	Total
Ind	3	2	3	8
Neg	13		1	14
Pos	19	13	26	58
% Pos/Tot	54%	87%	87%	
% Pos/Neg	**59%**	**100%**	**96%**	
Total Tested	*35*	*15*	*30*	*80*

Pos: *Positive;* ***Neg:*** *Negative;* ***Ind:*** *Undetermined*

Sensitivity Results More Than 10 Days After Symptoms Based on Severity

IgG	> 10 Days Between Onset of Symptoms and Specimen Collection		
	Non Hospi	Hospi	ICU
Ind	3		1
Neg	2	2	2
Pos	15	17	24
% Pos/Neg	**88%**	**89%**	**92%**

Non Hospi: *Patient with milder forms without hospitalization.*
Hospi: *Patient hospitalized in conventional units.*
ICU: *Patient hospitalized in Intensive Care Unit or deceased.*

In a study comparing the EDI IgG assay to three others, authors found the clinical sensitivity to be 100% and the specificity to be 88.7%.[3] The study measured 50 serum specimens from patients with known COVID-19 (positive PCR result in respiratory specimens) and 25 specimens collected from patients with a negative respiratory PCR specimen. Of all the assays, EDI's IgG assay showed the highest sensitivity.[3] Authors said it showed sufficient specificity and sensitivity for identifying individuals with past SARS-CoV-2 infection.

Sensitivity and Specificity of Four SARS-CoV-2 IgG Assays

Test Results				
Assay Manufacturer	Rate of Correct Positive Test Results	Rate of Correct Negative Test Results	Sensitivity	Specificity
Euroimmun	19/22	51/53	86.4%	96.2%
EDI	**22/22**	**47/53**	**100%**	**88.7%**
Mikrogen	19/22	53/53	86.4%	100%
Viramed	17/22	53/53	77.3%	100%

Anti-SARS-CoV-2 IgG antibody was measured in donor convalescent plasma with the EDI IgG assay. Subsequent treatment of two patients with the donated plasma improved acute respiratory distress syndrome.[4]

Performance at Diagnostic Solutions Laboratory

Our internal validations resulted in average IgG variation of 9–13% and IgM variation of 3–11%. A significant portion of the variation was related to sample age. We tested serum samples with more than overnight shipping and serum that was stored for longer than six months. The longer shipping and storage times had increased variation compared to shorter shipping and storage times.

Food and Drug Administration Status

The EDI IgG and IgM kits were submitted to the FDA under number PEUA200035 on March 05, 2020 for Emergency Use Authorization (EUA) and are pending approval. In compliance with the May 4th revisions of the Policy for Diagnostic Tests for Coronavirus Disease – 2019 during the Public Health Emergency, the kits were assigned the number EUA201310. EDI is in discussions with the FDA but does not have a timeline for approval. Please note that the kits are in compliance with all FDA regulations and are eligible for use and distribution in the United States.

On the FDA's website for FAQs on Testing for SARS-CoV-2 there is a section titled: ***"Q: What commercial manufacturers are distributing serology test kits under the policy outlined in Section IV.D of the Policy for Coronavirus Disease-2019 Tests? (Updated 6/10)."*** The products from Epitope Diagnostics are listed under this section, demonstrating that notification has been given to the FDA and an EUA has been submitted.

Limitations of the Assays

1. This test is only for qualitative detection. Test results should not be the sole basis for clinical diagnosis and treatment. The confirmation of infection with novel coronavirus (COVID-19) must be combined with the patient's clinical signs in conjunction with other tests.
2. Patients with low immunity or other diseases that affect immune function, failure of important systemic organs, and use of drugs that suppress immune function can also lead to negative results of SARS-CoV-2 IgG and IgM.
3. Negative results do not rule out SARS-CoV-2 infection, particularly in those who have been in contact with the virus.
4. Positive results may be due to past or present infection with other (non-SARS-CoV-2) coronavirus strains, such as coronavirus HKU1,NL63, OC43, or 229E.

Published Studies Using the EDI Kits

The following papers utilize the EDI™ Novel Coronavirus COVID-19 IgG or IgM ELISA Kits:

- Ahn JY et al. Use of Convalescent Plasma Therapy in Two COVID-19 Patients with Acute Respiratory Distress Syndrome in Korea. *J Korean Med Sci*. 2020;35(14):e149. Published 2020 Apr 13. doi:10.3346/jkms.2020.35.e149
- Ambroise Pare Hospital, Evaluation report of the EDI test at Ambroise Paré Hospital. Assistance Publique Hospitaux de Parix. Published 2020 May 14.
- Alexander Krüttgen et al. Comparison of four new commercial serologic assays for determination of SARS-CoV-2 IgG. Journal of Clinical Virology. 2020;128:104394. Published 2020 Apr 22. doi:10.1016/2020.104394
- Jeffrey D. Whitman, et al. Test performance evaluation of SARS-CoV-2 serological assays. MedRXiv. Published 2020 Apr 29. doi:10.1101/2020.04.25.20074856 [Not certified by peer review] — This study was conducted using deviations to the instructions for use and with inadequate conditions. For additional information, please view the statement from Epitope Diagnostics, Inc. on the validity of the study.
- Margo Egger, et al. Comparison of the Elecsys® Anti-SARS-CoV-2 immunoassay with the EDITM enzyme linked immunosorbent assays for the detection of SARS-CoV-2 antibodies in human plasma. Clinica Chimica Acta. Published 2020 May 28. doi/10.1016/j.cca.2020.05.049
- Renata Varnaite, et al. Expansion of SARS-CoV-2-specific Antibody-secreting Cells and Generation of Neutralizing Antibodies in Hospitalized COVID-19 Patients. BioRXiv. Published 2020 May 29. doi/10.1101/2020.05.28.118729
- Denise Roland. HEALTH Blood of Recovered Covid-19 Patients Is Becoming a Hot Commodity. Wall Street Journal. Published 2020 May 29.

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1. Egger M, Bundschuh C, Wiesinger K, et al. Comparison of the Elecsys(R) Anti-SARS-CoV-2 immunoassay with the EDI enzyme linked immunosorbent assays for the detection of SARS-CoV-2 antibodies in human plasma. *Clin Chim Acta.* 2020;509:18-21.
2. Evaluation report of the EDI test at Ambroise Pare Hospital. 2020; https://static1.squarespace.com/static/52545951e4b021818110f9cf/t/5ec2b09288c9ec04fa2ff41f/1589817494069/rapport+validation+EDi_anglais_2+%283%29.pdf. Accessed June 9, 2020.
3. Kruttgen A, Cornelissen CG, Dreher M, Hornef M, Imohl M, Kleines M. Comparison of four new commercial serologic assays for determination of SARS-CoV-2 IgG. *J Clin Virol.* 2020;128:104394.
4. Ahn JY, Sohn Y, Lee SH, et al. Use of Convalescent Plasma Therapy in Two COVID-19 Patients with Acute Respiratory Distress Syndrome in Korea. *Journal of Korean medical science.* 2020;35(14):e149.