CovIgG-Assay

(Version 3.0 July 07th, 2020)

For in vitro diagnostic use. For prescription use only. Emergency Use Authorization Submission Number: EUA201115

Instructions for Use

Contents:

The CovIgG-Assay kit is intended for the quantitative detection of human anti-COVID19 IgG antibody in human serum.

Intended Use:

The CovIgG-Assay kit contains an enzyme-linked immunosorbent assay intended for the quantitative detection of IgG class antibodies to SARS-CoV-2 in human serum or plasma. This test is intended for use in aiding the identification of individuals with an adaptive immune response to SARS-CoV-2, which indicates a current or past infection. For now, it is unknown how long the antibodies persist or if they confer protective immunity.

Results are for the detection of SARS-Cov-2 antibodies. IgG antibodies to SARS-CoV-2 are generally detectable in blood several days after an infection, but the duration of these after the infection has not yet been characterized. Individuals may have detectable virus present for several weeks following seroconversion.

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 may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

This product is for research use only

This product is intended for use by professional persons only

Clinical Significance:

SARS-CoV-2 is a member of the coronavirus family, specifically a member of the betacoronavirus genus. This new coronavirus originated in Wuhan, China.

SARS-CoV-2 is a positive-sense single stranded RNA virus that is predominantly transmitted by droplets during coughing or sneezing and through close contact with infected persons. Health care personnel and family members are especially at risk of infection.

The incubation time of SARS-CoV is three to seven, maximally 14 days and the most common symptoms include fever, coughing, breathing difficulties and fatigue, although many of the cases are asymptomatic. The most at risk population includes the elderly and immunocompromised. Reported case fatality rates depend on geographic location, age, and comorbidities. In February 2020, the disease caused by SARS-CoV-2 was named COVID-19 by the WHO.

The methods for the detection of SARS-CoV-2 include the detection of the viral RNA by reverse transcriptase polymerase chain reaction (RT-PCR) or the detection of antibodies specific to the virus using ELISA assays. The determination of antibodies enables confirmation of recent or prior SARS-CoV-2 infection in patients with typical symptoms and in suspected cases.

Cross reactions with antibodies within the genus Betacoronavirus have been described. Currently, there is no medication or vaccine available against infection with this new virus.

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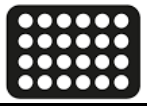
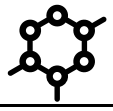
Test principle:

This ELISA kit is designed, developed, and produced for the quantitative measurement of the human anti-COVID-19 IgG antibody in serum. The test kit contains microplate strips coated with recombinant structural protein of SARS-CoV-2. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgG antibodies will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalyzing a color reaction.

Contents of the test kit:

Kit components	Volume/Quantity	Manufacturer	Symbol
Antigen-coated Polystyrene flat bottomed 96-wells	1	Plates (Fisher Scientific 07200721)	Corning® 96 Well EIA/RIA Assay Microplate Coated with CoV-2 Spike-RBD protein 
Recombinant Spike-1-RBD protein	N/A	SARS CoV-2 Spike-RBD protein (GenScript Z03483)	CoV-2 Spike-RBD protein 
IgG Positive Control (HPC) (20µg/ml)	1 x 1 ml	From the manufacturer	NC 
IgG Negative Control (0.078µg/ml)	1x 1 ml	From the manufacturer	HPC 
10X Sample Dilution / Wash Solution	2 x 50 ml	From the manufacturer	10X Sample Dilution / Wash  Solution
HRP conjugated mouse anti-Human IgG-Fc	1 x 10 ul	GenScript A01854-200	HRP conjugated mouse anti-human IgG-Fc 
OPD-tablet	1 x 10 mg	Sigma P8287	Substrate-OPD 

Citrate-Phosphate buffer solution	1 Tablet	Gibco 21300-058	Substrate bufferEs 
Stop solution	1 x 6 ml	HCL 10% , Sigma 258148	Stop Solution 
User manual	1	From the manufacturer	

Materials required but not provided:

- Microplate reader suitable for the measurement of absorbance at 492nm
- Automatic microplate washer
- Distilled water to dilute 10X Sample Solution / Wash Solution
- Graduate cylinder to prepare Wash Solution
- Incubator 37°C
- Precision pipettes to deliver volumes of 10µL, 100µL, 200µL and 1000µL
- 10µL, 100µL, 200µL and 1000µL pipette tips
- Multichannel pipettes
- Disposable reagent reservoir
- Paper towel
- Laboratory timer
- Refrigerator to store samples and kit components
- Disposable tubes
- 30% w/v Hydrogen Peroxide (H2O2)
- Centrifuge

Storage and stability:

This test kit must be stored at 2 – 8°C upon receipt. All components are stable until this expiration date.

Warnings and Precautions:

Wear gloves while performing this assay and handle these reagents as if they were potentially infectious. Avoid contact with reagents containing hydrogen peroxide, or sulfuric acid. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.

Preparation and stability of the samples:

Samples:

Human serum or EDTA, heparin or citrate plasma.

Stability of the patient samples:

The Clinical and Laboratory Standards Institute (CLSI GP44-A4) recommends the following storage conditions for samples: Samples should be stored at room temperature no longer than 8 hours. If the assay will not be completed within 8 hours, the samples should be refrigerated at +2°C to +8°C. If the assay will not be completed within 48 hours, or if the samples will be stored beyond 48 hours, samples should be frozen at -20°C or lower. Samples should not be repeatedly frozen and thawed. Frozen samples must be mixed well after thawing and prior to testing. Diluted samples should

be incubated within 8 hours. Do not use bacterially contaminated samples. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine its own specific stability criteria.

Human serum: Use a blood separator tube and allow sample to clot for 30 minutes, then centrifuge for 10 minutes at 1000g. Run assay immediately, otherwise store aliquot sample below -20°C. Avoid repeat freeze-thaw cycle. When the human serum is tested, it should be diluted 1:100.

Human plasma: Treat blood with an anticoagulant such as citrate, EDTA or heparin. Centrifuge for 10 minutes at 1000g within 30 minutes for plasma collection. Run assay immediately, otherwise store aliquot sample below -20°C. Avoid repeat freeze-thaw cycle. When the human plasma is tested, it should be diluted 1: 100.

Preparation and stability of the reagents:

All reagents must be brought to room temperature (+18°C to +25°C) approximately 30 minutes before use.

- **Coated wells**: Ready for use.
- **Controls:** Ready for use. Mix reagents thoroughly before use.
- **Wash Solution:** Dilute 10-fold the Wash Solution with distilled water. For example, dilute 50 mL of 10X Wash Solution with 900 mL of distilled water to make 500 mL of 1X Wash Solution. Store at 2-8ºC.
  Note: If any precipitate is found in the 10× Wash Solution, incubate the bottle in water bath (up to 50°C) with occasional mixing until all the precipitate is dissolved.
- **Conjugate Preparation:** Mix 2.5µl of HRP conjugated mouse anti-Human IgG-Fc with 25 mL of Wash Solution. Discard the unused conjugate solution.
- **Substrate Buffer Preparation:** 1 buffer tablet is dissolved in 100 mL distilled water yields a 0.05M phosphate-citrate buffer, pH 5.0, at 25ºC. Store at 4ºC.
- **Substrate Solution Preparation**: Immediately before use dissolve 10 mg OPD in 25 mL substrate buffer and add 10µl H_2O_2 (keep the solution in the dark)

Waste disposal:

All material should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.

Quality control:

Two controls are provided: A high positive control (HPC) and a negative control (NC). Both are supplied ready to use. Therefore, they do not require any preparation before using in the CoVIgG Assay. Controls should be storage at 4C.

Assay procedure:

1. Add 100µL of a set of positive and negative controls and samples to the corresponding wells (duplicates are recommended).

2. Leave wells A1-2 for the Substrate Blank. For blank sample use 100µL of Wash Solution (The position of Blank in the plate is arbitrary).

3. Cover wells with sealing tape

4. Incubate for 30 minutes at 37±1°C

5. After incubation, remove the sealing tape and wash the plate three times with 300µL of

1X Wash Solution. Remove any residual liquid by pat the plate on paper towel prior to the next step. Do not skip washing steps as insufficient washing results in poor precision and false results

6. Add 100µL of HRP-Conjugated antibody dilution to each well

7. Incubate for 30 minutes at 37±1°C.

8. Repeat step 5

9. Add 100µL Substrate Solution to each well

10. Incubate for 15-20 min at room temperature (20-25°C) in the dark.

11. Add 50µL of Stop Solution to each well to stop the reaction.

12. Read the absorbance at 492 nm in microplate reader immediately

Assay procedure summary:

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK											
B	BLK											
C	HPC											
D	HPC											
E	HPC											
F	NC											
G	NC											
H	NC											

Typical Assay Data:

IgG Positive Control (mg/ml)	OD 492		
	Duplicate-1	Duplicate-2	Average
HPC (20.00)	2.73	2.78	2.75
NC (0.078)	0.026	0.028	0.027

Interpretation of results:

Assessment of CovIgG-Assay 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.

Results can be reported as Negative or Positive as described below.

Negative result:

When the OD value of a serum or plasma sample at this working dilution (1:100) is equal or less than the cut-point (OD_{492} = 0.312), the CovIgG-Assay in the sample is inferred to **be negative**. A negative result in the CovIgG-Assay does not make impossible acute SARS-CoV-2 infection and should not be used as the sole basis for patients' management decisions.

IgG antibody generally become detectable beginning 10 to 14 days following infection but may occur later and patients may remain infectious during acute infection even if IgG antibody is present. For this reason results of CovIgG-Assay must be combined with clinical observations, patient history, and epidemiological information.

The presence of IgG antibodies following a previously negative testing, indicate IgG antibody seroconversion. Once IgG antibodies are elicited they may remain in circulation for long time period even after convalescence although, at this time it is unknown for how long time IgG antibodies may persists following SARS-CoV-2 infection.

Positive result:

When the OD492 of an specimen at the dilution 1:100 is greater than the cut-point (0.312) but lower than 0.49 the sample is reported as borderline and may require re-drawn at least 21 days apart and retested with CovIgG-Assay. Samples with OD492 >0.49 are inferred as positive and if their OD492 are equal or greater than the accepted HPC range, then the sample is inferred to be strongly positive.

The table below illustrate the accepted OD492 ranges for both controls provided:

Table-1. Accepted OD range for the Positive Controls

Controls	Valid OD Range
HPC	>2.0 < 3.0
NC	<0.1

Quantitative results:

CovIgG-Assay also provide the possibility to estimate the antibody IgG titer of a positive sample without need to be accomplishing the sample titration. The CovIgG-Assay antibody titer is defined as the maximal sample dilution that render OD values greater than the ROC cut-point (>0.312).

By using the lineal regression equation provided below it could easily estimate the IgG antibody titer of the sample. There is a lineal correlation between the OD492 values (at the working dilution 1:100) and the IgG antibody titer (Figure-1) and this correlation is maximal (r2=0.9946) at OD492 greater than 0.49.

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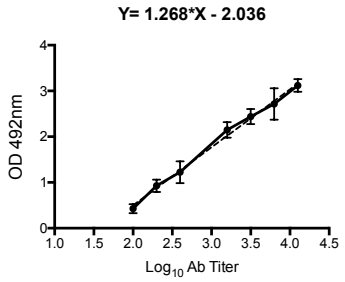

Best-fit values	
Slope	1.268 ± 0.04190
Y-intercept when X=0.0	-2.036 ± 0.1323
X-intercept when Y=0.0	1.606
1/slope	0.7888
95% Confidence Intervals	
Slope	1.160 to 1.375
Y-intercept when X=0.0	-2.376 to -1.696
X-intercept when Y=0.0	1.455 to 1.736
Goodness of Fit	
R square	0.9946

Figure-1. Correlation between OD492nm and the antibody IgG Titer. There is a strong lineal correlation between the OD492 determined by CovOgG-Assay in serum or plasma samples and the antibody titer.

Example illustrating how to use the Linear Regression Equation to estimate the antibody titer:

In the equation **Y= 2.036* X -1.268**

- Y= mean OD_{492}
  2.036 is the Y intercept when X is =0
  1.268 is the slope
  X= Log_{10} Ab Titer

Suppose that a determined specimen had a mean OD492 = 2.6

Step-1: Calculating the sum of OD_{492} (2.6) + Y-Intercept (2.036) = 4.636

Step-2: Divide the resulting sum (4.636) by the slope (1.268) = [4.636/1.268] = 3.656

Step-3: Calculating the anti-Log_{10} 3.656 = 4,528

Result: The Ab titter estimated for this specimen is 1:4,52

Precision

Intra-assay: Five different known levels of control were spiked into sample buffer as test samples. All samples were tested 10 times on the same plate to evaluate intra-assay precision of the kit. Intra-assay precision of this kit is less than 10%.

Inter-assay: Five different known levels of control were spiked into sample buffer as test samples. All samples were tested in 6 separate assays to evaluate inter-assay precision of the kit. Inter-assay precision of this kit is less than 10%.

			Repeatability (Within-Run)		Within-aboratory [a]	
Sample	**N**	**Mean A_{492}**	**SD**	**% CV**	**SD**	**% CV**
NC	30	0.022	0.021	N/A[b]	0.0026	N/A[b]
HPC	30	2.476	0.211	8.52	0.219	8.84
NS-1	6	0.049	0.011	N/A[b]	0.014	N/A[b]
NS-2	6	0.043	0.016	N/A[b]	0.032	N/A[b]
NS-3	6	0.042	0.024	N/A[b]	0.035	N/A[b]
NS-4	6	0.062	0.005	N/A[b]	0.006	N/A[b]
PS-1	6	2.085	0.011	0.527	0.075	3.59
PS-2	6	2.37	0.05	2.109	0.012	0.506
PS-3	6	2.235	0.015	0.671	0.15	6.71
PS-4	6	3.17	0.057	1.79	0.28	8.83

[a] Includes repeatability (Within-run), between-run and between-day variability
[b] Not applicable
HPC: High positive control
NS: Negative Serum
NC: Negative control
PS: Positive Serum

Troubleshooting:

Problem	Probable Cause	Solution
Poor Precision	Wells are not properly washed	Make sure that washer apparatus works properly and wells are well aspirated after each washing
	Samples have some particulates	Remove any particulate by centrifugation prior assay
	Improper preparation of Controls	Prepare new controls as described in the manual
	Pipetting error	Check pipette calibration and repeat assay
	Components are used from other lots or source	Never substitute any component from another kit
	Components are not brought to room temperature prior to assay	Repeat the assay with components that have been equilibrated at room temperature
	Incubation steps are not performed at wrong temperature	Perform incubation steps as described in the manual
Weak / No Signal	Substrate are not properly prepared, not added or added to wrong time	Follow the instruction in the manual for proper prepation and addition of substrate
	Volumen of reagents are not correct	Repeat the assay with the required volumes indicated in the manual
	The plate is not incubated for proper time or temperature	Follow the manual to repeat the assay
	The plate was not read immediately	Read the plate no later than 5 min after completing the assay
High Background	Plate is not washed properly	Make sure that washer apparatus works properly and wells are well aspirated after each washing
	Substrate buffer is contaminated	Prepare again the substrate and repeat the assay
	Evaporation of wells during incubation	Perform incubation steps with plate sealer in repeat assay or place the plate into an humidity chamber and then incubate at 37oC.

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Performance evaluation:

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 exceptionally limited at present).

Cross reactivity: We completed the cross-reactivity test by using two panels.

Panel 1 includes 40 de-identified samples collected from 2012 to 2018 stored in our samples bank. These samples came from subjects with known respiratory allergies (n=13) or previous Zika infection (n=7). This panel also includes 20 samples recently collected from individuals with known previous infection to other frequent pathogens circulating in Puerto Rico like dengue (n=6), chikungunya (n=2), influenza (n=2) or recent mycoplasma infection (n=9). One sample was positive for chikungunya, influenza and dengue. Summary panel is sowed in the table below.

Immnological Status	Sample ID	Date	OD1	OD2	Odx	Results
DENV+	6	2020415	0.0445	0.0413	0.0429	NEG
	9	20160616	0.0183	0.0201	0.0192	NEG
	8	20200415	0.0129	0.0132	0.0130	NEG
	116	20200428	0.0534	0.0529	0.0531	NEG
	117	20200504	0.0590	0.0550	0.0570	NEG
	118	20200504	0.0184	0.0281	0.0232	NEG
DENV+/Influenza+/CHIKV+	107	20200428	0.0289	0.0309	0.0299	NEG
Mycoplasma IgGM+	123	20200428	0.0472	0.0456	0.0464	NEG
	124	20200428	0.0313	0.0331	0.0322	NEG
	125	20200428	0.1966	0.1743	0.1854	NEG
	126	20200428	0.0499	0.0554	0.0526	NEG
	127	20200428	0.0641	0.0804	0.0722	NEG
	128	20200428	0.0516	0.0536	0.0526	NEG
	129	20200428	0.0286	0.0402	0.0344	NEG
	130	20200428	0.0323	0.0315	0.0319	NEG
	131	20200428	0.0631	0.0738	0.0684	NEG
Respiratory allergy	IB1	2012	0.0887	0.0826	0.0856	NEG
	IB2	2012	0.0166	0.0328	0.0247	NEG
	IB3	2012	0.0140	0.0064	0.0102	NEG
	IB4	2012	0.0583	0.0539	0.0561	NEG
	IB5	2012	0.0339	0.0366	0.0352	NEG
	IB6	2012	0.0104	0.0109	0.0106	NEG
	IB7	2012	0.0846	0.0900	0.0873	NEG
	IB8	2012	0.0351	0.0465	0.0408	NEG
	IB9	2012	0.0306	0.0360	0.0333	NEG
	IB10	2012	0.0035	0.0450	0.0242	NEG
	IB11	2012	0.1311	0.1399	0.1355	NEG
	IB12	2012	0.0000	0.0298	0.0149	NEG
	IB13	2012	0.0235	0.0185	0.0210	NEG
Influenza +	4	20200430	0.0000	0.1192	0.0596	NEG
	138	20200430	0.0229	0.0224	0.0226	NEG
Chikungunya +	119	20200504	0.0067	0.0445	0.0256	NEG
	20	20200415	0.1159	0.0982	0.1070	NEG
ZIKV+	VB83	2016	0.0143	0.0095	0.0119	NEG
	VB84	2016	0.0125	0.0162	0.0143	NEG
	RB	2016	0.0680	0.0648	0.0664	NEG
	FM	2016	0.0161	0.0221	0.0191	NEG
	VB82	2016	0.0267	0.0226	0.0246	NEG
	JN	20180811	0.0541	0.0559	0.0550	NEG
	JR	20160805	0.0354	0.0296	0.0325	NEG

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A second panel of 28 samples collected before 2019 with cross reactivity to other pathogens (Kindly provided by, CDC Dengue Branch) was also tested. From this panel we tested samples know to be positive for dengue (n=5), Zika (n=5), Influenza A (n=6), Influenza B (n=6), Respiratory Syncytial Virus (n=6). Results are showed in the table below.

Surveillance and Research Laboratory

CDC Dengue Branch Immunodiagnostics

Purpose:

UPR Medical Science Campus serum panel for COVID-19 antibody test specificity validation

Note: DENV and ZIKV specimens were tested and IgM positive. Flu A, B, and RSV have a paired acute sample that was RT-PCR+.

No IgM testing for Flu A, B, and RSV in convalescent sample provided.

Prepared by: F. Vila & F. Medina

	Date sent:	4/29/20			**CovIgG-Assay Results**			
Position in Box	Sample Type	DPO	Sample ID	Volume (µl)	**OD1**	**OD2**	**Odx**	**Results**
1	DENV IgM+	23	233	100	0.0131	0.0133	0.0132	**NEG**
2	DENV IgM+	11	255	100	0.0098	0.0092	0.0095	**NEG**
3	DENV IgM+	16	269	100	0.0135	0.0058	0.0097	**NEG**
4	DENV IgM+	13	713	100	0.0522	0.0501	0.0512	**NEG**
5	DENV IgM+	14	736	100	0.0822	0.0800	0.0811	**NEG**
6	ZIKV IgM+	17	315	100	0.0578	0.0523	0.0551	**NEG**
7	ZIKV IgM+	19	324	100	0.0173	0.0241	0.0207	**NEG**
8	ZIKV IgM+	10	432	100	0.0105	0.0123	0.0114	**NEG**
9	ZIKV IgM+	11	493	100	0.0750	0.0637	0.0694	**NEG**
10	ZIKV IgM+	15	518	100	0.0551	0.0622	0.0587	**NEG**

11	Influenza A rtPCR+	10	101	50	0.0113	0.0175	0.0144	**NEG**
12	Influenza A rtPCR+	11	102	50	0.0227	0.0228	0.0228	**NEG**
13	Influenza A rtPCR+	11	103	50	0.0424	0.0450	0.0437	**NEG**
14	Influenza A rtPCR+	12	104	50	0.0147	0.0195	0.0171	**NEG**
15	Influenza A rtPCR+	12	105	50	0.0640	0.0642	0.0641	**NEG**
16	Influenza A rtPCR+	13	106	50	0.2571	0.2548	**0.2560**	**NEG**
17	Influenza B rtPCR+	10	107	50	0.0766	0.1365	0.1066	**NEG**
18	Influenza B rtPCR+	11	108	50	0.0345	0.0363	0.0354	**NEG**
19	Influenza B rtPCR+	12	109	50	0.0136	0.0169	0.0153	**NEG**
20	Influenza B rtPCR+	12	110	50	0.0804	0.0219	0.0512	**NEG**
21	Influenza B rtPCR+	14	111	50	0.0930	0.1011	0.0971	**NEG**
22	Influenza B rtPCR+	14	112	50	0.0580	0.0628	0.0604	**NEG**
23	RSV rtPCR+	8	113	50	0.0580	0.0695	0.0638	**NEG**
24	RSV rtPCR+	9	114	50	0.0361	0.0338	0.0350	**NEG**
25	RSV rtPCR+	10	115	50	0.0496	0.0497	0.0497	**NEG**
26	RSV rtPCR+	11	116	50	0.0222	0.0254	0.0238	**NEG**
27	RSV rtPCR+	12	117	50	0.0982	0.1013	0.0998	**NEG**
28	RSV rtPCR+	13	118	50	0.0363	0.0367	0.0365	**NEG**

Clinical performance:

The Clinical Agreement Study includes 15 serum samples and 23 plasma samples confirmed positive by PCR-based Assay for a total of 48 PCR confirmed samples.

In addition one plasma sample was confirmed IgG positive by COVID-19 ELISA IgG Antibody Test developed by Mount Sinai for a total of 49 known positive samples.

Positive samples were confirmed by the following PCR-Based Assays:

Roche Molecular Systems, Inc. (RMS)

Laboratory Corporation of America (LabCorp) COVID-19 RT-PCR

Center for Diseases Control and prevention CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel (CDC)

Quest Quest SARS-CoV-2 rRT-PCR

Summary of the positive samples tested and their ODs are provided in the list below:

Dx status	Numeric ID	Confirmatory Method	Sample Date	Average OD
PCR+/ IgG/IgM Positive Local Laboratory	45	LabCorp	4/12/20	2.291
***BBMC PCR+**	102	Roche or LabCorp or CDC	4/27/20	2.555
	103	Roche or LabCorp or CDC	4/27/20	2.539
	104	Roche or LabCorp or CDC	4/27/20	0.973
	105	Roche or LabCorp or CDC	4/27/20	2.447
	106	Roche or LabCorp or CDC	4/27/20	0.826
Local Major Hospital PCR+	120	Cepheid	4/27/20	2.014
Hosp. Aux. Mutuo PCR+ / IgG/IgM Positive	121	Cepheid	4/24/20	2.372
Local Major Hospital PCR+/ IgG+/IgM-	143	CEpheid	4/28/20	2.851
PCR+ / IgG/IgM Positive Local Laboratory	122	LabCorp	4/16/20	1.981

***BBMC PCR+**	132	Roche or LabCorp or CDC	4/30/20	2.176
	133	Roche or LabCorp or CDC	4/30/20	1.081
	134	Roche or LabCorp or CDC	4/30/20	0.822
	135	Roche or LabCorp or CDC	4/30/20	1.981
	136	Roche or LabCorp or CDC	4/30/20	0.045
	137	Roche or LabCorp or CDC	4/30/20	1.037
****BSSM**	155	Quest PCR	5/11/20	1.371
	156	Quest PCR	5/11/20	1.759
	157	Quest PCR	5/11/20	2.483
	158	Roche	5/11/20	3.207
	159	ASEM PCR	5/11/20	3.056
	160	Quest PCR	5/11/20	1.897
	161	Quest PCR	5/11/20	2.958
	162	Roche	5/11/20	2.122
	163	Roche	5/11/20	1.437
	164	Roche	5/11/20	2.603
	165	Roche	5/11/20	2.196
	166	Roche	5/11/20	1.783
	167	Roche	5/11/20	1.798
	170	VA Orlando PCR	5/11/20	3.140
	171	Roche	5/11/20	2.477
	173	Dept Salud PCR	5/11/20	3.204
	176	VA San Juan PCR	5/11/20	2.512
	177	Dept Salud PCR	5/11/20	2.200
	178	Quest PCR	5/11/20	1.571
	181	Roche	5/11/20	1.715
	182	Quest PCR	5/11/20	2.906

	183	COVID-19 Ab Assay - Titer 2880 Mount Sinai	5/11/20	1.711
	184	Quest PCR	5/11/20	1.752
	185	Roche	5/11/20	1.430
PCR+ Local Laboratory	146	LabCorp	5/11/20	2.732
	147	LabCorp	5/11/20	2.636
***BBMC PCR+**	148	Roche or LabCorp or CDC	5/11/20	2.250
	149	Roche or LabCorp or CDC	5/11/20	2.092
	150	Roche or LabCorp or CDC	5/11/20	0.886
	151	Roche or LabCorp or CDC	5/11/20	2.186
	152	Roche or LabCorp or CDC	5/11/20	1.195
	153	Roche or LabCorp or CDC	5/11/20	2.260
	154	Roche or LabCorp or CDC	5/11/20	0.987

*BBMC: Blood Bank Medical Center **BMSM: Blood Bank Servicios Mutuos

Also we tested 104 negatives SARS-CoV-2-IgG samples collected in Puerto Rico from 1995 to June 2019 plus 28 samples collected before 2019 with cross reactivity to other pathogens (CDC kindly provided by Dr. Jorge Muñoz, CDC Dengue Branch) for a total of 132 known negative samples.

Summary of the negative samples tested and their ODs are provided in the list below:

Dx status	Numeric ID	Additional Information	Sample Date	Average OD
Virology Serum Bank	VB1		5/23/95	0.027
	VB2		12/7/95	0.202
	VB3		2/10/97	0.085
	VB4		5/25/97	0.054
	VB5		2/17/06	0.047
	VB6		6/28/16	0.056
	VB7		4/10/17	0.099
	VB8		8/15/16	0.084

	VB9		8/15/16	0.268
	VB10		6/27/17	0.067
	VB11		1/10/17	0.011
	VB12		4/28/17	0.068
	VB13		6/26/18	0.442
	VB14		6/26/18	0.025
	VB15		6/28/18	0.033
	VB16		9/8/00	0.045
	VB17		5/8/19	0.029
	VB18		6/6/19	0.073
Immunology Bank	IB95		2012	0.090
	IB96		2012	0.046
	IB97		2012	0.078
	IB100		2012	0.032
	IB144		2012	0.100
	IB145		2012	0.042
	IB146		2012	0.069
	IB147		2012	0.036
	IB148		2012	0.029
	IB149		2012	0.046
	IB135		2012	0.044
	IB136		2012	0.103
	IB137		2012	0.025
	IB138		2012	0.029
	IB139		2012	0.027
	IB140		2012	0.106

	IB141		2012	0.029
	IB142		2012	0.044
	IB143		2012	0.040
	IM		2012	0.030
	AO		2012	0.093
	OF		2012	0.072
	VB83		2012	0.062
	IB37		2012	0.074
	IB58		2012	0.057
	IB86		2012	0.015
	IB84		2012	0.015
Virology Serum Bank	RB	**ZIKV +**	2016	0.075
	FM		2016	0.020
	VB82		2016	0.055
	JN		8/11/16	0.060
	JR		8/15/16	0.095
	EXP		4/1/17	0.028
Immunology Bank	IB133		2012	0.017
	IB130		2012	0.067
	IB131		2012	0.024
	IB132		2012	0.252
Immunology Bank	IB1	**Resp. Allergies**	2012	0.084
	IB2		2012	0.035
	IB3		2012	0.029
	IB4		2012	0.073
	IB5		2012	0.065

	IB6		2012	0.030
	IB7		2012	0.096
	IB8		2012	0.061
	IB9		2012	0.063
	IB10		2012	0.024
	IB11		2012	0.163
	IB12		2012	0.040
	IB13		2012	0.090
Immunology Bank	BVF		2012	0.056
	VA		2012	0.061
	JJF		2012	0.073
	IB53		2012	0.065
	IB54		2012	0.059
	IB55		2012	0.050
	IB56		2012	0.169
	IB64		2012	0.089
	IB65		2012	0.057
	IB66		2012	0.066
	IB74		2012	0.063
	IB75		2012	0.238
	IB77		2012	0.063
	IB78		2012	0.231
	IB79		2012	0.129
	IB80		2012	0.106
	IB81		2012	0.091
	IB83		2012	0.091

	IB84		2012	0.104
	IB85		2012	0.057
	IB86		2012	0.067
	IB89		2012	0.063
	IB90		2012	0.120
	IB22		2012	0.065
	IB23		2012	0.068
	IB35		2012	0.062
	IB69		2012	0.069
	IB82		2012	0.060
CDC	CDC233	**DENV+**	4/28/20	0.013
	CDC255	**DENV+**	4/28/20	0.010
	CDC269	**DENV+**	4/28/20	0.010
	CDC713	**DENV+**	4/28/20	0.051
	CDC736	**DENV+**	4/28/20	0.081
	CDC315	**ZIKV +**	4/28/20	0.055
	CDC324	**ZIKV +**	4/28/20	0.021
	CDC432	**ZIKV +**	4/28/20	0.011
	CDC493	**ZIKV +**	4/28/20	0.069
	CDC518	**ZIKV +**	4/28/20	0.059
	CDC101	**Influenza A+**	4/28/20	0.014
	CDC102	**Influenza A+**	4/28/20	0.023
	CDC103	**Influenza A+**	4/28/20	0.044
	CDC104	**Influenza A+**	4/28/20	0.017
	CDC105	**Influenza B+**	4/28/20	0.064
	CDC106	**Influenza B+**	4/28/20	0.256

	CDC107	**Influenza B+**	4/28/20	0.107
	CDC108	**Influenza B+**	4/28/20	0.035
	CDC109	**Influenza B+**	4/28/20	0.015
	CDC110	**Influenza B+**	4/28/20	0.051
	CDC111	**Influenza B+**	4/28/20	0.097
	CDC112	**Influenza B+**	4/28/20	0.060
	CDC113	**RSV**	4/28/20	0.064
	CDC114	**RSV**	4/28/20	0.035
	CDC115	**RSV**	4/28/20	0.050
	CDC116	**RSV**	4/28/20	0.024
	CDC117	**RSV**	4/28/20	0.100
	CDC118	**RSV**	4/28/20	0.037
Virology Serum Bank	1		4/26/17	0.065
	3	**FluA H1N1+**	6/26/18	0.040
	5	**DENV +**	10/11/17	0.085
	7	**DENV +**	8/17/14	0.026
	9	**DENV +**	6/24/16	0.040
	29	**ZIKV +**	6/29/16	0.024
	VB83	**ZIKV +**	2016	0.012
	VB84	**ZIKV +**	2016	0.014

Results:		***Positive Samples by PCR-based assay (n=48) or COVID-19 ELISA IgG Antibody (n=1) / Negative historical samples before August 2019 (n=133)***		
		Positive	***Negative***	***Total***
CovIgG-Assay	***Positive***	*48*	*0*	*48*
	Negative	*1*	*132*	*133*
	Total	*49*	*132*	*181*

Positive Percent Agreement = 97.9 %

Negative Percent Agreement = 100%

<u>Limitations of the procedure</u>:

1) Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. The sensitivity of the 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. Samples with positive results should be confirmed with alternative testing method(s) and clinical findings before a diagnostic determination is made.

2) A negative result can occur if the quantity of antibodies for the SARS-CoV-2 virus present in the specimen is below the detection limit of the assay, or if the virus has undergone minor amino acid mutation(s) in the epitope recognized by the antibody used in the test.

3) 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.

4) Not for the screening of donated blood.

5) It is not known at this time if the presence of antibodies to SARS-CoV-2 confers immunity to re-infection.

6) Correct performance of sample collection and storage is crucial for the test results.

7) The test system is validated for the quantitative determination of anti-SARS-CoV-2 IgG in human serum or plasma only.

8) The binding activity of the antibodies and the activity of the enzyme used are temperature-dependent.

9) Insufficient washing can lead to false high OD readings.

10) Residual liquid in the reagent wells after washing can interfere with the substrate and lead to false low OD readings.

11) The partial or complete adjustment of the test system to the use of instruments for automated sample processing or other liquid handling devices may result in differences between the results obtained with automated processing and those obtained with manual procedure. It is the responsibility of the user to validate the instruments used so that they yield test result within the reliable range.