Validation and performance of a quantitative IgG assay for the screening of SARS-CoV-2 antibodies

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Abstract

The current COVID-19 epidemic imposed an unpreceded challenge to the scientific community in terms of treatment, epidemiology, diagnosis, social interaction, fiscal policies and many other areas. The development of accurate and reliable diagnostic tools (high specificity and sensitivity) is crucial in the current period, the near future and in the long term. These assays should provide guidance to identify immune presumptive protected persons, potential plasma, and/or B cell donors and vaccine development among others. Also, such assays will be contributory in supporting prospective and retrospective studies to identify the prevalence and incidence of COVID-19 and to characterize the dynamics of the immune response. As of today, only thirteen serological assays have received the Emergency Use Authorization (EUA) by the U.S. Federal Drug Administration (FDA). In this work we describe the development and validation of a quantitative IgG enzyme-linked immunoassay (ELISA) using the recombinant SARS-CoV-2 Spike Protein S1 domain, containing the receptor-binding domain (RBD), showing 98% sensitivity and 98.9% specificity. The assay showed to be useful to test for SARS-CoV-2 IgG antibodies in plasma samples from COVID-19-recovered subjects as potential donors for plasmapheresis. This assay is currently under review by the Federal Drug Administration for an Emergency Use Authorization request (Submission Number EUA201115).

Introduction

The current severe acute respiratory syndrome coronavirus 2 (SARS CoV-2) pandemic and the resulting unprecedented outbreak of coronavirus disease 2019 (COVID-19) have shifted the paradigm for viral research, epidemiology and diagnostic. Both molecular and serological methods have been developed at an extraordinary speed. As of April 2, 2020 only four months after the virus was detected for first time in Wuhan region, 28 companies obtained Emergency Use Authorization (EUA) approvals from US Federal Drug Administration (FDA) for their commercial Reverse Transcription-Polymerase Chain Reaction (RT-PCR) diagnostics. Those assays are intended to detect the virus during the acute phase of the infection, providing no information regarding the immunological status of these patients. By the same time, from the more than 25 rapid serological tests available only one had the EUA granted. These rapid tests are relatively simple to perform and interpret and therefore require limited test operator training. The main drawback of these rapid tests is that the specificity and particularly the sensitivity are lower than the standard Enzyme-linked Immunosorbent Assays (ELISA). As of June 1, 2020 FDA had received more than 198 notifications from manufacturers confirming they have validated and intend to distribute their tests in the market. However only 13 of those tests have indeed the EUA from FDA. Moreover, in May 2020, FDA removed 28 SARS-CoV-2 serological tests from the notification list of tests offered during the COVID-10 emergency for not having an EUA request. Choosing an appropriate test to screen for the presence of humoral immune response to SARS-CoV-2 is critical. Such serologic tests are expected to play a key role in the fight against COVID-19 by helping to identify individuals who had developed an adaptive immune response and may be at lower risk of infection. Also, validated serological tests are needed to confirm which subjects, being confirmed positive for COVID-19, truly developed a substantial humoral immune response and may be considered as plasma donors (1). Different antigens have been used to detect antibodies against other novel coronavirus such as SARS-CoV and MERS-CoV (2-5). From these previous works it can be concluded that spike-derived (S) antigens are more sensitive, specific

70 and accurate than nucleocapsid protein-derived (NP) antigens. Also results from assays using S
71 antigens correlated much better with the neutralizing titers than those using NP antigens (6). A
72 recent work showed the usefulness for the Receptor Binding Domain (RBD) and the full Spike
73 protein to detect SARS-CoV-2 specific antibodies (7) and their correlation with neutralizing
74 antibodies nAb (8). For these reasons we choose to use a recombinant SARS-CoV-2 Spike
75 Protein, S1 domain containing the RBD.
76 With this work we described the validation of a quantitative ELISA, CovIgG-Assay
77 (https://prsciencetrust.org/the-covigg-assay-kit/), showing a very low background and lack of
78 cross reactivity with other respiratory and non-respiratory pathogens in more than 132 samples
79 collected before June 2019. Also the limited correlation with a rapid test and another serology test
80 availables in the market is described. Finally, we confirm the usefulness of the assay detecting
81 anti-SARS-CoV-2 antibodies in plasma samples from potential plasma donors. CovIgG-Assay is
82 a useful tool to characterize, quantify and to study the dynamics of the humoral immune response
83 to SARS-CoV-2.
84

Materials and Methods

Study Design

The study population included 49 positive samples from individuals with symptomatic infection and positive SARS-CoV-2. Forty-eight (48) were confirmed by RT-PCR tests EUA authorized and one (1) diagnosed by COVID-19 ELISA IgG Antibody Test – Mount Sinai, also EUA authorized. De-identified serum or plasma specimens were obtained from local clinical laboratories and Blood Banks and no personal identifiers were retained. For specificity, we tested a total of 132 samples taken previously to 2019 from the Virology UPR MSC or the Immunology UPR-MSC serum bank. From these samples, 78 had no previous history of viral, allergic or bacterial infections according to our cross-reactivity panel. Nine (9) were previously diagnosed with Zika, three (3) with Dengue, thirteen (13) with history of respiratory allergies and one (1) with Influenza H1N1. We also included a cross reactivity panel with 28 samples kindly donated by the Centers for Disease Control and Prevention (CDC) Dengue Branch, San Juan, PR. These samples included six (6) positives for Respiratory Syncytial Virus (RSV)-IgM, twelve (12) RT-PCR positive for Influenza A or B, five (5) Zika-IgM positive and five (5) positive por Dengue-IgM. In addition, to complete our cross-reactivity panel, we tested nine (9) samples IgM positive for Mycoplasma and three (3) positives for Chikungunya. This cross-reactive panel was selected according to the most common viral and respiratory infections affecting our population. All samples were stored at -80°C until use.

For comparison with two other serological test (CoronaCheck and Abbott Architect) holding an EUA, we used a set of nine (9) samples assumed to be positive for IgG and IgM and eighteen (18) assumed to be IgG positive for SARS-CoV-2 antibodies. Those samples were also received de-identified from local laboratories.

113 **CovIgG-Assay**

114 CovIgG Assay is an indirect ELISA for quantitative determination of human IgG antibody class,
115 which was optimized by checkerboard titration. Disposable high bind flat-bottomed polystyrene
116 96-wells microtiter plates (Costar, Corning MA No. 3361) were coated overnight at 4°C with
117 2µg/ml of recombinant SARS-CoV-2 S1-RBD protein (GenScript No. Z03483-1) in carbonate-
118 bicarbonate buffer (Sigma Aldrich No. 08058). Plates were washed 3 times with phosphate
119 buffered saline (PBS) containing 0.05% Tween-20 (PBST) and blocked for 30 min at 37°C with
120 250µl/well of 3% non-fat, skim milk in PBST. Samples (serum or plasma) were diluted 1:100 in
121 PBST; 100µL/well was added in duplicates and incubated at 37°C for 30 min. The excess antibody
122 was washed off with PBST. Horseradish peroxidase (HRP) labeled-mouse anti-human IgG-Fc
123 specific (GenScript No. A01854) diluted 1:10,000 in PBST was added (100µl/well) and incubated
124 for 30 min at 37°C. After another washing step, the substrate solution (Sigma Aldrich No. P4809)
125 was added (100µl/well) followed by 15 min incubation in dark. The reaction was stopped by the
126 addition of 50µl/well 10% HCl and the absorbance was measured at 492nm (A_{492}) using a
127 Multiskan FC reader (Thermo Fisher Scientific). In every CovIgG-Assay determination two in-
128 house controls, a high positive control (HPC) and negative control (NC) were included. HPC and
129 NC were prepared by diluting an IgG anti-SARS-CoV-2 at a concentration of 30µg/ml and
130 0.070µg/ml, respectively in PBST containing 10% glycerol. The IgG anti-SARS-CoV-2 was
131 purified from plasma of a convalescent patient using a 5/5 HiTrap rProtein-A column (GE
132 Healthcare, USA) (see detailed information about this procedure in **Supplementary method**
133 **No.1**).

134

135 **Antibody class specificity**

136 To confirm that our assay accurately detects antibody IgG class and excludes the potential for
137 human IgM to cross-react with IgG, five (5) COVID-19 samples (1:100 diluted) were treated with
138 5mM DTT for 30 min at 37°C prior testing. After treatment, samples were added in duplicate

139 (100µl/well) followed by the addition of the anti-human IgG-Fc-HRP (GenScript No. A01854)
140 conjugate (diluted 1:10,000) or the addition of an anti-human IgM-HRP conjugate (Abcam No.
141 ab97205) diluted 1:8,000 in PBST and the assay progressed as described above.
142

143 **Estimation of Antibody Titer**

144 To estimate the IgG antibody titer, 26 COVID-19 RT-PCR-confirmed samples were subjected to
145 serial dilutions from 1:100 to 1:12,800, each dilution was tested in duplicate in the CovIgG-Assay
146 and each experiment was replicated twice. A standard curve was created in which the mean
147 individual absorbance (A_{492}) of each sample at 1:100 dilutions was correlated with its
148 corresponding IgG antibody titer. Antibody titer was defined as the highest serum dilution that
149 renders A_{492} values greater than the cut point estimated by the ROC analysis.
150

151 **Comparison with two serological assays approved for emergency use**

152 We tested a set of 9 samples reported as IgM/IgG positives and 18 reported as IgG positives for
153 SARS-CoV-2 antibodies by CoronaCheck (20/20 BioResponse, 20/20 Genesystems, Inc,
154 Rockville, MA, USA). The information provided by the manufacturer claims that this assay use
155 Roche's technology (Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim,
156 Germany). Same set of 18 samples reported as SARS-CoV-2 IgG positive were also tested by
157 Abbott Architect SARS-CoV-2 IgG (Abbott Laboratories Diagnostics Division Abbott Park, IL
158 60064 USA). For comparison, both set of samples (n=27) were tested with our CovIgG-Assay.
159

160 **Data analysis**

161 Each CovIgG-Assay determination was performed in duplicate and the results expressed as the
162 mean absorbance at 492 nm (A_{492}) for each determination. The optimal cut point for the assay
163 was established within a 95% confidence interval (CI) by receiver operating characteristic (ROC)
164 curve analysis using the EpiTools epidemiological calculator (http://epitools.ausvet.com.au).

165 Arbitrary guidelines were followed for analyzing the area under curve (AUC) as follows: non-
166 informative, AUC=0.5; low accurate, 0.5 < AUC < 0.7; moderately accurate, 0.7 < AUC < 1;
167 perfect, AUC = 1 (9). Intra-plate repeatability was evaluated for CovIgG-Assay by measuring the
168 coefficient variation (CV) of 60 repeats of a High Positive Control (HPC) and a Negative Control
169 (NC). For reproducibility evaluation, we completed three independent runs for the CovIgG Assay
170 using HPC, NC, four (4) negative and four (4) COVID-19 positive sera for each of the three runs.
171 Correlation between the A_{492} at 1:100 dilutions and the antibody titer as well as between the
172 results of CovIgG Assay and the RT-PCR test results were evaluated using the Pearson
173 correlation coefficient (with the 95% CI). To evaluate the agreement between the CovIgG-Assay
174 and the RT-PCR, CovIgG-Assay and CoronaCheck or Abbott Architect SARS-CoV-2 IgG, inter-
175 rater agreement (kappa) was calculated according to the method described by Thrusfield (10).
176 The Kappa values (κ) were considered as follows: poor agreement, κ<0.02); fair agreement,
177 κ=0.21 to 0.4; moderate agreement, κ=0.41 to 0.6; substantial agreement, κ=0.61 to 0.8; very
178 good agreement, κ=0.81 to 1.0.
179
180 **Results**
181 **Distribution of absorbance values of sera and ROC analysis**
182 We used the RT-PCR for COVID-19 positive samples, as recommended standard reference
183 method, to build ROC curves on the basis of the absorbance values (A_{492}) obtained with
184 specimens from two reference populations: subjects infected with SARS-CoV-2 that were all RT-
185 PCR positive (assumed infected population) and healthy subjects or subjects that had been
186 diagnosed with other respiratory or viral infections prior to the COVID-19 pandemic (uninfected
187 population). The A_{492} values of uninfected population ranged between 0.011 and 0.312 with a
188 mean ± SD A_{492} value of 0.075 ± 0.052 whereas samples from assumed infected population
189 showed A_{492} values that ranged between 0.045 (one sample) and 3.21 with a mean A_{492} value of
190 1.99 ± 0.727. The mean value of the infected population was significantly different from the mean

value of the uninfected population (p<0.0001). The distribution of A_{492} values of these two reference populations was very different. Approximately the 75% of infected population had A_{492} values between 0.828 and 2.5 (median 2.01), whereas that the 95% of uninfected population had A_{492} values between 0.011 and 0.176 (median 0.065) (**Figure-1**). Receiving operating characteristic analysis was used to determine the best cut-points for the CovIgG-Assay. The ROC optimized cut-point was 0.312. The selection of this cut-point derived from three different conditions: (a) maximum specificity at which the sensitivity was still 100%, (b) maximum sensitivity at which the specificity of the assay was also maximized, and (c) maximum value for Youden's J index (S + Sp-1) and test efficiency (**Table-1**).

Table-1. Specificity (Sp) and sensitivity (Se) of the CovIgG-Assay based on cut-points results obtained when compared infected and uninfected populations.

Target Sp	Cut-point	Specificity	Sp lower 95% CL	Sp Upper 95% CL	Sensitivity	Se Lower 95% CL	Se Upper 95% CL
0.999	0.745	1	0.961	1	0.98	0.893	0.996
0.995	0.745	1	0.961	1	0.98	0.893	0.996
0.990	0.745	1	0.961	1	0.98	0.893	0.996
0.98	**0.312**	**0.989**	**0.943**	**0.998**	**0.98**	**0.893**	**0.996**
0.95	0.202	0.958	0.897	0.984	0.98	0.893	0.996
0.9	0.129	0.905	0.83	0.949	0.98	0.893	0.996
0.8	0.099	0.8	0.709	0.868	0.98	0.893	0.996

The area under curve values (AUC) (accuracy) for the ROC curve was 0.985 (**Figure-2**). Based on the established cut-point only one seronegative was detected in the infected group whereas no seropositive was detected in the uninfected group. A sample from the uninfected group, collected between 1995 and June 2019, had A_{492} values equal to the cut-point and was considered negative (**Figure-3**).

To verify the cross reactivity of the assay we tested 67 samples know to be positive to common respiratory and non-respiratory infections (RSV, Flu A and B, Mycoplasma, Zika, dengue, Chikungunya) or allergies which are very common in the local population. As it is showed in figure

213 3, all those samples were negative showing no-cross reactivity in CovIgG-Assay. Based on these
214 results the estimated sensitivity if 98% (95% CI 89.35-99.95%) and the specificity is 100% (95%
215 CI 97.24-100%). Also the predictive positive value (PPV) and predictive negative value (NPV) for
216 CovIgG-Assay were estimated to be 100% and 99.25% (95% CI 94.99-99.89%), respectively
217 (**Table-2**). There was substantial agreement (κ=0.71) between CovIgG-Assay and RT-PCR.
218 Detailed optical densities (ODs) values of the positive and negative samples, including the cross-
219 reactivity panel are provided (**Supplementary tables 1 and 2 respectively**)

220
221
222 **Table-2. Agreement between test results of CovIgG-Assay and the PCR-based assay used as**
223 **reference method for COVID-19 diagnosis.**
224

		PCR-based assay		
		Positive	**Negative**	**Total**
CovIgG-Assay	**Positive**	48	0	48
	Negative	1	132	133
	Total	49	132	181

225 Positive Predictive Value (PPV)=100% is calculated as the number of individuals with a positive result by
226 CovIgG-Assay / the total of true positive individuals x 100. Negative Predictive Value (PNV)= 99.25% is
227 calculated as the number of individuals reported as negative by CovIgG-Assay / the total of true negative
228 individuals x 100.
229

230 We also assessed the reproducibility of the CovIgG-Assay by calculating the CV of data from 3
231 different assays and 30 repeats of controls and 6 repeats of selected samples. The intra-assay
232 and inter-assay reproducibility values were both lower than 15% (**Supplementary Table 3**).

233

234 **Class antibody specificity of CovIgG-Assay**

235 To confirm that the positivity showed by CovIgG-Assay with the COVID-19 samples was mostly
236 due to the presence of IgG antibody class and not due to potential cross-reactions with IgM
237 antibody, five samples treated with DTT were tested in parallel on the CovIgG-Assay using as

secondary antibody anti-human IgG- and anti-human IgM-HRP conjugates and the results obtained were compared with those obtained for the same samples previous to the DTT treatment. As expected, the A_{492} values of DTT-treated samples tested with the anti-IgM-HRP conjugate significantly dropped to values similar to the background. In contrast, the A_{492} values for the same DTT-treated samples tested with the anti-IgG-HRP conjugate were similar to those obtained with the untreated samples, confirming that positive results were from IgG antibodies. (**Table-3**).

Table 3. Samples before and after treatment with DTT (Dithiothreitol) showing IgG class specifity for CovIgG-Assay. Each result represent OD at 492nm absorbance.

Numeric ID	IgG			IgG DTT			IgM			IgM DTT		
	OD1	OD2	Average OD	OD1	OD2	Average OD	OD1	OD2	Average OD	OD1	OD2	Average OD
45	2.7594	2.6820	2.7207	1.7638	1.7889	1.7763	1.6503	1.5807	1.6155	0.0244	0.0235	0.0239
121	3.1175	2.9996	3.0585	2.7779	2.6761	2.7270	3.5162	3.5980	3.5571	0.3303	0.3293	0.3298
122	2.7393	2.3745	2.5569	1.9655	1.9516	1.9585	2.6131	2.5388	2.5759	0.1060	0.1019	0.1039
146	2.7958	2.7973	2.7965	2.3467	2.3361	2.3414	1.0818	1.0508	1.0663	0.0898	0.0876	0.0887
147	2.8958	2.7455	2.8206	2.4553	2.3915	2.4234	3.2084	3.2769	3.2426	0.8243	0.8349	0.8296
183	1.4743	1.4506	1.4624	0.8341	0.8457	0.8399	3.3603	3.3997	3.3800	0.0607	0.0519	0.0563

Samples tested were previously confirmed by PCR and IgG/IgM rapid tests. Sample 183 was previously confirmed by PCR.

Correlation between the A_{492} values and the IgG antibody titer

To determine whether the magnitude of the A_{492} values correlate with the antibody titer we selected 27samples from infected individuals with different A_{492} values, which were titrated at dilutions from 1:100 to 1:12,800 and each dilution was tested in duplicate in the CovIgG-Assay. The number of individuals with different antibody titers (defined as the maximal dilution that renders a positive result) is shown in Table-4. We found a lineal correlation (r^2=0.984) between the antibody titer (maximal dilution that render A_{492} = 0.312) and the individual A_{492} value at the working dilution (1:100). Thus, results reported by CovIgG-Assay could be quantitatively reported by estimating the titer, using the lineal equation (Y= 1.185*X -1.773) derived from the lineal correlation between antibody titer and the magnitude of absorbance values (**Figure-4**). Titers are reported from 1:100 to 1:12,800 which were the dilutions tested to establish the OD at A_{492} and the IgG titers. Positive samples rendering titers below or above of these values are reported as <1:100 or >1:12,800.

265

266

267 **Table 4. Experimental antibody titers of SARS-CoV-2 infected subjects in relation to**
268 **their absorbance value at 492nm (1:100 dilution).**
269

Antibody Titer*	Number of Individuals	Absorbance range at 492 nm (A_{492})	Mean A_{492} + SD
1:100	0	0	0
1:200	6	0.745-1.03	0.896 + 0.103
1:400	4	1.08-1.55	1.24 + 0.18
1:800	0	0	0
1:1600	7	1.98-2.52	2.2 + 0.203
1:3200	6	2.17-2.57	2.43 + 0.166
1:6400	2	2.37-3.05	2.71 + 0.34
1:12800	2	2.85-3.12	2.985 + 0.135

270 *Antibody titer is defined as the maximal serum dilution that renders A_{492} greater than the
271 optimized cut-point (A_{492} = 0.312) determined by ROC analysis.
272

273 **Agreement between CovIgG-Assay and other Rapid tests**

274 To evaluate the performance of CovIgG-Assay with other tests in the market, we analyzed a group
275 of samples that have been previously reported as positive for IgG/IgM (n=9) or only positive for
276 IgG (n=18) by CoronaCheck rapid test. CovIgG-Assay had 100% agreement with the
277 CoronaCheck results for the IgG/IgM positive samples. These samples were all reported as
278 positive by CovIgG-Assay with antibody titers that ranged between 1:100 and 1:3,251
279 (Supplementary table 4). However, the agreement was fair (38.8%) for samples only reported
280 positive for IgG by CoronaCheck since only 7 from 18 samples were reported as positive. From
281 these sera 3 had antibody titers <1:100 and 4 had antibody titters among 1:219 to 1:254 (**Table-**
282 **5**). Interestingly, all 18 presumptive IgG positive samples reported by CoronaCheck were found
283 negative by Abbot Architect SARS-CoV-2 IgG method, which might subject that these 18 could
284 be considered as false positive and reveal a better agreement between Abbot Architech SARS-

CoV-2 IgG method and our CovIgG-Assay. The correlation between CovIgG-Assay and Abbot Architect method was of 61% (**Table 5**). Detailed OD values for this experiment are provided in **supplementary table 4**.

Table 5. Comparison of two rapid tests against CoVIgG-Assay for the detection of anti SARS CoV-2 IgG antibodies.

Sample	CoronaCheck[1] (Rapid COVID-19 IgG)	CoVIgG-Assay[2] Estimated titer	CoV-2 IgG[3] (Abbott Architect) Index value
46	+	-	.05
47	+	†	.03
48	+	-	.03
49	+	254	.05
50	+	-	.03
51	+	-	.05
52	+	-	.02
53	+	-	.04
54	+	254	.05
55	+	-	.04
56	+	-	.07
57	+	-	.37
58	+	†	.08
59	+	†	.02
60	+	-	.05
61	+	-	.01
62	+	219	.69
63	+	220	.05

Serum samples obtained during April 2020 were tested for SARS CoV-2 IgG antibodies using two different assays. All samples tested positive with the Coronacheck test, when tested with the CoVIgG-Assay only 7 were positive for a 38.8% concordance. Same set of samples were tested with Abbott Architect CoV-2 (EUA) assay; all samples were negative for a 61% match when compared with CoVIgG-Assay. Positive results for CoVIgG-Assay are expressed as estimated titers from the average of duplicates OD_{492}.
[1]Lateral flow rapid test intended to qualitatively detect antibodies to SARS-CoV-2. Presence of IgG/IgM antibodies are indicated by a visible line in the specific region on the device. Positive results may be due to cross reactivity with other Coronavirus strains (non CoV-2). Information regarding antigen used is not available. [2]Quantitative ELISA for the detection SARS CoV-2 IgG antibodies using Spike S1-RBD (GenScript) as capture antigen. Samples with OD_{492}<0.312 are considered negative. †IgG titration of positive samples by CovIgG-Assay range from 1:100 to 1:12,800. Values below or above those limits are reported as < or > respectively.
[3]Chemiluminescent microparticle immunoassay for the qualitative detection of IgG antibodies against SARS CoV-2. Assay is designed to detect IgG antibodies to the nucleocapsid protein of SARS CoV-2. Samples with an index value <1.4 are considered negative.

Discussion

308 Since the circulation of SARS-CoV-2 was spillover outside of China, the global efforts to develop
309 serological assays have been unprecedently huge. The precise diagnostic of COVID-19 poses
310 multiples challenges. The proposed method reference is the molecular diagnostic which
311 determine the presence of an active infection. However the timing of viral replication and the
312 development of immune response is quite variable (reviewed in (11)) and the presence of IgM
313 and or IgG at the time of the molecular diagnostic is merely speculative. While the molecular
314 testing results are a guide, they should not be considered gold standards as it has been the
315 practice so far. Other factors as clinical presentation and epidemiological aspects need to be
316 considered at the time of selecting the appropriate samples to validate any assay. Here we
317 selected 48 samples reported positive by authorized molecular methods and 1 sample reported
318 as positive by an authorized serological assay which is not considered a rapid test (7). As negative
319 samples we used a set of 132 sera that were collected in the period of 1995 to June 2019, before
320 COVID-19 period. The CovIgG-Assay data were subjected to ROC curve analysis. During the last
321 two decades this type of analysis has become a popular method for evaluating the accuracy of
322 medical diagnostic systems and has been used not only to evaluate the ability of a test to
323 discriminate between infected and healthy subjects (12) but also to compare the diagnostic
324 performance of a number of tests (13). The ROC curve is obtained by plotting the true-positive
325 rate (sensitivity) as a function of the false-positive rate (100-specificity) that is associated with
326 each cut-point. The AUC is then used as a measure of the accuracy of the test. If the assay can
327 distinguish between infected and normal populations the AUC will be equal to 1 and ROC curve
328 will reach the upper left corner. As our results demonstrate, the AUC value obtained from the
329 ROC curve analysis conducted on the CovIgG-Assay data was very high, indicating the accuracy
330 of this test. The only sample that was not detected by CovIgG-Assay could be a false positive in
331 the RT-PCR. Currently, there are few reports addressing those discordant results. Several
332 molecular assays have been developed with high specificity and low limit of detections (14-17)
333 and are considered the reference method for SARS-CoV-2 diagnostic (18). However everyday

334 there are more reports addressing problems with the RT-PCR accuracy (14, 19-21). Otherwise
335 that sample may be collected within a window where the immune response was not developed
336 yet. Nevertheless, our results reinforce the complexity of the diagnostic of COVID-19 and the
337 need for prospective studies with more samples and better-characterized cohorts to better
338 understand the dynamics of the immune response to this novel coronavirus.
339 We also considered fundamental to develop a quantitative test, in addition to suggesting a
340 qualitative result. This would provide a guide about quantity or the dimension of the immune
341 response mounted by an individual. Up to today, few works on COVID-19 addressed the relation
342 between the titer of the IgG and the neutralizing capability of that sample. But all of them coincide
343 that there is a direct correlation (1, 21-23). While the scientific community develops safe (BSL-2)
344 and reproducible neutralization assays to determine nAbs against SARS-CoV-2, quantitative
345 assays like CovIgG-Assay are useful tools for a reliable serological characterization.
346 When we compared the performance of an available rapid test and a high throughput assay, both
347 with EUA by the FDA. The results are highly worrisome showing 100% and 0% of positive samples
348 reported by CoronaCheck and Abbott Architect SARS-CoV-2 IgG respectively while our assay
349 reported a 38.8% % of the samples as positive. These results reinforce the need for better assays
350 and for better validations in the context of clinical presentations (24).
351 These results reinforce the concept recently proposed by the CDC to implement an orthogonal
352 testing algorithm (i.e., employing two independent tests in sequence when the first test yields a
353 positive result (shorturl.at/AHUY2). However the best characteristics of CovIgG-Assay are its PPV
354 of 100% along a PNV of 99.2% which render highly trustable positive results. Theses
355 characteristics made this assay suitable to be implemented both in a high or low prevalence
356 settings. A subject confirmed as positive by this assay, with a high degree of confidence can be
357 reincorporating to a normal life in support of the rest of the community with a high degree of
358 confidence of having certain level of immunity. On the other hand the high NPV of the assay
359 increase its value to know which individuals may be still at risk to be infected.

360 Furthermore, the quantitative aspect of CovIgG-Assay, after a subject being reported as positive,
361 provides a second step of certainty of possible protection against SARS-CoV-2 infection. However
362 such correlation studies using a larger set of samples are under way.

363 **Statistical analysis**

364 The sensitivity of the developed ELISAs was calculated as (the number of true positive samples
365 / the total number of true positive and false negative samples) x 100. The specificity was
366 calculated as (the number of true negative samples / the total number of true negative and false
367 positive samples) x 100. As we do not know the prevalence of the tested population, the PPV
368 (probability that the disease is present when the test is positive) was calculated as the number of
369 individuals with a positive result by CovIgG-Assay / the total of true positive individuals x 100. The
370 NPV was calculated as the number of individuals reported as negative by CovIgG-Assay / the
371 total of true negative individuals x 100. Agreement was calculated as (the total number of true
372 positive and negative samples / the total number of samples) X 100 and evaluated with kappa
373 values. Receiver operating characteristic (ROC) analysis was calculated using GraphPad Prism
374 software.

375 **Authors Contribution**

376 AME and CAS conceived and supervised the studies. PP and AME performed the experiments.
377 CAS and AME drafted the manuscript. CAS, AME and PP reviewed the final version of the
378 manuscript. CAS and AME obtained the funds. CAS prepared and CAS and AME submitted the
379 Emergency Use Authorization request to the US Federal Drug Administration.

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459 **FIGURES**
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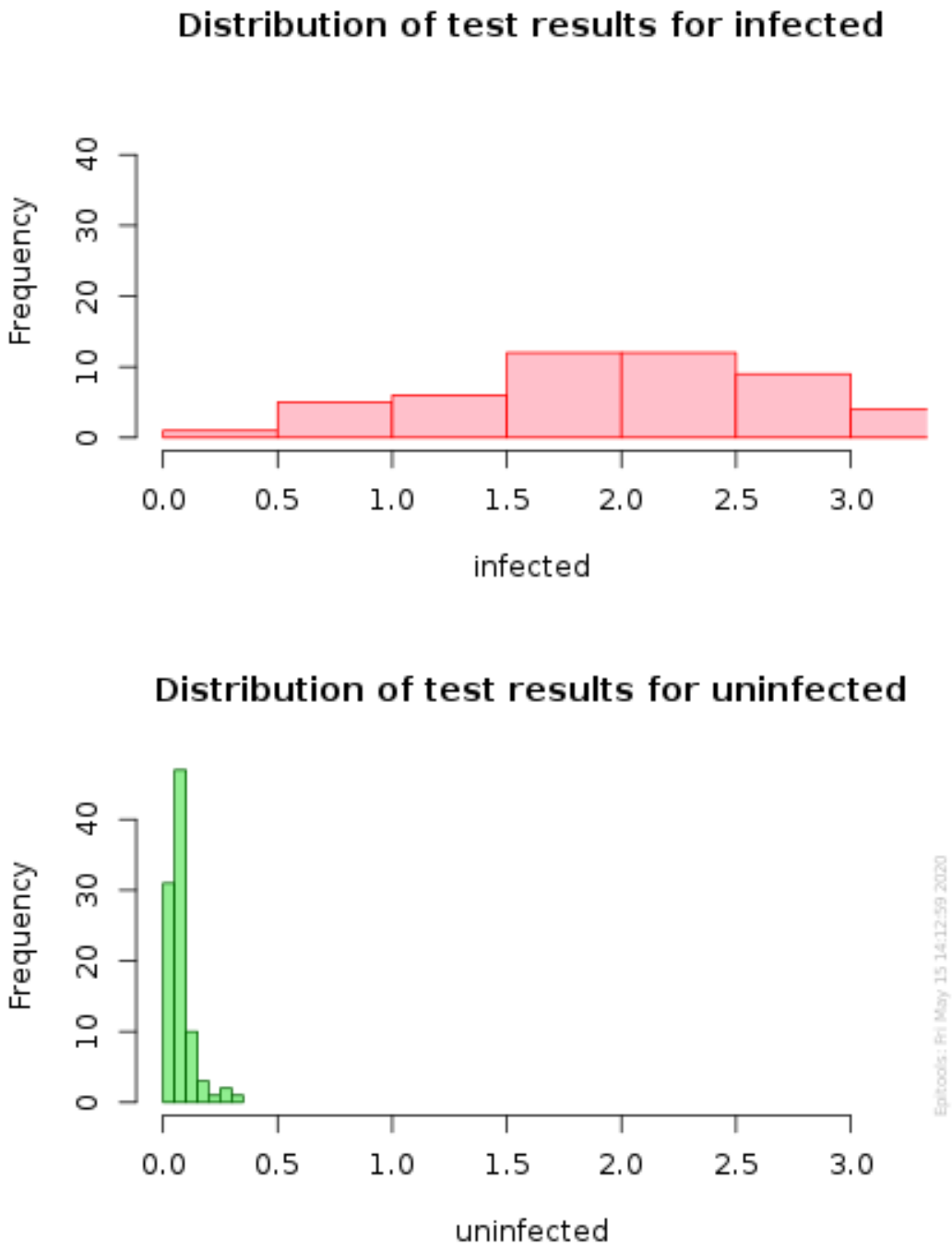

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464 **Figure 1: Distribution of absorbance values obtained with CovIgG-Assay.** The CovIgG-
465 Assay was optimized using as antigen recombinant Spike-S1-RBD from SARS-CoV-2.
466 Absorbance values were distributed in form of frequency histograms to clearly visualize the
467 separation between true positives (COVID-19 sera) (upper figure) and true negative populations

(sera from healthy subjects and subjects carrying other respiratory and viral infections) (lower figure).

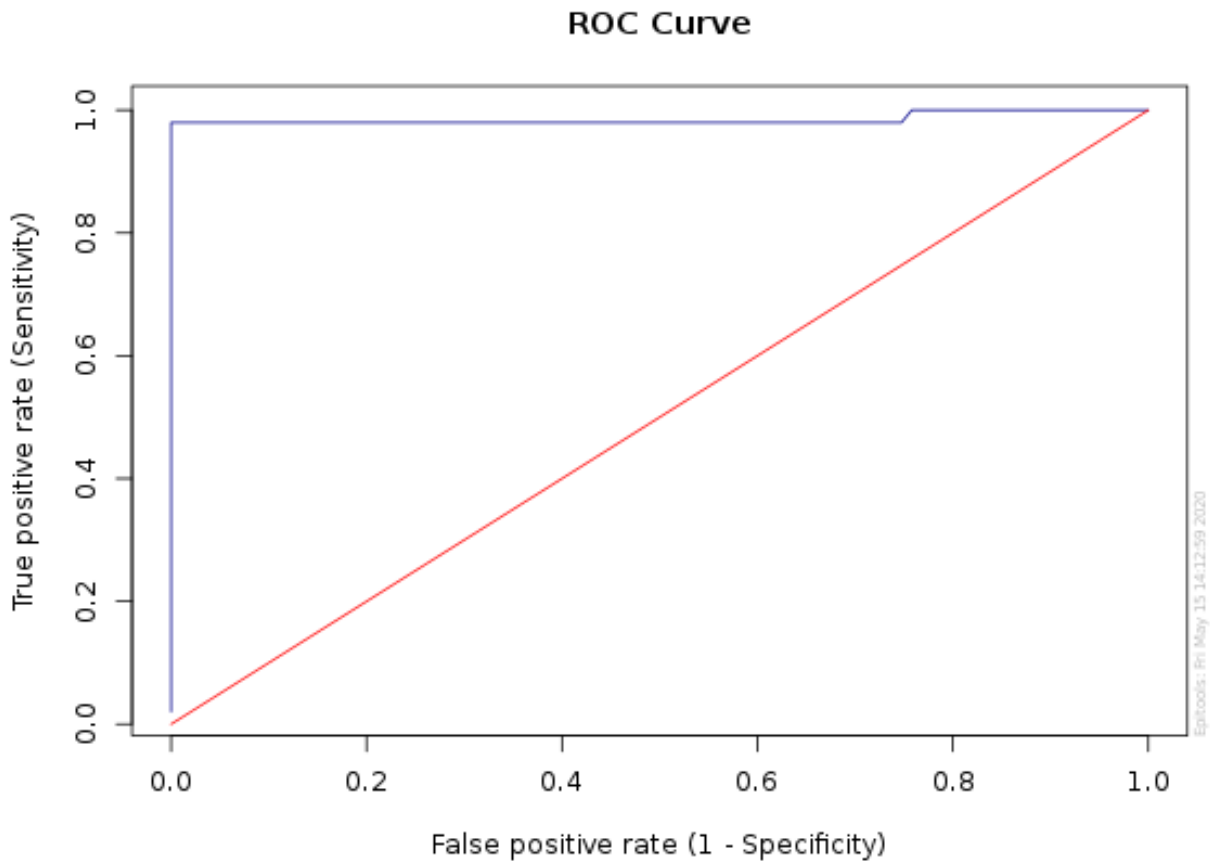

Figure 2: Receiver-operator characteristic (ROC) curve. The ROC curve was built for 132 sera from healthy subjects or subjects carrying other respiratory or other viral infections and 49 COVID-19 confirmed subjects. The area under the ROC curve (accuracy) was 0.985 and 95% for AUC= 0.954-1.

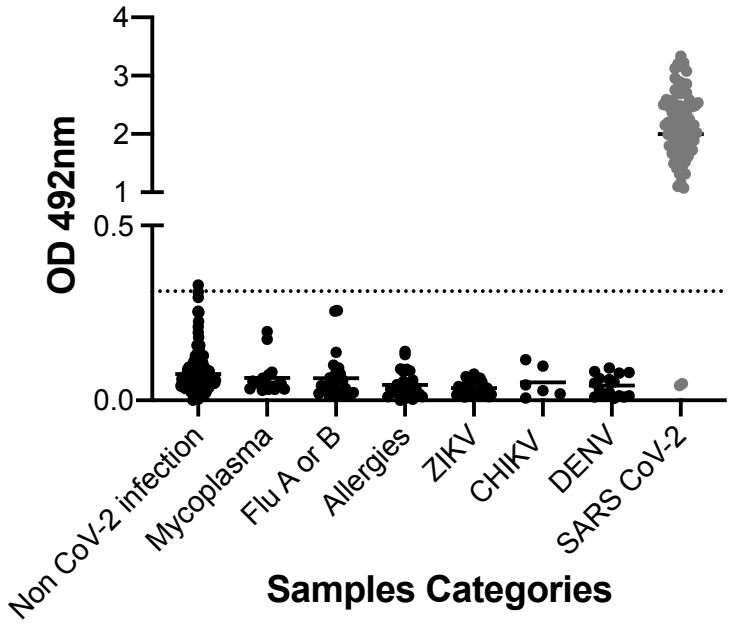

Figure 3: Validation of use of Spike S1-RBD ELISA for detection of SARS-CoV-2 IgG antibodies. Non-CoV-2 infection indicate negative cohorts from samples prior 2019 with no previous history of selected viral infections or respiratory allergies (n= 78). Black dots indicate samples which tested positive for Mycoplasma IgM, (n = 9), Influenza A or B (n=13), respiratory allergies (n=13), ZIKV (n=14), CHIKV (n=3), DENV (n=8). Gray dots indicate SARS CoV-2 infections (n=49). Dotted horizontal line indicate CoVIgG ELISA cutoff value (OD_{492}= 0.312). S, spike; RBD, receptor-binding domain; ZIKV, Zika virus; CHIKV, Chikungunya virus; DENV, Dengue virus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2. ODs in duplicate for every sample are showed.

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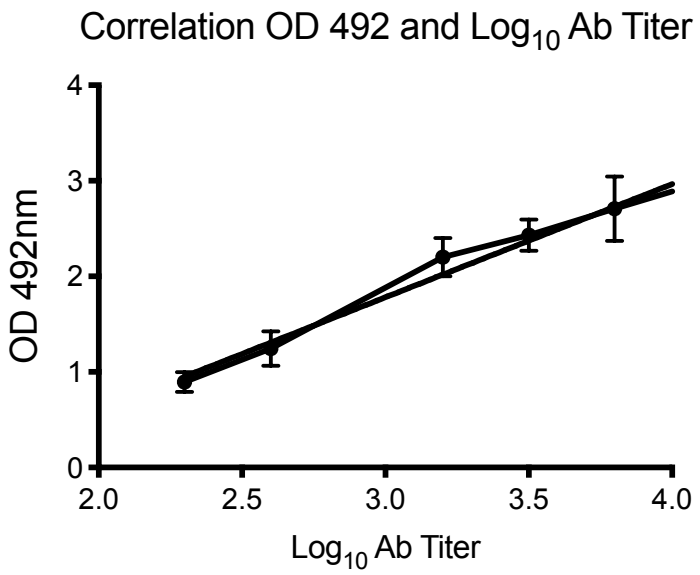

Best-fit values	
Slope	1.185 ± 0.07537
Y-intercept when X=0.0	-1.773 ± 0.2496
X-intercept when Y=0.0	1.497
1/slope	0.8440
95% Confidence Intervals	
Slope	0.9756 to 1.394
Y-intercept when X=0.0	-2.466 to -1.080
X-intercept when Y=0.0	1.101 to 1.779
Goodness of Fit	
R square	0.9841

Equation: Y= 1.185* X- 1.773

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497
498 **Figure 4: Correlation between absorbance at 492nm (A_{492}) and antibody titter.** A
499 total of 27 sera from confirmed COVID-19 subjects that resulted positive by CovIgG-
500 Assay were titrated at dilutions among 1:100 to 1:12,800. A lineal regression analysis
501 was then done in which the mean A_{492} of sera with similar antibody titer were plotted
502 with their corresponding A492 values. We found a lineal correlation (r^2=0.984) between
503 the antibody titer (maximal dilution that render A_{492} = 0.312) and the individual A_{492}
504 value at the working dilution (1:100). From this analysis the following lineal equation
505 (Y= 1.185*X -1.773) was obtained, which was further used to estimate the antibody titer
506 of all sera reported as seropositive by CovIgG-Assay.