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Comparative Evaluation of Immunoassays for Anti-Spike and Anti-Nucleocapsid Antibodies to SARS-CoV-2 Against a Commercial Surrogate Serological Viral Neutralization Test in COVID-19 Convalescent Samples

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ABSTRACT

Objective: assess five EIA tests aimed to quantify specific antibodies to SARS-CoV-2 and compared to a surrogate viral neutralization for assessing neutralizing antibodies.

Methods: We conducted a cross-sectional study during the period from July to October 2020, in which the sera of 96 participants whose ages were between 18 and 65 years were evaluated, all recovered from COVID-19 and obtained between 28 and 212 days after the onset of symptoms. ELISA tests used for testing include measurement of total antibodies (IgG & IgM); IgG antibodies against S protein (IgG S1/S2, IgG S, IgG S-RBD); IgG antibodies against nucleocapsid antigens, and IgM antibodies against S-RBD antigen. Pearson's linear correlation coefficient was used to know the degree of correlation between the values of the viral neutralization antibody titer and the values of the titer level of antibodies evaluated by each of the immunoassays performed.

Results: Of 96 participants, 46 were women (48%), with a mean age of 40.8 years. In the surrogate viral neutralization test, 85 samples (89.4%) were positive, and the positive neutralization rate ranged from 30% to 97%. In the correlation analysis to evaluate each of the tests that detect antibodies against the viral neutralization test, a positive correlation is observed in the tests for the detection of IgG antibodies against protein S, while the tests based on the detection of antibodies IgG against the nucleocapsid antigen showed a lower correlation.

Conclusions: Correlation analysis between each EIA test against the surrogate viral neutralization test showed better results for IgG antibodies against protein S and this is used to measure immunogenicity at the time of vaccination. This study assures us that serological assays can be used to monitor neutralizing antibody responses.

Key words: COVID-19, SARS-CoV-2, Pandemic, Immunoassay, Surrogate;

INTRODUCTION

COVID-19 epidemic begun in December 2019 in China and rapidly affected all countries around the world, becoming the most recent and serious pandemic threatening humankind [(1)]. COVID-19 is caused by a new Coronavirus, the SARS-CoV-2, apparently transmitted from wild animals to humans and now easily transmitted from person to person through respiratory droplets [(1-3)]. Laboratory diagnosis of COVID-19 has been very challenging since the beginning of the pandemic, due to the fastest development of serologic tests based on the detection of IgG and/or IgM and the waiver of compliance with quality standards for most regulatory agencies around the globe [(4-6)].

Surrogate tests for detecting neutralizing antibodies have been developed and validated in order to fill the gap of regular viral neutralization in public health [(7, 8)]. Serological assays mimicking results of virological assays were used widespread, pressuring the community to measure their level of neutralizing antibodies in order to assess vaccine efficiency without strong evidence [(9, 10)].

The need for developing and validating surrogate serological tests for assessing neutralizing antibodies rates among vaccinated and previously infected COVID-19 patients required the implementation of several assays. In this study we assess at least five EIA tests aimed to quantify specific antibodies to SARS-CoV-2 that can serve as a surrogate serological test for assessing neutralizing antibodies.

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Materials and Methods

Participants

A cross-sectional study was implemented during the period from July to October 2020, in which the sera of 96 participants whose ages were between 18 and 65 years were evaluated, all recovered from COVID-19 and obtained between 28 and 212 days after the onset of symptoms. Is important to point out that there was no specific requirement of time after-infection, due to the ongoing epidemic and the intention to measure immune response based on antibodies. A total of 88 participants had results for the confirmatory RT-PCR test performed at the National Institute of Health of Peru (INS).

As an initial diagnosis with complete resolution of symptoms after 28 days and 8 participants were diagnosed by clinical diagnosis and additionally by a positive serological test. Participants who were hospitalized and not hospitalized of both sexes were included. The RT-PCR (Reverse Transcription Polymerase Chain Reaction) test is a molecular test used for detecting the presence of specific genetic material in any pathogen, including a virus.

In your context, it seems to be used as an initial diagnostic tool. The National Institute of Health of Peru conducting these tests indicates a high standard of testing, likely for a significant health concern, possibly COVID-19.

Table 1: Demographic characteristics of participants included in the study (N=95)

Characteristic		n	(%)
Sex	Female	46	(48)
Age	Median (Range)	40.8	(21-61)
Diagnostic criteria for COVID-19	Positive RT – PCR test and positive rapid test	88	(92.3)
		8	(7.7)
Time post-diagnostics	28 – 47 days	33	(34)
	48- 81 days	31	(33)
	82 – 212 days	30	(33)
Classification according to severity of disease	Asymptomatic	4	(4.21)
(Source: WHO)	Mild	66	(69.5)
	Moderate	22	(23.2)
	Severe	3	(3.2)

Biological Specimens

Whole blood samples were collected in tubes without anticoagulant. The serum was immediately separated by centrifugation at 3500 rpm for 10 minutes and 8 aliquots of 500 uL each were prepared, kept at -50 ° C for a period of no more than 3 months and only thawed 30 minutes before the respective analysis.

Serological assays

Each sample was analyzed by duplicate, using a single aliquot, thawed 30 minutes before processing, with each of the seven different immunoassays whose characteristics are shown in table 2: This means that each sample was tested twice (in duplicate) to ensure accuracy and reliability of the results.

Table 2: Serologic tests used in the assessment

Name of the assay	Diasorin Liaison®S1/S2	Diasorin Liaison® IgM	BioMerieux Vidas®	Roche Elecsys®	Beckman Coulter Acces®	Euroinmun® S1	Euroinmun® NCP
Antibody detected	IgG	IgM	IgG	Ig Totales	IgG	IgG	IgG
Revombinant protein in solid phase	S1 + S2	RBD	S1	NC	RBD	S1	NC
Methodology	QL	QL	ELFA	EQL	QL	ELISA	ELISA
Sample volume (uL)	20	20	100	100	50	10	10
Instrument	Liaison® XL	Liaison® XL	Mini Vidas®	Cobas®	Acces®	Euroinmun Analyzer®	Euroinmun Analyzer®
Testing time	35 min	35 min	27 min	20 min	30 min	3 horas	3 horas
Requirement for Cut Off calculation	Calibrador	Calibrador	Estandar	Calibrador	Calibrador	Calibrador	Calibrador
Umbral value	AU/ml: 15	Ratio: 1.1	Ratio:1.1	Ratio: 1	Ratio: 1.1	Ratio: 1.1	Ratio: 1.1
Grey zone	AU/ml: 12 - 15	ND	ND	ND	ND	≥ 0.8 to <1.1	≥ 0.8 to <1.1
Interval measurement	3.8 - 400	3.8 - 400	NR	NR	NR	0 – 8.45	0 – 8.45
Reported sensitivity	97.6%	91.8%	100%	100%	96.8%	90%	86.7%
Reported specificity	99.3%	99.3%	99.9%	99.8%	99.6%	100%	99.6%

Footnotes: ND= Not Determined, NR=non-reactive

Surrogate Viral Neutralization Assay

All samples were also analyzed using a surrogate viral neutralization test based on the ability of neutralizing antibodies to inhibit the binding of the RBD region to the ACE-2 protein in a competitive ELISA format (GS-cPass; GenScript, Piscataway, New Jersey, USA). The assay was performed in the EVOLIS™ System (Bio-Rad), according to the manufacturer's instructions: serum samples and positive and negative controls were diluted 1:10 in a sample dilution buffer and then mixed in an equal volume with the HRP-RBD conjugate. After 30 minutes of incubation at 37°C, 100 uL of the mixture was transferred to a 96-well plate fixed with the recombinant ACE-2 protein. After incubation at 37 ° C for 15 minutes, the supernatant was removed by washing the plate 4 times. 100 uL of TMB was added as a substrate and incubated for 15 minutes in the dark, then the reaction was stopped by adding 50 uL of a H2SO4 solution. The plate was read in a spectrophotometer at 450 nm and the values were expressed as a percentage of reduction according to the established formula, a value greater than or equal to 30% was considered positive as indicated by the manufacturer. The plaque reducing neutralization test (PRNT), which is the gold standard, requires the use of live cells active viruses and a biosafety level 3 laboratory and several days to produce results; unavailable at the hospital setting were the tests were performed. The cPass SARS-CoV-2 Neutralizing Antibody Detection Kit is a neutralizing antibody test which assesses the presence of circulating antibodies that block the interaction of RBD-HRP with hACE2 with high correlation with the gold standard technique.

Ethical issues

All participants were informed of the objectives of the study and agreed to participate voluntarily by signing the informed consent for their inclusion before providing samples. The study was implemented in accordance with the Declaration of Helsinki, and the protocol was approved by the EsSalud Special IRB for COVID-19 Pandemic.

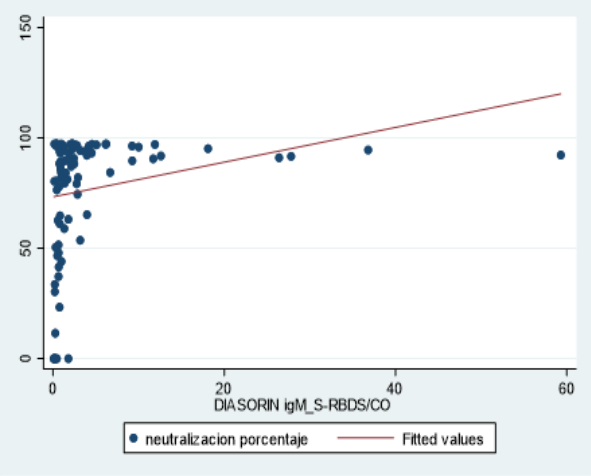
Results

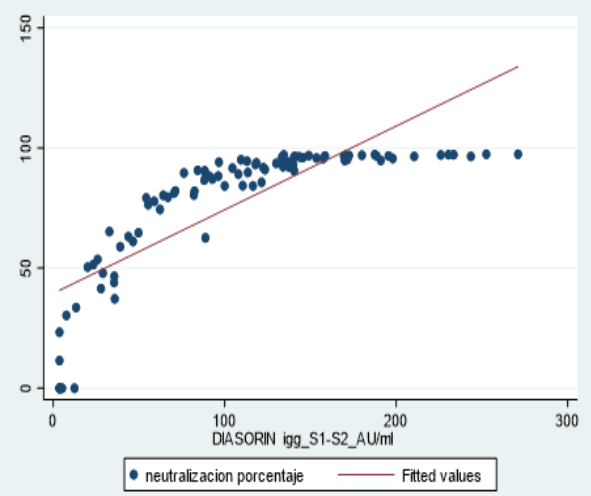
Participants

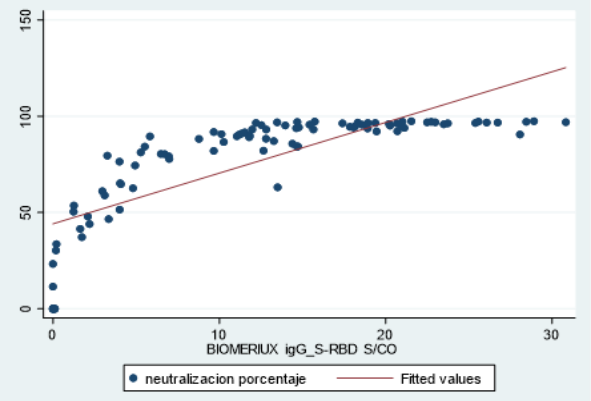
Samples of 95 participants among 96 recruited were included, of which 46 were female (48%), with a mean age of 40.8 years (21-61). A total of 88 participants (92%) had a positive RT-PCR test and 8 (8%) of the participants accessed the study with a clinical diagnosis plus a positive rapid serological test. The time after diagnosis of the disease was established between the date of the test that confirms the COVID-19 disease and the day of the obtention of the sample for the present study, with participants who had from 25 to 212 days of COVID-19 post diagnosis, which for analytical purposes were divided into tertiles of time (25-47, 48-81 and 82-212. Considering the criteria of the World Health Organization (WHO) to classify the degree of disease, there were 4 asymptomatic participants, 66 mild, 22 moderate and 3 severe. For the respective analyzes, the degree of the disease was grouped into two variables, asymptomatic/mild (n = 70) and moderate/severe (n = 25); See Table 1.

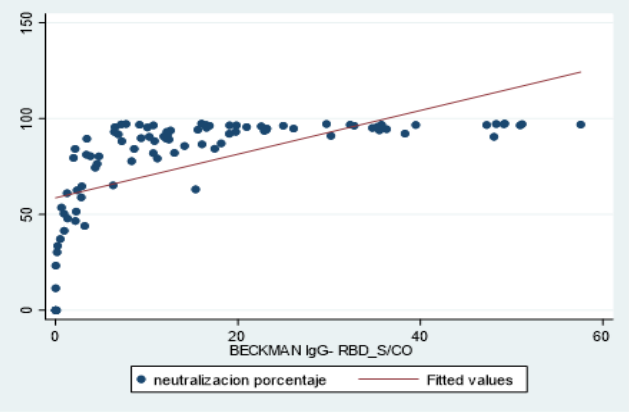
Correlation analysis

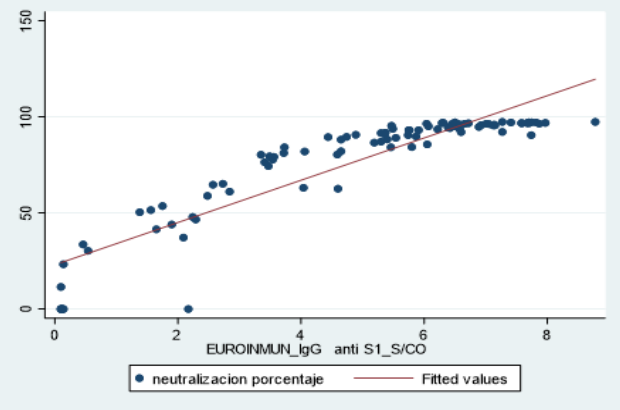
With the obtained results, the Spearman correlation analysis was used to evaluate each of the tests that detect antibodies (against the spike protein or against the nucleocapsid of the virus) against the surrogate viral neutralization test (table 2). A positive correlation was evidenced highlighting in descending order the test of DiaSorin IgG S1/S2 which was strongly correlated; EUROIMMUN IgG, BioMérieux IgG S and Beckman IgG S-RBD were moderately correlated (Figure 1). While the tests based on the detection of IgG antibodies against the nucleocapsid antigen were moderately correlated as shown by the EUROIMMUN IgG NCP test and the Roche total antibody test (Figure 1). The test aimed at detecting IgM antibodies against the S-RBD antigen (DiaSorin) was poor correlated with the neutralizing antibody test (Figure 1).

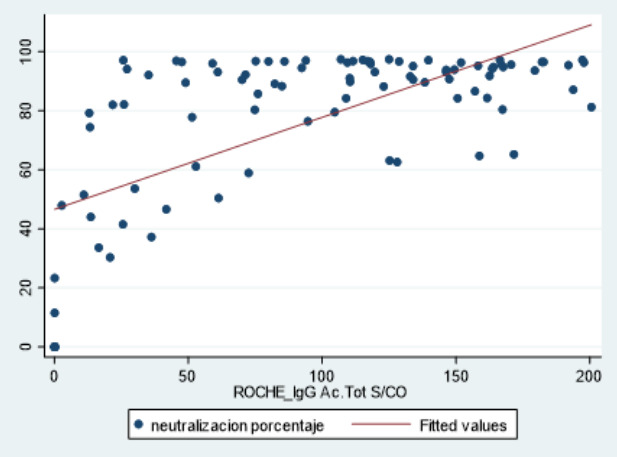


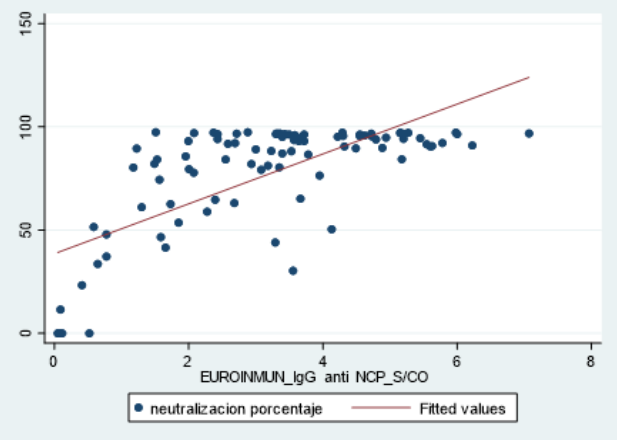












Qualitative interpretation of immunoassays

According to the criteria established by the assay manufacturers based on the cut-off point to consider a positive or negative result, the frequency of the results was determined, ranging from 86 participants (91%) who were positive for DiaSorin IgG S1/S2, 86 (91%) for Roche, 85 (90%) for EUROIMMUN IgG, 83 (87%) for bioMérieux, 82 (86%) for EUROIMMUN IgG NCP, 80 (84%) for Beckman and 48 (51%) for DiaSorin IgM; see table 2.

Surrogate Neutralization Assay, post-diagnosis time, and disease severity

In the results of the surrogate neutralization test, 85 samples (89.4%) were positive in a positive neutralization range that was 30% to 97% (30% cutoff value and 100% maximum possible value, ≤30% means no neutralization and >30% means there is neutralization). 10 samples had values lower than 30%, being considered negative (10.6%). Statistical analysis of the relationship between the level of neutralization (%) was carried out as a function of the post-diagnosis time (days), which was divided into tertiles (25-47, 48-81 and 82-212), showing that there are no statistically significant differences between the analysis groups. An analysis was also made according to the severity of the disease, which was divided into two groups (asymptomatic/mild and moderate/severe), determining a higher percentage of neutralization in the moderate/severe group compared to the asymptomatic/mild, ($p < 0.05$).

Discussion

Sera from 96 participants were collected and assayed using several EIA tests for the measurement of antibodies to SARS-CoV-2 and compared to a surrogate viral neutralization test to assess their comparability and performance. Sociodemographic correlates indicate equal distribution of participants regarding sex and most were diagnosed of having COVID-19 by positive RT-PCR testing. Survey and sampling were post-diagnosis and ranked from less than month to almost 7 months. Most participants reported having a mild to moderate disease, with few being asymptomatic and/or severe. Our correlation analysis between each of the tests against the surrogate viral neutralization test, showed better results when IgG antibodies against protein S was assessed, evidencing the elapsed time between the infection and testing.

Antibodies against protein S and/other spicula antigens seems to be more long-lasting among other antibodies, and are the base of immunogenicity measures when vaccination and/or infection occurs [11]. Lower correlation between the assessed tests and tests that detect IgG against nucleocapsid antigen reveals the weak immunogenicity caused by the core of the SARS-CoV-2 virus, making it inadequate for both, diagnostics tests and vaccines development, due to the poor response when generating correspondent antibodies [12]. The lowest correlation was found when we tried to assess detection of IgM antibodies against S-RBD antigen, again, most sera were from prolonged past infections making it more difficult to detect IgM, which in the new infection, would reveal a shift to a more chronic stage of the disease, even when COVID-19 has shown different and confuse patterns in terms of antibody response [13].

Among the 96 sera analyzed by all the tests from different manufacturers, we found a better performance when using the DiaSorin test which is focused in the detection of IgG anti S1/S2 antigens; in contrast, the lowest performance was reached by the same brand when detecting total IgM against SARS-CoV-2[14]. The use of calibrators and standards reveals, at this stage of the epidemic, that there is no clear way to stablish reliable cut-offs for detecting antibodies for the new coronavirus [15, 16]. Is important to annotate that performance of the tests used in the study were similar between them with ranges for sensitivity from 86% to 98.3%; and for specificity from 95% to 100% according to manufacturer's package insert; and definitely since performance could be affected by sensitivity and specificity we should consider additional factors such a time after infection, immune response of the host, accuracy of measurement among others [1].

When assessing the surrogate neutralization test, we found a very wide positive neutralization range, which includes a low cut of value, and based on this most sera were considered as positives[17]. There were no differences between level of neutralization and days post-infection, revealing that neutralizing antibodies, tested by surrogate tests, can be present and detected at any time in the majority of sera tested [18]. When Rathe [16], tested neutralization assays for SARS-CoV-2 antibodies the performance of IgG against RBD were superior, which correlates well with our findings. Also, the performance of the surrogate neutralization test assessed ranks like Peterhoff et al., [19]which in certain way assure that serological. Assays can be used for monitoring naturalizing antibody response. Serological tests usually detect antibodies against the spike protein (S) and/or nucleoprotein (N), since they are the most immunogenic proteins of SARS-CoV-2, it has been shown that antibodies directed against the S1 subunit of the SARS-CoV-2 S protein, specifically against the receptor binding domain (RBD), strongly correlates with virus neutralization[20].

In our study, the DiaSorin IgG S1/S2 test showed better correlation compared to the surrogate viral neutralization test, which was expected since both assays target the RBD region of the S1 protein. Clinically speaking, this assay has the potential to be used for the detection of IgG antibodies that correlate with the development of protective immunity against SARS-CoV-2 infection. This study assures that serological assays can be used to monitor neutralizing antibody responses as a strategy for monitoring individual's immune response to COVID-19 infection and vaccination, reducing costs, times, and facilitating access to testing for the population.

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