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EXTREME ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2 SPECIAL ANTIBODY REACTION IN CORONAVIRUS ILLNESS CASES

[1]Sidratul Muntaha, [2]Aqsa Khaild, [3]Dr Sania Hussain

[1]Fatima Jinnah Medical University, [2]Fatima Jinnah Medical University, [3]Shalamar Medical and Dental College, Lahore.

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Abstract:

Another Covid, Covid 2 (SARS-CoV-2), an extremely intense respiratory disorder, has recently emerged to cause a human pandemic. While atomic indicator tests have evolved rapidly, serological measurements are still lacking, but are desperately needed. Approved serological tests are needed for contact tracing, virus pool recognition and epidemiological investigations. Our current research was conducted at Jinnah Hospital, Lahore from February 2020 to October 2020. We have developed serological tests for the discovery of antibodies to SARS CoV-2, explicit antibodies to peak proteins and explicit antibodies to nucleocapsules. Using serum assays from patients with PCR-confirmed SARS CoV-2 contaminations, other Covid, or other pathogenic respiratory diseases, we have also approved, assayed different antigens in various in-house and commercial ELISAs as well. We have shown that most PCR-confirmed SARS CoV-2 infected individuals seroconverted approximately 14 days after the onset of infection. We found that the IgG or company S1 IgA ELISAs were less specific, and that the affectability varied between the two tests; the IgA ELISAs indicated higher affectability. In general, the approved tests described may be useful for the discovery of explicit antibodies to SARS-CoV-2 in the context of demonstrative and seroepidemiologic antibody evaluation.

Keywords: *Extreme Acute Respiratory Syndrome, Coronavirus 2–Special Antibody Reaction.*

Corresponding author:

Sidratul Muntaha,

Fatima Jinnah Medical University.

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INTRODUCTION:

In December 2019, another Covid appeared in China and caused an intense respiratory infection, now known as Covid 2019 infection (COVID-19). The infection was recognized as a beta-coronavirus related to Covid intense respiratory illness (SARS-CoV) and was therefore named SARS-CoV [1]. In <2 years, this infection is the third known Covid infection to cross the species boundary and cause extreme respiratory infections in humans after SARS-CoV in 2003; in addition, Middle East Respiratory Covid Disease (MERS-CoV) in 2012, but with exceptional contrasted spread and the 2 previous infections. Given the rapid rise in the number of cases, the uncontrolled spread and the magnitude of the disease worldwide, the World Health Organization has announced SARS-CoV- 2 as a pandemic [2]. As of March 14, 2020, the infection had infected more than 130,000 people in 121 countries, 4.8% of whom had died. Rapid evidence of the etiology and shared hereditary arrangement of the infection, followed by the global community's efforts begun following the development of SARS-CoV-2, provided rapid access to continuous indicative PCR measurements that help determine the case and track the episode. The availability of these tests contributed to patient recognition and efforts to contain the infection [3]. Nevertheless, approved serological tests are still lacking and are seriously needed. Approved serological tests are essential for the follow-up of persistent contacts, as the viral repository also has epidemiological tests available. Epidemiological investigations are desperately needed to help reveal the burden of disease, particularly the rate of asymptomatic illness, and to improve measures of illness and death. Among the 4 underlying Covid proteins, the peak and nucleocapsid proteins are the main immunogens [4]. We describe the improvement of serological assays for the localization of infection-killing antibodies and antibodies to the N protein and to the different zones of the S protein, including the S1 subunit, and the receptor-bounding space (RBD) of CoV-2-SARS from an ELISA model. Using a well-described partner of PCR-confirmed serum assays of CoV-2-SARSS and PCR-confirmed patients as being contaminated with occasional Covid and other respiratory microorganisms, we approved and tested different antigens at different stages created in-house, as well as a commercial step [5].

METHODOLOGY:

We used serum tests (n = 10) collected from 3 patients confirmed by PCR: 2 with COVID-19 mild and 1 with COVID-19 severe (Table 1) from France according to the recommendations of neighboring morality. Our current research was conducted at Jinnah Hospital, Lahore from February 2020 to October 2020. For test approval, we used examples obtained from persons with PCR determined contaminations with human covids (HCoV-229E, NL63 or OC43), SARS-CoV, MERS-CoV or other respiratory infections (Table 1) as detailed (6). We have also included examples of patients who had late cytomegalovirus disease, Epstein-Barr infection, or Mycoplasma pneumonia, on the grounds that these microbes have a higher probability of causing fictitious positive results. As negative controls, we used serum tests from 48 solid blood contributors (accomplice A). We also tested serum tests from SARS patients. All examples were stored at -20°C until use. The Sequin Blood Bank obtained informed consent for the research on blood donor testing. The use of serum tests from the Netherlands was confirmed by the local clinical morality committee. All serum tests (n = 31) of patients with COVID-19 PCR confirmed cases were recently examined by a recombinant immunofluorescence test based on the SARS-CoV-2 S protein and neutralization of plaque decline. We tested the serum assays as part of an overall indicative routine after obtaining informed patient consent. We obtained SARS-CoV-2 free serum tests (n = 31) from the serum assortment of the National Coronavirus Detection Laboratory at Mayo Hospital, Lahore. The tests were collected after obtaining informed consent.

Figure 1:

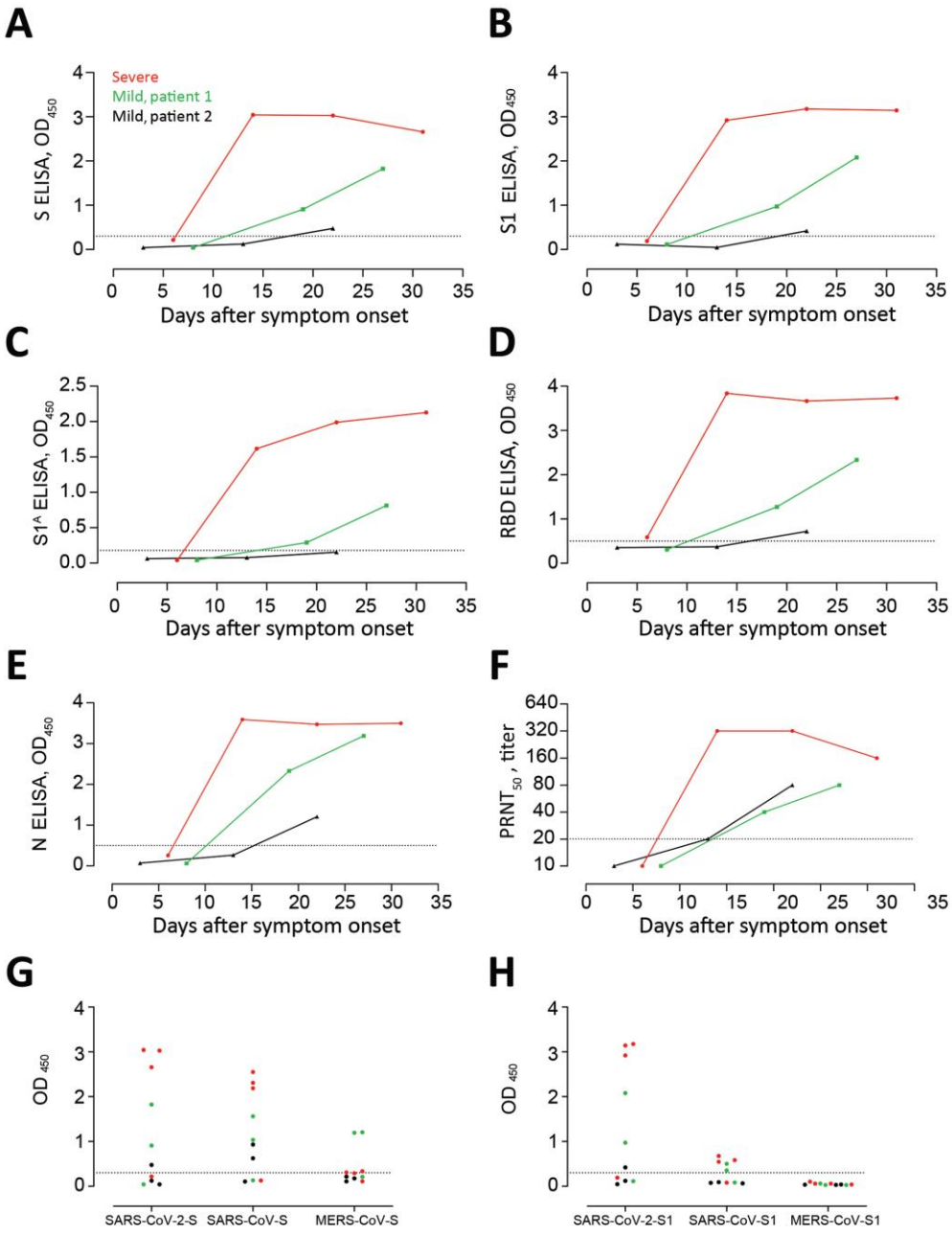

A
B
C
D
E
F
G
H
Severe
Mild, patient 1
Mild, patient 2
S ELISA, OD450
S1 ELISA, OD450
S1^ ELISA, OD450
RBD ELISA, OD 450
N ELISA, OD450
PRNT50, titer
Days after symptom onset
OD 450
SARS-CoV-2-S
SARS-CoV-S
MERS-CoV-S
SARS-CoV-2-S1
SARS-CoV-S1
MERS-CoV-S1

Figure 2:

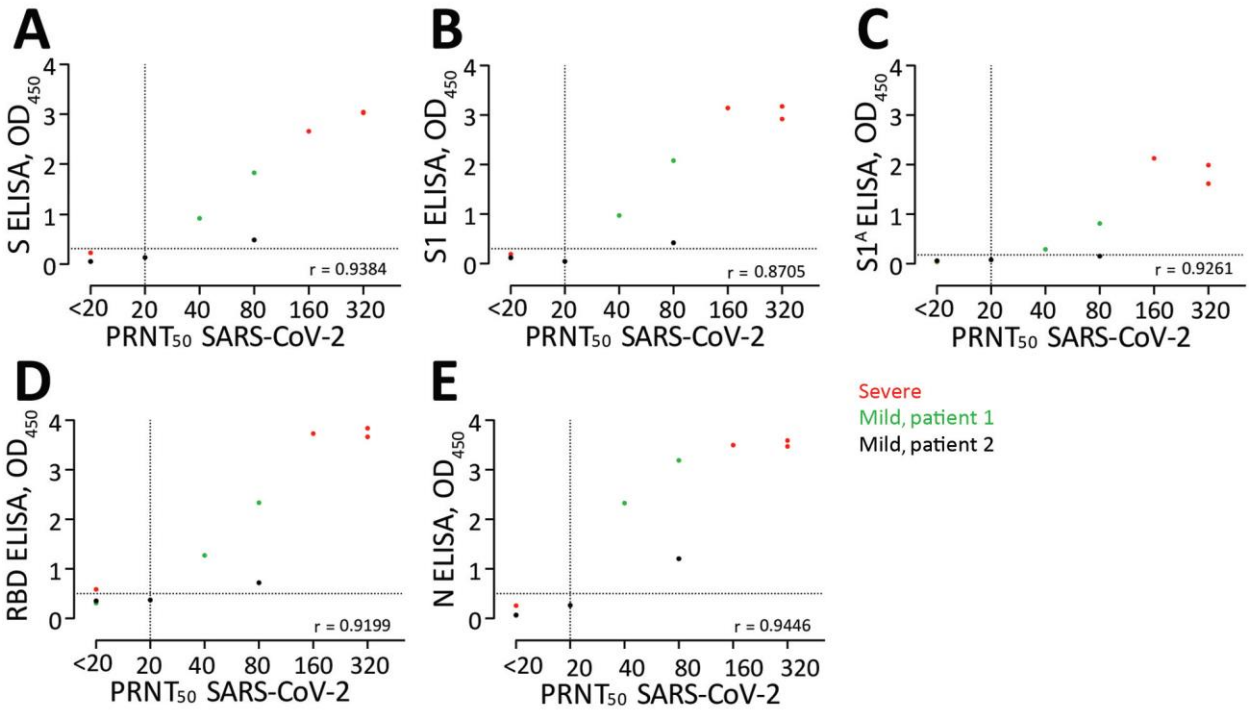
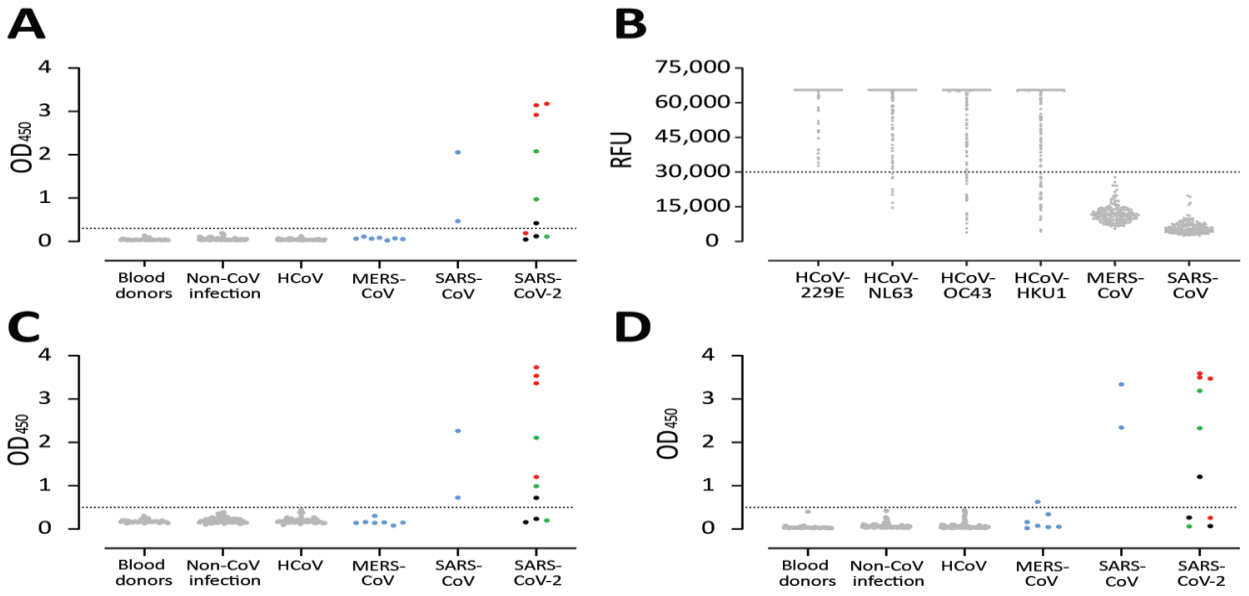


Figure 3:



RESULTS:

We evaluated reactions to explicit CoV-2 neutralizers in severe and mild cases using serum tests collected on various occasions after onset of disease in 3 French patients with PCR-confirmed COVID-19. We tested serum tests for CoV-2 antibodies using different ELISAs. After infection, each of the 3 patients underwent seroconversion between days 16 and 23 after the onset of infection (Figure 1), and antibodies were raised against CoV-2 S, S1 subunit and DBR, but only 2/3 of the patients had antibodies detectable in the N-terminal area. Since the CoV-2-SARS N protein is

92% similar to CoV-SARS (Table 2), we used CoV-SARS N protein as an antigen to test for antibodies coordinated to CoV-2-SARS N protein in an ELISA model. We found that antibodies were raised against the N protein in all three patients. At the time of the trial in PRNT, serum tests from each of the three patients killed the CoV-2-SARS disease. Neutralizing reactions recognized by various measures strongly associated with neutralizing immunization reactions (Figure 2). We found cross-reactivity with the SARS-CoV S and S1 proteins, and to a lesser degree with MERS-CoV S, but not with MERS-CoV S1 (Figure 1, Tables G, H). This finding appeared when dissecting the level of similarity of the various areas of the Covid S protein with the SARS-CoV-2 related proteins (Table 2). This examination indicated that the S2 subunit is more rationed and subsequently assumes a role in the cross-reactivity observed when whole S was used as an antigen. In this way, S1 is more explicit than S as an antigen for the serological conclusion of SARS-CoV-2.

Table 1:

Name	Antigenicity and functionality	Adjuvant	Route	Animal models	Antibody response	Cellular immune response	Protection	References
Subunit vaccines based on SARS-CoV full-length or trimeric S protein								
FL-S and EC-S proteins	Bind to SARS-CoV S1, NTD, RBD, and S2-specific mAbs	MPL + TDM	S.C.	BALB/c mice	Elicit SARS-CoV S-specific Abs (IgG, > 1:2 × 10^5), neutralizing (> 1:2.4 × 10^4) pseudotyped SARS-CoV (Tor2, GD03, and SZ3 strains)	N/A	N/A	He et al., 2006a
S andS-foldon proteins	N/A	TiterMax Gold; Alum Hydro + MPL	S.C. or I.M.	BALB/c mice	Elicit SARS-CoV S-specific Abs (IgG, > 1:10^4) in mice, neutralizing (~2.4 × 10^2 for S; ~1:7 × 10^2 for S-foldon) live SARS-CoV (Urbani strain)	N/A	Protect vaccinated mice from challenge of SARS-CoV (Urbani strain, 10^5 $TCID_{50}$) with undetectable viral load in lungs	Li et al., 2013
trlSpike protein	N/A	Alum hydro	I.P. or S.C.	BALB/c mice; Hamsters	Elicits SARS-CoV S-specific mucosal and serum Abs (IgA and IgG) in mice and hamsters, blocking S-ACE2 receptor binding and neutralizing live SARS-CoV (HKU-39849 strain); induces ADE	N/A	Protects vaccinated hamsters from challenge of SARS-CoV (Urbani strain, 10^5 $TCID_{50}$) with undetectable or reduced viral load in lungs	Kam et al., 2007; Jaume et al., 2012
Subunit vaccines based on SARS-CoV RBD protein								
RBD-Fc protein	N/A	Freund's	I.D. orI.M.	BALB/c mice; Rabbits	Elicits SARS-CoV S/RBD-specific Abs (IgG) in mice and rabbits, neutralizing pseudotyped (rabbits: ≥ 7.3 × 10^4) and live (mice: 1:4 × 10^2; rabbits: > 1:1.5 × 10^4) SARS-CoV (BJ01 strain)	N/A	Protects majority (4/5) of vaccinated mice from challenge of SARS-CoV (BJ01 strain, 10^5 $TCID_{50}$), with one mouse showing mild alveolar damage in lungs	He et al., 2004; Du et al., 2007
RBD193-CHO; RBD219-CHO proteins	Binds to SARS-CoV RBD-specific mAbs (neutralizing 24H8, 31H12, 35B5, 33G4, 19B2; non-neutralizing 17H9)	Freund's	S.C.	BALB/c mice	Elicit SARS-CoV RBD-specific Abs, neutralizing pseudotyped (< 1:10^4 for RBD193-CHO; 1:5.8 × 10^4 for RBD219-CHO) and live (< 1:10^3 for RBD193-CHO; 1:10^3 for RBD219-CHO) SARS-CoV (GZ50 strain)	Induce SARS-CoV RBD-specific cellular immune responses (IFN-γ, IL-2, IL-4, IL-10) in mice	Protect all (for RBD219-CHO) or majority (3/5, for RBD219-CHO) of vaccinated mice from challenge of SARS-CoV (GZ50 strain, 100 $TCID_{50}$ for RBD193-CHO; 5 × 10^5 $TCID_{50}$ for RBD219-CHO) with undetectable viral RNA or no, to reduced, viral load in lungs	Du et al., 2009c, 2010
RBD-293T protein	Binds to SARS-CoV RBD-specific mAbs (neutralizing 24H8, 31H12, 35B5, 33G4, 19B2; non-neutralizing 17H9)	SAS	S.C.	BALB/c mice	Elicits SARS-CoV RBD-specific Abs (IgG), neutralizing pseudotyped (1:6.9 × 10^5) and live (1:1.6 × 10^3) SARS-CoV (GZ50 strain)	N/A	Protects all vaccinated mice from challenge of SARS-CoV (GZ50 strain, 100 $TCID_{50}$) with undetectable viral RNA and viral load in lungs	Du et al., 2009b
S318-510 protein	N/A	Alum; Alum + CpG	S.C.	129S6/SvEv mice	Elicits SARS-CoV-specific Abs (IgG, IgG1, and IgG2a) in mice. Reduces neutralization after removing glycosylation	Induces SARS-CoV S-specific cellular immune responses (IFN-γ) in mice	N/A	Zakhartchouk et al., 2007
Subunit vaccines based on non-RBD SARS-CoV S protein fragments								
S1 and S1-foldon proteins	N/A	TiterMax Gold; Alum Hydro + MPL	S.C. or I.M.	BALB/c mice	Elicit SARS-CoV S-specific Abs (IgG, > 1:10^4) in mice, neutralizing (1:1.7 × 10^2 for S1; 1:90 for S1-foldon) live SARS-CoV (Urbani strain)	N/A	Protect vaccinated mice from challenge of SARS-CoV (Urbani strain, 10^5 $TCID_{50}$) with undetectable viral load in lungs	Li et al., 2013
S2 protein	N/A	Freund's	S.C.	BALB/c mice	Elicits SARS-CoV S2-specific Abs (IgG, 1:1.6 × 10^3) in mice with no neutralizing activity	Induces SARS-CoV S2-specific cellular immune responses (IFN-γ and IL-4) in mice	N/A	Guo et al., 2005
Subunit vaccines based on SARS-CoV non-S structural proteins (i.e. N and M)								
rN protein	N/A	Freund's	I.P.	BALB/c mice	Elicits SARS-CoV N-specific Abs (IgG (1:1.8 × 10^3), IgG1, and IgG2a) in mice	Induces cellular immune responses with up-regulated IFN-γ and IL-10 cytokines in mice	N/A	Zheng et al., 2009
rN protein	N/A	Montanide + CpG; Freund's	S.C.	BALB/c mice	Elicits SARS-CoV N-specific Abs (IgG) in mice	Induces SARS-CoV N-specific cellular immune responses (IFN- γ) in mice	N/A	Liu et al., 2006
M1-31 and M132-161 peptides	Bind to sera from SARS patients or immunized mice and rabbits	Freund's	I.D.	BALB/c mice; NZW rabbits	Induce SARS-CoV M-specific Abs (IgG) in rabbits	N/A	N/A	He et al., 2005b

[a]Abs, antibodies; ADE, antibody-dependent enhancement; Alum hydro, aluminum hydroxide; CHO, Chinese hamster ovary; CpG, cysteine-phosphate-guanine; I.D., intradermal; I.M., intramuscular; IFN-γ, interferon gamma; IL-2, interleukin 2; IL-4, interleukin 4; IL-10, Interleukin 10; I.P, intraperitoneal; mAbs, monoclonal antibodies; Montanide, Montanide ISA-51; MPL + TDM, monophosphoryl lipid A and trehalose dicorynomycolate; N/A, not reported; NTD, N-terminal domain; NZW rabbits, New Zealand White rabbits; RBD, receptor-binding domain; SAS, Sigma adjuvant system; S.C., subcutaneous; $TCID_{50}$, median tissue culture infectious dose.

Table 2:

	Patient 1		Patient 2		Patient 3	
Age	46		19		43	
Sex	Male		Female		Female	
No. symptomatic days	28		29		20	
Lowest Ct-value[a]	23		24.9		15.9	
No. positive viral PCR	6		3		3	
Total serum antibody concentrations[b]	*IgG*	8.1	*IgG*	10	*IgG*	9.7
	IgA	1.8	*IgA*	1.2	*IgA*	0.9
	IgM	0.48	*IgM*	1.4	*IgM*	1.5
α-SARS-CoV-2 IgG	Neg		Neg		Neg	
Neutralizing ab titer[c]	12		8		48	
α-RBD antibodies	*IgG*	Pos	*IgG*	Neg	*IgG*	Pos
	IgA	Neg	*IgA*	Neg	*IgA*	Neg
	IgM	Neg	*IgM*	Neg	*IgM*	Neg

Blood samples analyzed for specific antibodies and total serum antibodies collected at day 76 (patient 2) and 91 (patient 1 and 3) post symptom onset.

[a]Ct values <38 are considered positive.

[b]Normal range: IgM 0.27–2.1, IgG 6.7–15, IgA 0.88–4.5 g/L. RDB = Receptor binding domain.

[c]Titers ≥4 are considered positive.

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DISCUSSION:

Approved serological tests for SARS CoV-2 are desperately needed for follow-up contact examinations, epidemiological testing and antibody evaluation [6]. Since N and S proteins are the major immunogenic Covid proteins, we have developed ELISA-based tests that identify antibodies to these two proteins and to the two S spaces, S1A and RBD [7]. The results of these assays are now highly correlated with the consequences of PRNT50 henceforth. As most people have antibodies against the 4 endemic human Covid, there was an urgent need to confirm the specificity of these measurements to maintain a strategic distance from false positive results [8]. Similarly, the two zoonotic covid, SARS-CoV and MERS-CoV, are added to the beta-coronaviruses, raising the potential for cross-reactivity. Of the S antigens tested, S1 was more explicit than S in distinguishing antibodies to the SARS-2 CoV, as cross-reactive MERS-CoV S antibodies were identified in the serum of one of the COVID-19 patients, which was not observed when the MERS S1 CoV was used for testing [9]. This finding was clarified by the high level of preservation in the Covid S2 subunit compared to S1 (Table 2). Thus, in line with our previous findings for the serological examination of the MERS CoV, S1 is a particular antigen for the diagnosis of the SARS-2 CoV [10].

CONCLUSION:

In general, the tests developed and approved in this survey could be useful for follow-up of tolerant contacts, sero-surveillance studies and evaluation of the proposed immunization. In any case, given that different tests will be performed in different laboratories, there is an urgent need to align and standardize the tests created by the various research facilities using standard references characterized on all sides as a characteristic of the approval of analytical tests. This alignment should not only reduce the fluctuation between tests, but also combine the results obtained by different laboratories using different tests (15). This relationship is important to better review and translate the results of various investigations, to evaluate immunization preliminaries, to allow a consistent assessment of immunogenicity, viability, and a better understanding of the relationship between safety and security. Hence, the establishment of reference boards is an essential component of our preparedness to deal with infections that do occur.

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