



CAN IgG ANTIBODIES DIRECTED AGAINST DIFFERENT TARGETS SOLVE THE PROBLEM OF FALSE-POSITIVE RESULTS IN SARS-CoV-2 SEROLOGY?

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SUMMARY – In addition to RT-PCR assays, serology testing that has been recognized as a useful tool to assess the spread of infection in the population is considered successful and important strategy in the control of the global pandemic of SARS-CoV-2 infection. Now, a great number of manufacturers offer their serologic tests on the market. When interpreting the results, the rate of seroprevalence should be taken in consideration because it may influence the positive predictive value, as well as cross-reactivity with other coronaviruses in case of assays with poorly designed antigens. We present results of 11 patients with different clinical background and tested with two different serologic tests, DIAPRO (ELISA; Sesto San Giovanni, Italy) and VIDAS (ELFA; BioMerieux, Marcy l’Etoile, France). The results obtained by the former test showed ten of these patients to be IgG positive and one patient was IgG weakly positive with different confidence index. The latter test discriminated positive results with medium confidence index on the former test as negative. The results obtained with two serology tests were concordant with the observation that the results with medium confidence index may indicate cross-reactivity.

Key words: SARS-CoV-2; Serology; Anti-SARS-CoV-2 IgG; False-positive results; ELISA; ELFA

Introduction

According to previous studies, serologic testing has been suggested to play three roles in the COVID-19 pandemic, i.e. diagnosis, identification of convalescent plasma donors, and detecting seropositive persons to determine exposure and immunity¹. So far, due to several issues, such as whether they are not properly evaluated, the results can be potentially misinforming and misdiagnosing. These are as follows: false-positive and false-negative results, effect of low seroprevalence rate in a certain population on positive predictive value (PPV), and absence of proof that the antibodies are neutralizing and ensure protection at the same level at which they are detected.

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The aim of this study was to analyze and compare the results of two different serology tests for the diagnosis of SARS-CoV-2 infection in 11 patients with different clinical background.

Patients and Methods

In order to get a more precise serologic answer, we performed two enzyme immunoassays, DIA.PRO (enzyme-linked immunosorbent assay, ELISA; Sesto San Giovanni, Italy) and VIDAS (enzyme-linked fluorescent assay, ELFA; BioMerieux, Marcy l’Etoile, France) for determination of IgG antibodies to SARS-CoV-2 in human plasma and serum. The manufacturer of the first test claims that it can be used to identify the specific antibodies to the major immunodominant SARS-CoV-2 antigens (spike glycoprotein 1, spike glycoprotein 2, and nucleocapsid) with module-based ELISA for the confirmation of samples positive for IgG antibodies to SARS-CoV-2 in the first screening.

Table 1. Serology tests used for SARS-CoV-2 antibody detection

Test	Manufacturer	Reference values
Enzyme immunoassay:		
COVID-19 ELISA IgG	DIA.PRO, Sesto San Giovanni, Italy	IgG (S/Co) <0.9 negative; 0.9-1.1 equivocal; >1.1 positive
Confirmation of IgG antibodies to COVID-19 ELISA	DIA.PRO, Sesto San Giovanni, Italy	IgG (S/Co) <0.9 negative; 1-1.2 equivocal; >1.2 positive
		Confidence index:
		Very high – antibodies to all the 3 antigens
		High – antibodies to 2 antigens
		Medium – antibodies to nucleocapsid only
VIDAS SARS-CoV-2 ELFA IgG	bioMerieux, Marcy l'Etoile, France	IgG i= <1 negative; >=1 positive

Table 2. Antibodies against specific antigen determinants in eleven patients with positive IgG

Patient		IgG screening DIA.PRO	S1 ^a	S2 ^b	N ^c	Confidence index	IgG screening bioMerieux
1	Close contact with COVID-19 positive PCR persons	+/-					-
2		+	+	-	+	High	+
3		+	+	+	+	Very high	+
4	No contact, no clinical signs or symptoms	+	-	-	+	Medium	-
5	SARS-CoV-2 PCR positive	+	+	-	+	High	+
6		+	+	+	+	Very high	+
7		+	-	-	+	Medium	-
8		+	+	-	+	High	+
9		+	+	-	+	High	+
10	Sera obtained in September 2019 for other indication	+	-	-	+	Medium	-
11		+	-	-	+	Medium	-

^aIgG against spike glycoprotein S1; ^bIgG against spike glycoprotein S2; ^cIgG against nucleocapsid

The results are interpreted according to confidence index of the confirmatory assay that defines the level of reliability of the given result, which is reported as very high (antibodies to all the 3 antigens), high (antibodies to 2 antigens), and medium (antibodies to nucleocapsid only) (Table 1). According to the manufacturer of the other test, it can be used to measure the presence of anti-SARS-CoV-2 IgG antibodies to the major immunodominant SARS-CoV-2 antigens (spike glycoproteins) in human plasma and serum from people who have been infected with SARS-CoV-2, described as the target of neutralizing antibodies (Table 1).

We tested 11 patients with different clinical background of which 10 were all IgG positive and one was

weakly IgG positive when tested with DIA.PRO test, with consecutive confidence index results shown in Table 2, along with the IgG results obtained by bioMerieux test. Patients 1-3 were in close contact with COVID-19 polymerase chain reaction (PCR) positive persons, had no clinical signs or symptoms, and tested negative on PCR after 14 days of self-isolation. Patient 4 had no contact and no COVID-19 clinical signs or symptoms, and his serum was available because other tests were ordered. Patients 5-9 were all SARS-CoV-2 PCR positive and sera samples from patients 10 and 11 were obtained for some other indications in September 2019 before the pandemic outbreak.

Results

Our results obtained by DIA.PRO test showed that all IgG positive patients were positive for nucleocapsid antigen. In four patients, it was the only positive type of IgG having medium confidence index according to our test interpretation. These included one patient with no contact and no COVID-19 clinical signs or symptoms whose serum was available because of other tests, two patients with sera obtained in September 2019, and one patient with positive PCR result.

In all other six patients including those with positive PCR and those in contact with PCR positive persons, IgG antibodies to spike glycoprotein 1 and/or spike glycoprotein 2 were also positive yielding a very high or high level of reliability that the antibodies were directed to SARS-CoV-2.

The results obtained by bioMerieux test showed that patient 1 was negative although on DIA.PRO test the result was equivocal. Patients 4, 7, 10, and 11 were negative while on DIA.PRO test the result was positive having medium confidence index (Table 2). All patients that were positive and yielding a very high or high level of reliability that antibodies were directed to SARS-CoV-2 were positive on anti-SARS-CoV-2 IgG bioMerieux test as well.

Discussion

According to the manufacturer (DIA.PRO), about 10% of the reactive normal population sera collected before the outbreak show reactivity to nucleocapsid antigen. In our study patients that were positive only to nucleocapsid antigen on this test were IgG negative on bioMerieux test. At the moment, it is not known whether such positive reactivity is due to cross-reaction with other members of the Coronavirus family that generated an immune response in the past, or is due to some unknown nonspecific interferences/cross-reactions due to some components of the sample. Concerning patient 7 who was PCR positive, it is not clear why there were no IgG antibodies to other antigens because we could not get any detailed history on this patient regarding the time of the symptom onset.

It is considered that viral spike protein mediates entry of SARS-CoV-2 into host cells and the nucleocapsid is a highly immunogenic structural protein^{2,3}.

So far, it has been speculated that the viral nucleocapsid may be a better target for earlier detection of immunoglobulins than viral spike protein⁴. The S1 domain is considered strain-specific, while the N protein shows cross-reactivity across strains of coronaviruses. This poses an obstacle and explanation for false-positive results since the seroprevalence of common human coronaviruses is known to increase throughout childhood to near 100% by adolescence⁵.

Our results showed good concordance in patients whose samples were IgG positive on both tests with a very high or high level index of confidence on DIA.PRO test.

In conclusion, although based on a small number of patients, our observation shows the potential use of detecting IgG antibodies directed against different antigens on interpretation of serology testing results, preferably with two different tests. This might be a suggested future approach when using serologic testing, having in mind that it is crucial for laboratories firstly to validate assays in the context of clinical background. Further research on a large number of people who have been infected with SARS-CoV-2 should be performed.

A limitation of the study was that confirmatory, neutralization test was not performed.

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Sažetak

MOGU LI IgG PROTUTIJELA USMJERENA PROTIV RAZLIČITIH ANTIGENA RIJEŠITI PROBLEM LAŽNO POZITIVNIH REZULTATA U SEROLOGIJI SARS-CoV-2?

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Uz RT-PCR testove, serološko testiranje koje je prepoznato kao korisno sredstvo za određivanje širenja infekcije u populaciji smatra se uspješnom i važnom strategijom u kontroli globalne pandemije infekcijom SARS-CoV-2. Velik broj proizvođača nudi svoje serološke testove na tržištu. Pri interpretaciji rezultata treba uzeti u obzir učestalost infekcije u populaciji budući da ona utječe na pozitivnu prediktivnu vrijednost, kao i na križnu reaktivnost s drugim koronavirusima ako se radi o testovima sa slabije dizajniranim antigenima. Prikazujemo rezultate 11 ispitanika s različitom kliničkom slikom koje smo testirali s dva različita serološka testa, DIAPRO (ELISA; Sesto San Giovanni, Italija) i VIDAS (ELFA; BioMerieux, Marcy l'Étoile, Francuska). Rezultati dobiveni prvim testom pokazali su da je njih 10 bilo IgG pozitivno, a jedan slabo pozitivan s različitim indeksom pouzdanosti, dok je drugi test rezultate sa srednjim indeksom pouzdanosti u prvom testu prikazao negativnima. Rezultati dobiveni pomoću dva serološka testa bili su sukladni sa zapažanjem da rezultat sa srednjim indeksom pouzdanosti može biti križna reaktivnost.

Ključne riječi: *SARS-CoV 2; Serologija; Anti- SARS-CoV-2 IgG; Lažno pozitivni rezultati; ELISA; ELFA*