Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Diagnostic Microbiology and Infectious Disease 102 (2022) 115561

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Virology

Importance of anti-SARS-CoV-2 assay antigenic composition as revealed by the results of the Belgian external quality assessment (EQA) scheme

Alena Moerman[a,*], Kris Vernelen[b,c], Bernard China[b,c], Arnaud Capron[b,c], Dorien Van Den Bossche[b,d], Joachim Mariën[d], Kevin K. Ariën[d,e], Jos Van Acker[b,f], Marie-Luce Delforge[b,g], Marijke Reynders[b,h], An Boel[b,i], Melissa Depypere[b,j], Natasja Van Gasse[b,k], Sara Vijgen[b,l], Jonathan Brauner[b,m], Barbara Dujardin[b,m], Elizaveta Padalko[a,b]

[a] *Laboratory of Medical Microbiology, Ghent University Hospital, Ghent, Belgium*
[b] *Experts Committee EQA Infectious serology, Quality of Laboratories, Sciensano, Brussels, Belgium*
[c] *Quality of Laboratories, Sciensano, Brussels, Belgium*
[d] *Institute of Tropical Medicine, Antwerp, Belgium*
[e] *University of Antwerp, Antwerp, Belgium*
[f] *Laboratory of Clinical Microbiology, AZ Sint-Lucas, Ghent, Belgium*
[g] *CUB-Hôpital Erasme, ULB, Brussels, Belgium*
[h] *AZ St. Jan, Bruges-Ostend, Belgium*
[i] *Department of Medical Microbiology, OLVZ Aalst, Aalst, Belgium*
[j] *Department of Laboratory Medicine, University Hospitals Leuven, Leuven, Belgium*
[k] *Hospital Network Antwerp, Antwerp, Belgium*
[l] *Department of Laboratory Medicine, Jessa Hospital, Hasselt, Belgium*
[m] *University Centre Tivoli, La Louvière, Belgium*

ARTICLE INFO

Article history:
Received 27 July 2021
Revised in revised form 16 September 2021
Accepted 23 September 2021
Available online 11 October 2021

Keywords:
SARS-CoV-2
serology
external quality assessment scheme
Nucleocapsid protein
Spike protein
receptor-binding domain

ABSTRACT

We report on sample IS/17575 since it generated highly divergent results in the Belgian SARS-CoV-2 serology external quality assessment scheme. Sample IS/17575 was serum originating from a 30 years old male patient. 124 diagnostic laboratories analysed this sample. A total of 168 results was returned (including 5 doubles). Overall, 38 were positive. All tests against S1 were positive except the Euroimmun IgG ELISA and the Ortho clinical Diagnostics VITROS IgG CLIA. All tests against S1/S2 (Liaison, Diasorin) resulted in a signal above cutoff. Assays against RBD, mostly generate a negative result. An exception are the Wantai SARS-CoV-2 ELISA's. All tests targeting N protein were negative. The survey shows, when >6 months post-infection, assays targeting at least S1, and preferably S1 combined with S2, are the most sensitive. This finding accentuates the necessity of external quality assessment schedules and importance of antigenic composition of serologic SARS-CoV-2 assays.

1. Background

All Coronaviruses are enveloped, positive-stranded RNA viruses. Being an enveloped virus means that membrane fusion is essential for entrance in host cells and virulence. The fusion protein used is Spike (S) protein, which is present on the virion's surface. This is also the protein that gives rise to the neutralizing antibody response and is hence targeted by vaccines (Min and Sun, 2021). It initially occurs in the form of a trimer, that will be cleaved into receptor-binding unit S1 and fusion unit S2. S1 consists of 4 domains, the N-terminal domain, the receptor-binding domain (RBD), and 2 C-terminal domains (Cai et al., 2020).

Full commitment to diagnostic methods is especially important considering there are, at present, no curative medicines available. Serologic assays are the most important auxiliary tools to complement Nucleic Acid Amplification Tests (NAATs) (Plebani et al., 2020). The creation of these tests at an unprecedented speed consequently creates the need for a thorough assessment of their clinical performance.

An extra hurdle to overcome here is the fact that the commercially available serologic assays are anything but uniform, differing in the method of the immunoassay, the antibody class detected, the targeted viral components and the required specimen types

* Corresponding author. Tel.: +32-491 88 26 98, fax: 003293324985.
E-mail address: alena.moerman@uzgent.be (A. Moerman).

https://doi.org/10.1016/j.diagmicrobio.2021.115561
0732-8893/

(Theel et al., 2020). At present there are tests on the market detecting total antibody (TAB) as well as IgA, IgM and IgG separately. Targeted antigens include Nucleocapsid (N) or S protein alone next to combined N and S proteins. Viral S protein targeted immunoassays can make use of the monomeric S protein (spike subunit 1 and/or 2) or the S protein in its native trimer form (spike receptor binding domain). Assay formats used comprise enzyme-linked immunosorbent assays (ELISA), chemiluminescent immunoassays (CLIA), electrochemiluminescence immunoassay (ECLIA) and lateral flow immunoassays (LFIA) (Lassaunière et al., 2020).

Almost all patients will develop detectable antibodies against SARS-CoV-2. It is generally assumed they appear 3 to 14 days post-onset (Lin et al., 2020). The recommendation to test from day 14 after the alleged start of infection is the consequence of studies to reach the highest sensitivity (Interim Guidelines 2021).

Sciensano (formerly, the scientific public health institute for Belgium) and its department Quality of Laboratories routinely organizes external quality assessment (EQA) for a broad range of laboratory analyses under accreditation (ISO17043:2010). In order to ensure a scenically correct organization and evaluation of the results and to obtain useful and, if possible, commutable samples, Quality of Laboratories is assisted by a panel of experts. The members of these panels are chosen in function of their expertise in a given domain and work in different types of laboratories (university, smaller hospitals, private laboratories) to ensure a link with the actual situation amongst Belgian patients and population in general.

EQA is an important tool for the assessment of a method's performance among the different participants. It aims to determine the possible differences in characteristics of the multiple available assays as a means to help harmonize the results generated by different methods and platforms (Haselmann et al., 2020). Participating is mandatory for the licensed Belgian laboratories and contributes to ensuring and improving the quality of serological testing and providing the best patient care possible. The final goal is to ensure a reliable result, independent of the analyzing laboratory. EQA is the best way to compare the proficiency of the different assays for the same analysis. EQA also allows to put in evidence possible differences between different assays since all samples are identical.

The results were evaluated by comparison with a target value. This target value is the consensus of the panel of experts. Since it is a well-known fact that in infectious serology quantitative results between different methods and assays may differ even if the qualitative result (i.e., positive, negative or ambiguous) is the same, the target values were qualitative. Laboratories could however compare their quantitative results within their peer group (consisting of laboratories using the same method).

Each laboratory is indeed invited to compare its results with the expected result (target value) and with the results of its peer group. In case of a discordant result, a laboratory has the opportunity to demand a “repeat sample” to perform a second analysis in order to search for the reason for the discordant result. Each error in an EQA result should be considered a nonconformity in the laboratory's quality system.

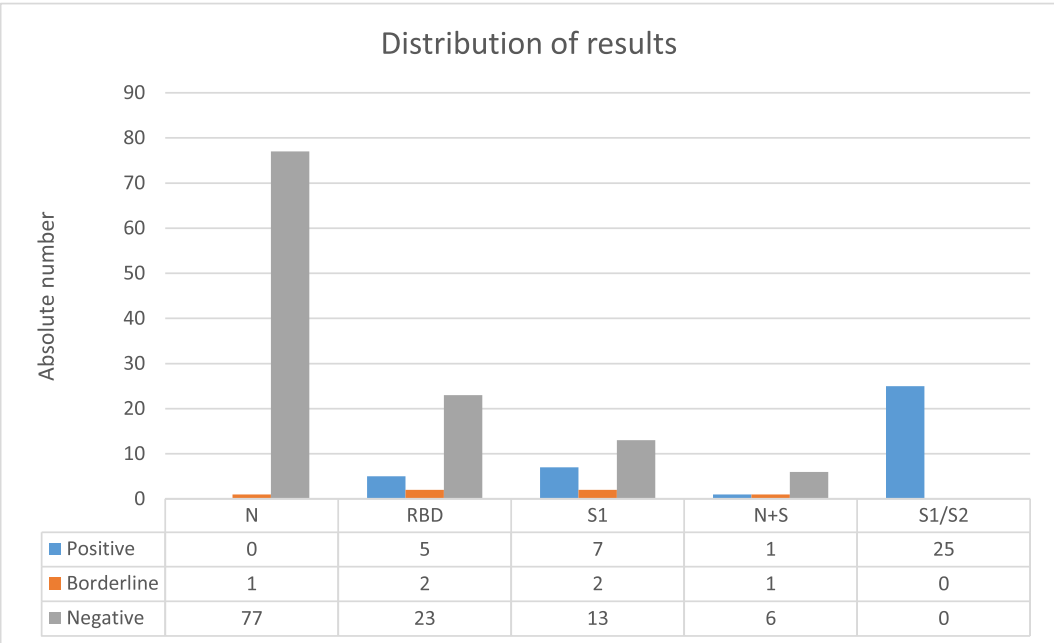
We report here on the Sciensano SARS-CoV-2 serology. A particular sample (IS/17575), which draw attention during analysis of the results, will be commented. This sample was 1 of 3 that were sent out in survey 2020/2 Fig. 1.

2. Methods

2.1. Samples and participants

The request to participate in the analysis was sent to all laboratories involved in the analysis of SARS-CoV-2 antibodies in Belgium and Luxembourg. This survey is part of the mandatory EQA program for SARS-CoV-2 serology in Belgium. In Luxembourg participating in the survey is voluntary. Three different serum samples, of whom 2 tested positive for SARS-CoV-2 through NAAT, were selected and delivered by CHU Tivoli (Centre Hospitalier Universitaire de Tivoli) and divided by Sciensano. The underlying clinical information was withheld so all laboratories performed the analysis without prior knowledge. The laboratories were asked to analyze the samples on the platform(s) they routinely use for analysis of SARS-CoV-2 antibodies. 124 Belgian and Luxembourg laboratories participated. An overview of the distribution of the tests used in function of the technique for determining anti-Covid antibodies is provided in Table 1.

Sample IS/17575 generated highly discordant results whilst the results for sample IS/17576 and IS/17577 were fully consistent across all participating labs. Background information was collected in order to get a better understanding of the discordant results. Sample IS/



	N	RBD	S1	N+S	S1/S2
Positive	0	5	7	1	25
Borderline	1	2	2	1	0
Negative	77	23	13	6	0

Fig. 1. Graphic representation of the number of positive, borderline and negative results, recovered from the EQA (sample IS/17575), stratified per antigen.

Table 1
Overview of the number of participating lab's and their type of antibodies tested.

Tested antibodies	N laboratories reporting results
TAB	44
TAB and IgG	7
TAB and IgM	2
TAB and IgG and IgM	6
TAB and IgG and IgA	1
IgG	54
IgG and IgM	8
IgG and IgA	1
IgG and IgM and IgA	1

17575 was serum originating from a 30 years old male patient, developing minor Covid-19 symptoms on April 12, 2020. There was fatigue, headaches, muscle pains, a cough, a sore throat, nasal course, dyspnea, some abdominal complaints, ageusia and ansomia. There were no abnormalities on CT-scan neither was there pneumonia, fever, conjunctivitis, vomiting or skin lesions. The patient tested positive (low viral load/ high Cycle threshold [Ct] value) through SARS-CoV-2 NAAT testing on April 16, 2020. At October 19, 2020, serum was taken for serological analysis of SARS-CoV-2 IgG which was found to be positive with the Diasorin S1/S2 IgG kit, and negative with the Euroimmun IgG ELISA.

2.2. Assays

To gain a good insight in the results, it is important to understand the different immunoassay-methods, know which antibody class is detected and what is the targeted viral component. Therefore, we provided an overview of the different serological platforms used in the EQA in Table 2 (Bryan et al., 2020; Egger et al., 2020; Garritsen et al., 2021; GeurtsvanKessel et al., 2020; Gutiérrez-Cobos et al., 2021; Jääskeläinen et al., 2020; Lippi et al., 2020; Mahajan et al., 2020; Maine et al., 2020; Manthei et al., 2020; National SARS-CoV-2 Serology Assay Evaluation Group 2020; Padoan et al., 2020; Pieri et al., 2020; Plebani et al., 2020; Renard et al., 2021; Ruscio et al., 2021; San Tang et al., 2020; Steensels et al., 2020; Van Elslande et al., 2020).

After performing the EQA and analyzing all results, it was decided to perform additional analyses in view of the inconsistency of the data. The first additional analysis was the SARS-CoV-2 ELISA by Vircell. Furthermore, a Luminex immunoassay targeting RBD, N Protein, monomeric S1 protein and native S protein trimer was performed for analysis of IgG. Luminex is a type of immunoassay that is able to precisely measure multiple analytes in 1 sample. This platform is currently used in research setting only (Mariën et al., 2021). Last, the sample was run on the Abbott SARS-CoV-2 IgG II Quant assay.

2.3. Evaluation of results

All laboratories sent the results of their analysis to Sciensano. Data were classified per antibody-type detected as well as stratified per platform and specific kit used, where rapid tests were listed separately. The viral target used in the kits was not taken into account. Sciensano had access to the quantitative data but interpreted these qualitatively according to the lab's used cutoff. Equivocal or borderline results were considered as such. The complete panel of results was sent to the participants in an anonymous manner.

3. Results

The 124 laboratories participating in the survey returned a total of 168 results for sample IS/17575. 96 lab's performed 1 analysis (77.42%), the others 2 or even multiple. Sciensano received 61 sets of TAB results (36.31%), 84 IgG determinations (50%), 20 IgM results (11.90%) and 3 IgA analysis (1.79%). Techniques used to screen the sera included ELISA (12.27%), CLIA (85.27%) and LFIA (2.45%). An overview of the results is provided in Tables 2 and 3.

Table 2
Overview of the results obtained with the different assays used in the questionnaire, stratified per manufacturer (Bryan et al., 2020; Egger et al., 2020; Garritsen et al., 2021; GeurtsvanKessel et al., 2020; Gutiérrez-Cobos et al., 2021; Jääskeläinen et al., 2020; Lippi et al., 2020; Mahajan et al., 2020; Maine et al., 2020; Manthei et al., 2020; National SARS-CoV-2 Serology Assay Evaluation Group 2020; Padoan et al., 2020; Pieri et al., 2020; Plebani et al., 2020; Renard et al., 2021; Ruscio et al., 2021; San Tang et al., 2020; Steensels et al., 2020; Van Elslande et al., 2020).

Manufacturer	Kit	Assay type	Target	N tests	Results (positive/borderline/negative)		
Abbott	SARS-CoV-2 IgG Assay (Architect)	CMIA	N	23			23-
	SARS-CoV-2 IgG Assay (Alinity)		N	9			9-
	SARS-CoV-2 IgM Assay (Architect)		RBD	2			2-
Beckman (Coulter)	Access SARS-CoV-2 IgG	CLIA	RBD	2			2-
Bejing Wantai Biological Pharmacy	Wantai SARS-CoV-2 Ab ELISA	ELISA	RBD	5	3+	2+/-	
	Wantai SARS-CoV-2 IgM ELISA		RBD	1	1+		
bioMérieux	VIDAS SARS-CoV-2 IgG	ELFA	RBD	4			4-
	VIDAS SARS-CoV-2 IgM		RBD	6			6-
Diasorin	LIAISON SARS-CoV-2 S1/S2 IgG tests	CLIA	S1/S2	25	25+		
	LIAISON SARS-CoV-2 IgM		RBD	3			3-
Epitope Diagnostics (EDI)	Novel Coronavirus COVID-19 IgM ELISA Kit	ELISA	N	1			1-
Euroimmun	Anti-SARS-CoV-2 ELISA IgG	ELISA	S1	8			8-
	Anti-SARS-CoV-2 NCP EI 2606-9601-2G		S1	2			2-
	Anti-SARS-CoV-2 ELISA IgA		S1	3	1+	2+/-	
Healgen Diagnostics	COVID-19 IgG/IgM Rapid Test Cassette	LFIA	N+S	1			1-
Multi-G	Covid-19 IgM/IgA Ab test cassette	LFIA	N	1			1-
Ortho clinical Diagnostics	VITROS Immunodiagnostic Products Anti-SARS-CoV-2 Total	CLIA	S1	6	6+		
	VITROS Immunodiagnostic Products Anti- SARS-CoV-2 IgG		S1	3			3-
Roche	Elecsys Anti-SARS-CoV-2 Test (Cobas)	ECLIA	N	44		1+/-	43-
Shenzen Yhlo Biotech	iFlash- SARS-CoV-2 IgG	CLIA	N+S	1	1+		
	iFlash- SARS-CoV-2 IgM		N+S	1			1-
Siemens	SARS-CoV-2 Total Antibody Test	CLIA	RBD	6	1+		5-
	SARS-CoV-2 IgG Assay		RBD	1			1-
Snibe	2019-nCoV IgG (CLIA)	CLIA	N+S	1		1+/-	
	MAGLUMI SARS-CoV-2 IgM/IgG Test		N+S	1			1-
	2019-nCoV IgM (CLIA)		N+S	1			1-
Xiamen Boson Biotech	Rapid 2019-nCoV IgG/IgM Combo Test Card	LFIA	N+S	2			2-

Table 3
Classification of the results into positive, borderline and negative and further into antibody detected per antigen.

	N				RBD			S1			N+S			S1/S2
	IgM	IgG	IgM/IgA	TAB	IgM	IgG	TAB	IgA	IgG	TAB	IgM	IgG	IgM/IgG	IgG
+					1		4	1		6		1		25
+/-				1			2	2				1		
-	1	32	1	43	11	7	5		13		2		4	

Table 4
Overview of the results obtained with the additional assays performed after the questionnaire, stratified per manufacturer.

Manufacturer	Kit	Assay type	Target	N tests	Results (positive/borderline/negative)
Abbott	SARS-CoV-2 IgG II Quant	CMIA	N	1	1+
	SARS-CoV-2 IgG Assay (Architect)		RBD	1	1-
bioMérieux	VIDAS SARS-CoV-2 IgG	ELFA	RBD	1	1-
	VIDAS SARS-CoV-2 IgM		RBD	1	1-
Luminex multiplex assay	In house assay	ELISA	RBD+ N+ S+ S1/S2	4	2+ 2-
Vircell	SARS-CoV-2 ELISA	ELISA	N+RBD	1	1+

For the TAB results, 10 out of 61 were positive (16.39%). 3 results were borderline or equivocal (4.92%) and 48 were negative (78.69%). Considering IgG CLIA and ELISA, 26 out of 80 results were positive (32.5%), 1 result was borderline/equivocal (1.25%) and 53 were negative (66.25%). All IgG results generated by rapid test analysis were negative. When looking at the results for IgM, only 1 out of 14 lab's (7.14%) or 1 out of 16 tests had a positive result (6.25%). All rapid IgM tests were negative. For IgA, 2 tests were borderline and 1 was positive.

When stratifying the results according to viral target, 77 out of 78 analysis targeting N-antigen were negative, 1 was borderline/equivocal. 6 out of 8 tests targeting N- and S-antigen together were negative, 1 was borderline/equivocal and 1 was positive. Twenty-three out of 30 analysis against RBD were negative, 2 were borderline/equivocal, 4 were positive. Something that immediately catches the eye, and which made IS/17575 such an interesting sample, is the fact that 32 out of 47 results generated by testing against S1 were positive (68.09%). 2 results were borderline/equivocal (4.26%) and 13 were negative (27.66%).

In the light of these particular findings, the decision was made to perform some additional tests. The sample was reran with the Abbott Architect IgG and IgG II Quant assays. The IgG assay yielded a negative result, whilst the result with the IgG II Quant assay was positive. The bioMérieux Vidas IgG and IgM assay, which had already been performed during the survey, yielded again a negative result for both antibodies. The sample was also additionally analyzed with the SARS-CoV-2 ELISA by Vircell, which provided a positive result. The Luminex analysis yielded a positive IgG result for the N protein and the native S protein trimer. Targeting RBD and monomeric S1 protein resulted in a signal below cutoff. An overview of the additional tests characteristics and results is provided in Table 4.

4. Discussion

Taking a closer look at the results, it stands out that when stratifying the results according to viral target, almost all tests against the S1 moiety and all tests against S1/S2 resulted in a signal above cutoff. This emphases the importance of the choice of antigenic composition when performing a serological test in order to investigate the patient's immune status (Fenwick et al., 2021). Choosing the most adequate assay is particularly important for pauci- or asymptomatic people, who are known to have a lower antibody response (Milani et al., 2020). Remarkable is the fact that assays against RBD, which is after all part of the S1 moiety, mostly generate a negative result. An exception is the Wantai SARS-CoV-2 ELISA's. One could expect them to give a negative result when extrapolating from the other assays. On the other hand, the Euroimmun IgG ELISA gives negative results, while we would expect a signal above cutoff since this is an S1-based assay. A possible explanation may be found in the tested lower sensitivity of the Euroimmun ELISA, while the Wantai ELISA provides excellent sensitivity and specificity and was tested superior to other ELISA's (Acro biosystems 2021; Harritshøj et al., 2021; Herroelen et al., 2020). Research on 30 NAAT positive patients showed that the Wantai assay detected antibodies in 28 cases, whilst the Euroimmun IgG assay picked up antibodies only 20 times. Remarkably, the Euroimmun IgA assay performed a lot better, with detecting 28 out 30 positives (Acro biosystems 2021). This is also reflected in the results of the EQA, where the Euroimmun IgA assay did detect a positive or borderline signal.

The Ortho clinical Diagnostics VITROS TAB CLIA (just like the Diasorin Liaison S1/S2 IgG) performs excellent, with all laboratories using this test finding sample IS/17575 positive. The similar VITROS IgG assay then again appears to be less accurate. This difference could possibly be explained by the fact that TAB assays are in general more sensitive comparing to detecting only a single class of antibodies (Harritshøj et al., 2021).

Another characteristic that can be deduced from this study is the fact that assays targeting the trimeric S protein combined with N protein, usually produce negative results. These assays seem to be noteworthy less sensible. The obvious explanation is that the vast majority of these assays are LFIA's (Lisboa Bastos et al., 2020). This assumption is reinforced by the fact that the Shenzen iFlash IgG assay, which is a CLIA, generates a positive result. None of the LFIA rapid tests was positive.

A possible explanation for this observed difference in N- and S protein based serological SARS-CoV-2 assays can be found in the recent insight that antibody response against the N protein appears to wane post-infection (Fenwick et al., 2021). As a result, N protein assays could underestimate the true seroprevalence when tested on patients who were infected some time ago (in the order of magnitude of months rather than weeks). S protein directed antibody response tends to persist over time (Fenwick et al., 2021). After all, in this particular case, the patient's antibody response was tested a little over 6 months after infection. In the acute phase of infection both antibody responses are equally sensitive, although IgG seroconversion for S protein would appear 2 days after this for N protein (Van Elslande et al., 2020).

Then we still have to look for an explanation for the observed difference in sensitivity of RBD vs S1 vs S protein based immunoassays. Since these are all S protein based one could expect for all the assays to generate a positive result. However, there appears to be a difference. It has already been stated that the use of the S protein in its trimer form is more sensitive when compared to monomeric S proteins. This would be due to antibodies binding to the S2 subunit and the

conservation of conformational epitopes within a higher order structure (Infantino et al., 2020). This theory seems to be confirmed by the Diasorin Liaison S1/S2 IgG tests. This higher sensitivity however comes at the expense of the specificity. S1 would be more specific compared to S since the spike S2 subunit is conserved among Coronaviruses. It is known that the specificity of the Diasorin Liaison S1/S2 IgG is lower when compared to its competitors (Harritshøj et al., 2021). If you reason that RBD is only 1 of the 4 subdomains of S1 (Yuan et al., 2021) it seems plausible that this will be even less sensitive (Tian et al., 2020). This seems confirmed by this EQA, with almost all assays targeting RBD being negative, including the Diasorin Liaison IgM test. An exception to this "rule" is the Wantai ELISA, which seems exceptionally more sensitive in comparison to its competitors (Acro biosystems 2021; Harritshøj et al., 2021; Herroelen et al., 2020). Finally, it is important to note that the data concern a single sample and however this EQA reveals some very interesting findings, more data and expansion of sample size are needed to be able to draw definitive conclusions.

5. Conclusion

In conclusion, the survey shows, when >6 months post-infection, assays targeting S1 are the most sensitive. This can be explained by the recent insight that antibody response against the N protein appears to wane post-infection (Fenwick et al., 2021). The highly divergent results highlight the importance of taking into account the antigenic composition in the light of intended use of the particular assay. Our findings also accentuate the necessity of EQA schedules for SARS-CoV-2 serology and use of sample drawn at different timepoints after Covid-19 episode.

Authors' contributions

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript.

Declaration of competing interest

The author stated that there are no conflicts of interest regarding the publication of this article.

Appendices

A. Figure

Fig. 1

B. Tables

Table 1,2,3,4

References

Acro biosystems. How does the COVID-19 antibody ELISA test function? 2021; Available at: https://www.acrobiosystems.com/A1125-How-does-the-COVID-19-antibody-ELISA-test-function.html. Accessed February 22, 2021.

Bryan A, Pepper G, Wener MH, Fink SL, Morishima C, Chaudhary A, et al. Performance characteristics of the Abbott Architect SARS-CoV-2 IgG assay and seroprevalence in Boise, Idaho. J Clin Microbiol 2020;58(8):e00941–20.

Cai Y, Zhang J, Xiao T, Peng h, Sterling SM, Walsh Jr. RM, et al. Distinct conformational states of SARS-CoV-2 spike protein. Science 2020;369:1586–92.

Egger M, Bundschuh C, Wiesinger K, Gabriel C, Clodi M, Mueller T, et al. Comparison of the Elecsys® Anti-SARS-CoV-2 immunoassay with the EDI™ enzyme linked immunosorbent assays for the detection of SARS-CoV-2 antibodies in human plasma. Clin Chim Acta 2020;509:18–21.

Fenwick C, Croxatto A, Coste AT, Pojer F, André C, Pellaton C, et al. Changes in SARS-CoV-2 spike versus nucleoprotein antibody response impact the estimates of infections population-based seroprevalence studies. J Virol 2021;95(3):e01828–20.

Garritsen A, Scholzen A, van den Nieuwenhof DWA, Smits APF, Datema ES, van Galen LS, et al. Two-tiered SARS-CoV-2 seroconversion screening in the Netherlands and stability of nucleocapsid, spike protein domain 1 and neutralizing antibodies. Infect Dis (Lond) 2021;53:498–512.

GeurtsvanKessel CH, Okba NMA, Igloi Z, Embregts CWE, Laksono BM, Leijten L, et al. Towards the next phase: evaluation of serological assays for diagnostics and exposure assessment. Nat Commun 2020;11:3436.

Gutiérrez-Cobos A, Gómez de Frutos S, García DD, Lara EN, Carrión AY, García-Rodrigo LF, et al. Evaluation of diagnostic accuracy of 10 serological assays for detection of SARS-CoV-2 antibodies. Eur J Clin Microbiol Infect Dis 2021;40:955–61.

Harritshøj LH, Gybel-Brask M, Afzal S, Kamstrup PR, Jørgensen CS, Kragh Thomsen M, et al. Comparison of 16 serological SARS-CoV-2 immunoassays in 16 clinical laboratories. J Clin Microbiol 2021;59(5) 20.

Haselmann V, Özçürümez MK, Klawonn F, Ast V, Gerhards C, Eichner R, et al. Results of the first pilot external quality assessment (EQA) scheme for anti-SARS-CoV2-antibody testing. Clin Chem Lab Med 2020;58(12):2121–30.

Herroelen PH, Martens GA, De Smet D, Swaerts K, Decavele AS. Humoral immune response to SARS-CoV-2. Am J Clin Pathol 2020;154(5):610–9.

Infantino M, Damiani A, Gobbi FL, Grossi V, Lari B, Macchia D, et al. Serological assays for SARS-CoV-2 infectious disease: benefits, limitations and perspectives. Isr Med Assoc J 2020;22(4):203–10.

Interim guidelines for COVID-19 antibody testing CDC. 2021

Jääskeläinen AJ, Kuivanen S, Kekäläinen E, Ahava MJ, Loginov R, Kallio-Kokko H, et al. Performance of six SARS-CoV-2 immunoassays in comparison with microneutralisation. J Clin Virol 2020;129:104512.

Lassaunière R, Frische A, Harboe ZB, Nielsen ACY, Fomsgaard A, Krogfelt KA, et al. Evaluation of nine commercial SARS-CoV-2 immunoassays. medRxiv 2020 https://doi.org/10.1101/2020.04.09.20056325.

Lin Q, Zhu L, Ni Z, Meng H, You L. Duration of serum neutralizing antibodies for SARS-CoV-2: lessons from SARS-CoV infection. J Microbiol Immunol Infect 2020;53(5):821–2.

Lippi G, Salvagno GL, Pegoraro M, Militello V, Caloi C, Peretti A, et al. Assessment of immune response to SARS-CoV-2 with fully automated MAGLUMI 2019-nCoV IgG and IgM chemiluminescence immunoassays. Clin Chem Lab Med 2020;58(7):1156–9.

Lisboa Bastos M, Tavaziva G, Abidi SK, Campbell JR, Haraoui LP, Johnston JC, et al. Diagnostic accuracy of serological test for covid-19: systematic review and meta-analysis. BMJ 2020;370:m2516.

Mahajan S, Redlich CA, Wisnewski AV, Fazen LE, Rao LV, Kuppusamy K, et al. Performance of Abbott architect, Ortho Vitros, and Euroimmun assays in detecting prior SARS-CoV-2 infection. medRxiv 2020 https://doi.org/10.1101/2020.07.29.20164343.

Maine GN, Lao KM, Krishnan SM, Afolayan-Oloye O, Fatemi S, Kumar S, et al. Longitudinal characterization of the IgM and IgG humoral response in symptomatic COVID-19 patients using the Abbott Architect. J Clin Virol 2020;133:104663.

Manthei DM, Whalen JF, Schroeder LF, Sinay AM, Li SH, Valdez R, et al. Differences in performance characteristics among four high-throughput assays for the detection of antibodies against SARS-CoV-2 using a common set of patient samples. Am J Clin Pathol 2020;155(2):267–79.

Mariën J, Ceulemans A, Michiels J, Heyndrickx L, Kerkhof K, Foque N, et al. Evaluating SARS-CoV-2 spike and nucleocapsid proteins as targets for antibody detection in severe and mild COVID-19 cases using a Luminex bead-based assay. J Virol Methods 2021;288:114025.

Milani GP, Dioni L, Favero C, Cantone L, Macchi C, Delbue S. Serological follow-up of SARS-CoV-2 asymptomatic subjects. Sci Rep 2020;10(1):20048.

Min L, Sun Q. Antibodies and vaccines target RBD of SARS-CoV-2. Front Mol Biosci 2021;8: 671633.

National SARS-CoV-2 Serology Assay Evaluation Group. Performance characteristics of five immunoassays for SARS-CoV-2: a head-to-head benchmark comparison. Lancet Infect Dis 2020;20(12):1390–400.

Padoan A, Bonfantec F, Pagliaric M, Bortolamic A, Negrinia D, Zuina S, et al. Analytical and clinical performances of five immunoassays for the detection of SARS-CoV-2 antibodies in comparison with neutralization activity. EBioMedicine 2020;62:103101.

Pieri M, Ciotti M, Carlozzi N, Frassanito ML, Meloni A, Cistera A, et al. SARS-CoV-2 infection serology validation of different methods: usefulness of IgA in the early phase of infection. Clin Chim Acta 2020;511:28–32.

Plebani M, Padoan A, Negrini D, Carpinteri B, Sciacovelli L. Diagnostic performances and thresholds: the key to harmonization in serological SARS-CoV-2 assays?. Clin Chim Acta 2020;509:1–7.

Plebani M, Padoan A, Negrini D, Carpinteri B, Sciacovelli L. Diagnostic performances and thresholds: the key to harmonization inserological SARS-CoV-2 assays?. Clin Chim Acta 2020;509:1–7.

Renard N, Daniel S, Cayet N, Pecquet M, Raymond F, Pons S, et al. Performance characteristics of the VIDAS® SARS-COV-2 IgM and IgG serological assays. J Clin Microbiol 2021;8:e02292–20.

Ruscio M, D'Agnolo E, Belgrano A, Plebani M, Lippi G. Analytical assessment of Beckman Coulter Access anti-SARS-CoV-2 IgG immunoassay. J Lab Precis Med 2021;6.

San Tang M, Hock KG, Logsdon NM, Hayes JE, Gronowski AM, Anderson NW, et al. Clinical performance of the Roche SARS-CoV-2 serologic assay. Clin Chem 2020;66(8):1107–9.

Steensels D, Oris E, Coninx L, Nuyens D, Delforge ML, Vermeersch P, et al. Hospital-wide SARS-CoV-2 antibody screening in 3056 staff in a tertiary center in Belgium. JAMA 2020;324(2):195–7.

Theel E, Slev P, Wheeler S, Couturier MR, Wong SJ, Kadkhoda K. The role of antibody testing for SARS-CoV-2: is there one?. J Clin Microbiol 2020;58(8):e00797–20.

Tian Y, Lian C, Chen Y, Wei D, Zhang X, Ling Y, et al. Sensitivity and specificity of SARS-CoV-2 S1 subunit in COVID-19 serology assays. Cell Discov 2020;6:75.
Van Elslande J, Decru B, Jonckheere S, Van Wijngaerden E, Houben E, Vandecandelaere P, et al. Antibody response against SARS-CoV-2 spike protein and nucleoprotein evaluated by four automated immunoassays and three ELISAs. Clin Microbiol Infect 2020;26(11):1557.
Yuan M, Liu H, Wu NC, Wilson IA. Recognition of the SARS-CoV-2 receptor binding domain by neutralizing antibodies. Biochem Biophys Res Commun. 2021;538:192–203.