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FULL PAPER

Evaluation and comparison of eighteen SARS-CoV-2 antibody assays from seven different companies to assess its diagnostic role in SARS-CoV-2 infections

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SUMMARY

The diagnostic performance of reverse transcriptase polymerase chain reaction (RT-PCR) decreases during the late acute stage of the corona virus disease (COVID-19) infection; hence, serological assays can be used for disease diagnosis in patients non-protected through vaccinations at this stage. The objective of this study was to assess the diagnostic accuracy of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibody tests in current/past infections, determine proper testing time, and check the accuracy of cutoff values. In this study, 18 Ig (immunoglobulin) G, IgM, IgA, and total antibody serological assays were performed using 839 samples. Positive sera (n=132) were collected during the first 5 months after the patients were symptomatic and tested positive for the SARS-CoV-2 RT-PCR test; they were grouped as 0-10, 10-15, >15 days according to the symptom onset. Negative sera (N=707) were obtained from patients with lupus before the pandemic. The performance of IgG and total antibody assays was better than those of IgA, IgM, and IgA-IgM for all post-symptom groups except for 0-10 days, which showed lower Ig assay sensitivity. During 10-15 and >15 days, >70% sensitivity to IgA, IgM, IgM-IgA assays and lower sensitivity were noted, respectively. The sensitivities of IgG and total antibody assays for group C were slightly lower than that of group B. There were no significant differences, but there were higher correlations between the methods or antigenic structures. Receiving operating characteristics (ROC) analysis revealed better cutoff values. For the diagnosis of late acute/past SARS-CoV-2 infection, serological tests can be performed on unvaccinated patients showing symptoms for ≥10 days. SARS-CoV-2 IgG and total antibodies were better diagnostic markers than IgM, IgA, and IgM+IgA, which were restricted to group B.

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INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA Reverse Transcription Polymerase Chain Reaction (RT-PCR) is the method of choice to diagnose coronavirus disease 2019 (COVID-19) (WHO 2020; Corman *et al*., 2020; Opota *et al*., 2020). During the late course of acute infection, the diagnostic performance of RT-PCR progressively decreases owing to viral clearance (Long *et al*., 2020; Peeling *et al*., 2020; He *et al*., 2020; Liu *et al*., 2020). Serological assays can be efficiently used in the late course of infection when the RT-PCR results are not convincing (Poland *et al*., 2020). Specific antibodies (Ig [immunoglobulin] M, IgA, and IgG) can be used as markers for diagnosis during the late course of infection as they develop within 5-15 days, and while IgM and IgA persist for at least 3-6 weeks, IgG persists for several months (Peeling, *et al*., 2020; Poland *et al*., 2020; Sethuraman *et al*., 2020; Ghaffari *et al*., 2020; Lee *et al*., 2020; Zhao *et al*., 2020; Krammer *et al*., 2020; Longchamp *et al*., 2020). Therefore, IgG antibodies may be useful for the diagnosis of past infections.

Usually, total/specific domains of the spike (S protein) or nucleocapsid (N protein) proteins form the basis of serological tests. The spike envelope protein is composed of two subunits: the S1 subunit contains the receptor-binding domain (RBD) required for binding to the host angiotensin-converting enzyme 2 (ACE2) receptor, and the S2 subunit contains

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an element for cell membrane fusion. Nucleocapsid protein is the most abundant viral protein (Cantuti-Castelvetri *et al*., 2020; Daly *et al*., 2020; Bangaru *et al*., 2020; Chen *et al*., 2007).

Serological tests are based on different methodologies, such as enzyme-linked immunosorbent assay (ELISA), chemiluminescent immunoassay (CLIA), and lateral flow immunoassay (LFIA). LFIA cassettes are easy to use and fast, but have low sensitivity (Mekonnen *et al*., 2021; Maxim *et al*., 2019). ELISA and CLIA are fast methods that enable simultaneous testing of multiple samples with better sensitivities. CLIA provides an absolute measurement but is more expensive (Carter *et al*., 2020; Cinquanta *et al*., 2017; Wong, *et al*., 2008). The accuracy of these tests may be highly dependent on the chosen subdomain, the resulting successful protein folding, and the conserved protein glycosylation (for some proteins).

In this study, we independently evaluated 18 CE certified SARS-CoV-2 serological tests intended for use in the late course of acute COVID-19 infection (current and past). We reported the technical performance, sensitivity relative to days after the onset of symptoms, correlations, and re-evaluated cut-offs.

METHODS

Study Design and Sample Collection

The study included 839 samples (132 positive and 707 negative). All sera were collected from Acibadem Labmed Laboratory, Turkey. A total of 132 positive sera samples were collected during the first 5 months post-symptom onset from patients with a documented positive SARS-CoV-2 RT-PCR result and presenting with mild to severe symptoms. The date of the first symptom was noted from their medical electronic records. Positive sera were stored for up to 5 days at 4°C before analysis. SARS-CoV-2 negative sera were collected from patients with lupus before February 10, 2020, which was assumed to be prior to the SARS-CoV-2 pandemic (the first confirmed case of COVID-19 in Turkey was on March 11, 2020). Possible cross-reactivity was assessed by testing sera known to be positive for autoimmune diseases (antinuclear antibodies (ANA) and dsDNA antibody tests were positive). SARS-CoV-2 negative sera were stored at -80°C before testing.

The SARS-CoV-2 positive cases were confirmed by RT-PCR (Bio-speedy® Direct RT-qPCR SARS-CoV-2, Bioeksen, Turkey) using an RT-PCR instrument (Biorad CFX96, USA) with nasopharyngeal and oropharyngeal swabs (vNAT® Transphere Tube) (Can Kaptan, Turkey).

All analyses were performed by a pathologist who was blinded to sample identity at the Acibadem Labmed Laboratory.

Based on the number of days after symptom onset, positive sera were split into three groups: 0-10 (n=40), 10-15 (n=55), and >15 (16-150) (n=37) days. For all three groups, half of the sera were taken from outpatients with mild clinical symptoms and the other half were taken from inpatients with moderate to severe clinical symptoms. Outpatient numbers in the 0-10, 10-15, and >15 days groups were 20, 23, and 19, respectively. The number of group samples for each kit was variable; however, two conditions were followed: half of the sera were taken from outpatients and the rest from inpatients, and B was the most crowded group.

Study protocol was approved by Acibadem Mehmet Ali Aydınlar University, School of Medicine, Clinical Microbiology and Infectious Diseases Departments.

Ethical Approval

The study protocol was approved by the local ethics committee, in accordance with the Declaration of Helsinki. The ethical approval protocol code of the study was 2020-26/18.

Serological Assays

CE marked 18 IgG, IgM, IgA, and total antibody tests were performed using varying numbers of samples from seven different manufacturers: Siemens Healthineers (Siemens Healthcare GmbH, Germany), Abbott (Abbott, USA), Roche Diagnostics (Roche Diagnostics, Switzerland), Euroimmun (EUROIMMUN Medizinische Labordiagnostika AG, Germany), Aesku (AESKU.GROUP GmbH & Co. KG, Germany), Vircell S.L. (Vircell S.L., Spain), and Healgen Scientific LLC. (Healgen Scientific LLC, USA) with their technical performances and references listed in *Table 1*. Each assay was performed in accordance with the manufacturers' instructions.

According to the manufacturers' instructions, some firms reported the result as negative and positive, while others reported the result as negative, equivocal, and positive. The equivocal results interval was restrained and ranged between negative and positive. All equivocal results were considered negative in the study.

Statistical Analysis

All statistical analyses were performed using Analyse-it for Microsoft Excel (Analyse-it Software, Ltd, Leeds, United Kingdom). Normal distribution of the data was confirmed using the Shapiro-Wilk test prior to further analysis. Descriptive statistics are presented as mean ± standard deviation (SD) and median (interquartile range, IQR). Intergroup comparisons of biochemical and clinical parameters were assessed using analysis of variance (ANOVA) and post-hoc Tukey's test. Correlations among all parameters were determined using Pearson's and Spearman's correlations. Receiver operating characteristics (ROC) and corresponding area under the curve (AUC) analyses

Table 1 - *Characteristics of different SARS-CoV-2 assays used in this study as reported by firms.*

Firms	*Methodology*	*Specimen type*	*Instrument used*	*Threshold*	*Recombinant labelled protein*	*Antibody detected*	*Sensitivity % (Days from symptoms)*	*Specificity %*	*NPV %*	*PPV %*
Siemens	CLIA	Serum/ plasma	Centaur	Ratio; 1	Spike	Total Antibody	61.05 (Day 0-6)	99.82	100	96.7
							97.50 (Day 7-13)			
							100 (Day ≥14)			
Roche	CLIA	Serum/ plasma	Cobas®	Ratio; 1	Nucleocapsid	Total Antibody	65.50 (Day 0-6)	99.8	100	96.5
							88.10 (Day 7-13)			
							100 (Day ≥14)			
					Spike	Total Antibody	60.2 (Day 0-6)	99.81	100	86.4
							85.3 (Day 7-13)			
							99.5 (Day ≥14)			
Abbott	CLIA	serum/ plasma	Architect	Ratio; 1.4	Recombinant full length nucleocapsid protein	IgG	25 (Day 3-7)	99.6	100	93.4
							86.40 (Day 8-13)			
							100 (Day ≥14)			
Euroimmun	ELISA	Serum/ plasma	Manual or automated ELISA instrument	Ratio; Negative <0.8, Positive >1.1	S1-Domain of Spike protein including receptor-binding domain	IgG	43.7 (Days <10)	99.6	99.7 (99.2-99.9)	93.2 (84.7-97.7)
							94.4 (Days >10)			
						IgA	50 (Days <10)	92.5	*	*
							100 (Days >10)			
Aesku	ELISA	Serum/ plasma	Manual or automated ELISA instrument	U/mL; Negative <8, Positive >12	Nucleocapsid	IgG	95.5	> 99	96.7	> 99
						IgA	98.3	> 99	98.6	> 99
						IgM	95.7	> 99	99.4	> 99
					Spike	IgG	98.6	> 99	99.1	> 99
						IgA	94.6	> 99	98	> 99
						IgM	> 99	> 99	> 99	> 99
Vircell	ELISA	Serum/ plasma	Manual or automated ELISA instrument	Index; Negative <4, Positive >6	Recombinant antigens from Spike glycoprotein and nucleocapsid protein	IgG	85 (Days 10-19)	98	99	99
						IgA	88 (Days >5) (compared to PCR)	99	100	99
						IgM	88 (Days >5) (compared to PCR)	99	100	99
	CLIA	Serum/ plasma	Virclia	AI, Negative <1.4, Positive >1.6	Recombinant antigens from Spike glycoprotein and nucleocapsid protein	IgG	92 (Days >9) (compared to PCR)	99	98	100
						IgA	78 (Days: >5) (compared to PCR)	99	96	100
						IgM	78 (Days: >5) (compared to PCR)	99	96	100
Healgen	LFIA	Serum/ plasma			Nucleocapsid	IgG	97.2	100		
						IgM	87.9	100		

Abbreviations: ELISA: Enzyme-linked immunosorbent assay, CLIA: Chemiluminescent immunoassay, LFIA: Lateral flow immunoassay, NPV: Negative predictive value, PPV: Positive predictive value. Days implies after symptoms onset.

for all the parameters were performed against the PCR test. Statistical significance was set at p value <0.05.

Limitations

Only SARS-CoV-2 RT-PCR positive patients were included in the positive control group. Patients diagnosed with computed tomography were excluded from this study. The study design did not include longitudinal sera or probability of infectious cross-reactivity. The number of tests performed using each antibody detection kit varied. Post SARS-CoV-2 vaccination, antibody production kinetics and protection analysis were not considered.

RESULTS

The study group's mean age was 45.96 years (20-91), and 52.84% of the group was female.

Table 2 - *Sensitivity, specificity, Positive Likelihood Ratio, Negative Likelihood Ratio, Odds Ratio, PPV and NPV values for SARS-CoV-2 assays based on the detection of total antibody, IgG, IgM and IgA and number patients tested.*

	N (pos/ neg)	*Sensitivity (%) (CIa)*	*Specificity (%) (CIa)*	*(+) LR (CIb)*	*(-) LR (CIb)*	*Odds Ratio (CIb)*	*PPV (CIc)*	*NPV (CIc)*
Total, Siemens, S	839 (132/707)	72.0 (63.8 - 78.9)	98.6 (97.4 - 99.2)	50.883 (27.590 - 94.247)	0.284 (0.214 - 0.368)	178.959 (86.848 - 368.027)	0.905 (0.836 - 0.947)	0.950 (0.935 - 0.961)
Total, Roche, N	178 (130/48)	69.2 (60.8 - 76.5)	100.0 (92.6 - 100.0)	+∞ (9.334 - +∞)	0.308 (0.235 - 0.392)	+∞ (27.516 - +∞)	1.000 ()	0.946 (0.931 - 0.957)
Total, Roche, S	178 (97/81)	69.1 (59.3 - 77.4)	97.5 (91.4 - 99.3)	27.974 (8.012 - 102.141)	0.317 (0.231 - 0.419)	88.217 (22.2201 - 346.011)	0.839 (0.569 - 0.954)	0.944 (0.926 - 0.958)
IgG, Abbott, N	191 (89/102)	57.3 (46.9 - 67.1)	100.0 (96.4 - 100.0)	+∞ (15.770 - +∞)	0.427 (0.329 - 0.531)	+∞ (34.903 - +∞)	1.000 ()	0.926 (0.908 - 0.941)
IgG, Euroimmun, S	134 (88/46)	68.2 (57.9 - 77.0)	100.0 (92.3 - 100.0)	+∞ (8.832 - +∞)	0.318 (0.230 - 0.421)	+∞ (24.840 - +∞)	1.000 ()	0.944 (0.925 - 0.958)
IgG, Aesku, N	139 (91/48)	57.1 (46.9 - 66.8)	100.0 (92.6 - 100.0)	+∞ (7.693 - +∞)	0.429 (0.332 - 0.531)	+∞ (16.235 - +∞)	1.000 ()	0.926 (0.908 - 0.941)
IgG, Aesku, S	162 (97/65)	73.2 (63.6 - 81.0)	96.9 (89.5 - 99.2)	23.789 (6.902 - 86.693)	0.277 (0.196 - 0.377)	86.019 (21.390 - 340.441)	0.816 (0.530 - 0.946)	0.951 (0.933-0.964)
IgG, Vircell , ELISA, N+S	161 (96/65)	69.8 (60.0 - 78.1)	98.5 (91.8 - 99.7)	45.365 (8.459 - 257.302)	0.307 (0.222 - 0.407)	147.862 (24.381 - 880.725)	0.895 (0.547 - 0.983)	0.946 (0.928 - 0.959)
IgG, Vircell, CLIA, N+S	162 (97/65)	70.1 (60.4 - 78.3)	96.9 (89.5 - 99.2)	22.784 (6.604 - 83.088)	0.308 (0.223 - 0.411)	73.862 (18.477 - 291.160)	0.810 (0.520 - 0.944)	0.945 (0.927 - 0.959)
IgG, Healgen, N	836 (129/707)	65.1 (56.6 - 72.8)	96.2 (94.5 - 97.4)	17.051 (11.571 - 25.166)	0.363 (0.283 - 0.452)	47.012 (27.752 - 79.639)	0.761 (0.683 - 0.825)	0.937 (0.921 - 0.949)
IgA, Euroimmun S	116 (79/37)	64.5 (47.2 - 68.5)	97.3 (86.2 - 99.5)	21.544 (4.166 - 122.405)	0.429 (0.323 - 0.549)	50.182 (8.160 - 302.509)	0.801 (0.366 - 0.966)	0.926 (0.905 - 0.942)
IgA, Aesku, N	140 (90/50)	23.3 (15.8- 31.3)	100.0 (92.9-100.0)	+∞ (3.241 - +∞)	0.767 (0.669 - 0.843)	+∞ (3.8 - +∞)	1.000 ()	0.875 (0.862 - 0.887)
IgA, Aesku, S	164 (99/65)	48.5 (38.9 - 58.2)	96.9 (89.5 - 99.2)	15.758 (4.524 - 57.874)	0.532 (0.430 - 0.636)	29.647 (7.521 - 115.705)	0.746 (0.425 - 0.921)	0.910 (0.892 - 0.925)
IgM, Aesku, N	130 (89/41)	40.4 (30.9 - 50.8)	100.0 (91.4 - 100.0)	+∞ (4.697 - +∞)	0.596 (0.492 - 0.691)	+∞ (7.060 - +∞)	1.000 ()	0.900 (0.883 - 0.914)
IgM, Aesku, S	165 (100/65)	55.0 (45.2 - 64.4)	96.9 (89.5 - 99.2)	17.875 (5.152 - 65.464)	0.464 (0.366 - 0.569)	38.500 (9.767 - 150.255)	0.769 (0.457-0.930)	0.920 (0.902-0.935)
IgM, Healgen, N	836 (87/749)	35.7 (27.9 - 44.2)	97.5 (96.0 - 98.4)	14.006 (8.433 - 23.221)	0.660 (0.572 - 0.740)	21.214 (11.806 - 38.120)	0.723 (0.611 - 0.813)	0.890 (0.877 - 0.902)
IgM-IgA, Vircell, ELISA, N+S	162 (97/65)	54.5 (44.8 - 64.0)	87.7 (77.5 - 93.6)	4.432 (2.357 - 8.747)	0.518 (0.405 - 0.649)	8.550 (3.732 - 19.509)	0.453 (0.297 - 0.619)	0.912 (0.891 - 0.929)
IgM-IgA, Vircell, CLIA, N+S	162 (97/65)	48.5 (38.8 - 58.3)	95.4 (87.3 - 98.4)	10.498 (3.730 - 31.117)	0.540 (0.435 - 0.651)	19.427 (6.007 - 62.329)	0.662 (0.389 - 0.858)	0.908 (0.890 - 0.924)

Abbreviations: S: Spike protein, N: Nucleocapsid protein, n: Number of samples, pos: Positive number of samples, n: negative number of samples, CI: Confidence Interval, (+) LR: Positive Likelihood Ratio, (-) LR: Negative Likelihood Ratio, (-) LR: Negative Likelihood Ratio, PPV: Positive Predictive Value, NPV: Negative Predictive Value. a: Wilson 95% Confidence Interval, b: Miettinen-Nurminen 95% Confidence Interval, c: Mercado-Wald 95% Confidence Interval. Note: All statistical calculations involve all samples done regardless of days post-symptom onset.

Total evaluation of all 18 SARS-CoV-2 serologic tests was performed, and values of sensitivity and specificity were reported with 95% confidence interval (CI) (*Table 2*).

We studied the specificity of the pre-pandemic negative control samples; the results obtained were as follows:

Specificity of IgG and total antibody assays (*96.2% lowest; IgG, Healgen, N - 100.0% highest; IgG Abbott N, Total Roche N, IgG Euroimmun S, IgG Aesku N*) was higher than those of IgA, IgM, and IgM-IgA assays (*87.7% lowest; IgM-IgA, Vircell, ELISA, N+S - 100.0% highest; IgA Aesku N and IgM Aesku N*).

Sensitivity of IgG and total antibody assays (*57.1% lowest; IgG, Aesku, N-73.2% highest; IgG, Aesku, S*) was higher than those of IgA, IgM, and IgM-IgA assays *(23.3% lowest; IgA, Aesku, N - 64.5% highest; IgA, Euroimmun, S*).

(+) LR (= probability of a positive diagnosis) of IgG and total antibody assays was higher than those of IgA, IgM, and IgM-IgA. All assays were >10.0, except for IgM-IgA Vircell ELISA N+S (4.432). The values of (+) LR of Total Roche N, IgG Abbott N, IgG Euroimmun S, IgA Aesku N, IgM Aesku N, IgG Aesku N were calculated as +∞ because of 100% specificity values.

(-) LR (probability of a negative diagnosis) of IgG and total antibody assays (*lowest 0.277, IgG Aesku S*) was lower than those of IgA, IgM, and IgM-IgA (*highest 0.767, IgA Aesku N*). All assays were >0.1.

The SARS-CoV-2 IgG and total antibody assays were better diagnostic markers than the IgA, IgM, and IgM-IgA assays.

Sensitivity of SARS-CoV-2 assays for 0-10 days: was low and varied between 3.23% (lowest; IgA, Aesku, N) and 38.09% (highest; IgA, Euroimmun, S).

Sensitivity of SARS-CoV-2 assays for 10-15 days: sensitivity of IgG and total antibody assays (*highest, Total Roche S, IgG Aesku S: 100%*) was higher than those of the IgA, IgM, and IgM-IgA assays (*lowest, IgM Healgen N: 72.72%)*.

Sensitivity of SARS-CoV-2 assay for >15 days: sensitivity of IgG and total antibody assays *(highest, Total Roche S: 97.30%)* was higher than those of the IgA, IgM, and IgM-IgA assays (*lowest, IgA Aesku N: 40.00%*).

Before 10 days of symptom onset, the sensitivity of all assays, regardless of antibody type, was low, and none could be used for diagnostic purposes. Within 10-15 days of symptom onset, the sensitivity of IgG and total antibody assays was 70% higher than that of IgA, IgM, and IgM-IgA assays. After 15 days of symptom onset, the sensitivity of IgG and total antibody assays decreased slightly but were sensitive enough to be used for diagnosis; however, IgA, IgM, and IgM-IgA assays could not be used.

Table 3 - *Specificity and sensitivity values for SARS-CoV-2 assays based on the detection of total, IgG, IgM and IgA and number patients tested according to symptoms onset day.*

	SYMPTOM ONSET, DAYS			
	Negative, Specificity % (n)	*0-10 days, Sensitivity % (n)*	*10-15 days, Sensitivity % (n)*	*>15 days, Sensitivity% (n)*
Total, Siemens, S	98.59 (697/707)	12.5 (5/40)	98.18 (54/55)	97.30 (36/37)
Total, Roche, N	100 (48/48)	12.82 (5/39)	94.54 (52/55)	91.67 (33/36)
Total, Roche, S	97.53 (79/81)	11.11 (3/27)	100 (33/33)	97.30 (36/37)
IgG, Abbott, N	100 (102/102)	10.00 (2/20)	84.86 (28/33)	86.11 (31/36)
IgG, Euroimmun, S	100 (46/46)	5.00 (1/20)	90.30 (28/31)	94.59 (35/37)
IgG, Aesku, N	100 (48/48)	10.00 (2/20)	85.29 (29/34)	86.49 (32/37)
IgG, Aesku, S	96.92 (63/65)	14.81 (3/27)	100 (33/33)	94.59 (35/37)
IgG, Vircell , ELISA, N+S	98.46 (64/65)	7.41 (2/27)	93.94 (31/33)	94.44 (34/36)
IgG, Vircell, CLIA, N+S	96.92 (63/65)	7.41 (2/27)	96.97 (32/33)	94.59 (34/37)
IgG, Healgen, N	96.18 (680/707)	15 (6/40)	90.91 (50/55)	82.35 (28/34)
IgA, Euroimmun S	97.30 (36/37)	38.09 (8/21)	78.26 (18/23)	74.29 (26/35)
IgA, Aesku, N	100 (50/50)	3.23 (1/31)	91.18 (31/34)	40.00 (10/25)
IgA, Aesku, S	96.92 (63/65)	10.34 (3/29)	77.79 (26/33)	51.35 (19/37)
IgM, Aesku, N	100 (41/41)	4.76 (1/21)	72.72(24/33)	54.29 (19/35)
IgM, Aesku, S	96.92 (63/65)	13.33 (4/30)	87.88 (29/33)	62.16 (23/37)
IgM, Healgen, N	96.11 (746/749)	9.52 (2/21)	72.72 (24/33)	72.73 (24/33)
IgM-IgA, Vircell, ELISA, N+S	87.69 (57/65)	13.79 (4/29)	84.85 (28/33)	62.86 (22/35)
IgM-IgA, Vircell, CLIA, N+S	95.38 (62/65)	17.24 (5/29)	81.82 (27/33)	48.57 (17/35)

Abbreviations: S: Spike protein, N: Nucleocapsid protein, n: Number of samples.

No statistical difference was noted between the three groups with mild and moderate symptoms except for IgG in the 10-15 days group, IgM in >15 days, and IgA in the 0-10 days group ($p<0.05$). For these three exceptions, the group with moderate symptoms had higher sensitivity than the one with mild symptoms (*Table 3*).

Correlations: For comparison of quantitative responses, Pearson's correlation coefficients (r) were color-coded, with higher values displayed in darker blue and lower values in lighter blue (*Table 4*). All correlations were significant ($p\leq0.0005$). The correlation values were higher for the same antigenic epitope and method. Correlations between IgG and total antibody assays (strongest between two nucleocapsids, IgG assays Abbott-Aesku, r value: 0.905) were higher than those within IgA, IgM, and IgM-IgA assays (strongest between Vircell ELISA N+S- Vircell CLIA N+S, r value: 0.757).

AUC: According to the ROC curves and analysis results, we recommend a cutoff setting in which the calculated sensitivity and specificity are increased (*Table 5* and *Figure 1*). Based on the revised cutoff, IgA, IgM, and IgM-IgA assays were affected. The highest AUC-ROC value of 0.971 was detected with the total, Siemens, and S systems. The lowest AUC-ROC value was for IgA, Aesku, and N (0.539). According to the ROC analysis in our study, by changing the sensitivity cutoff recommended by the manufacturer, the AUC of the assays was increased.

DISCUSSION

In order to identify patients with ongoing SARS-CoV-2 infection, it is crucial to rule out people without infection and identify people in need of isolation, their past infections, and immune responses. Failure

Table 4 - *Correlation between SARS-CoV-2 serology assays: r (n).*

	Total, Roche, N	*Total, Roche, S*	*IgG, Abbott, N*	*IgG, Euroimmun, S*	*IgG, Aesku, N*	*IgG, Aesku, S*	*IgG, Vircell , ELISA, N+S*	*IgG, Vircell, CLIA, N+S*	*IgA, Euroimmun S*	*IgA, Aesku, N*	*IgA, Aesku, S*	*IgM, Aesku, N*	*IgM, Aesku, S*	*IgM-IgA, Vircell, ELISA, N+S*	*IgM-IgA, Vircell, CLIA, N+S*
Total, Siemens, S	0.760 (178)	0.828 (178)	0.639 (191)	0.790 (134)	0.750 (139)	0.788 (162)	0.769 (161)	0.790 (162)	0.711 (116)	0.346 (146)	0.777 (164)	0.483 (130)	0.762 (164)	0.520 (164)	0.622 (162)
Total, Roche, N		0.817 (178)	0.775 (178)	0.705 (134)	0.775 (178)	0.712 (162)	0.717 (161)	0.736 (162)	0.306 (116)	0.505 (146)	0.637 (164)	0.425 (130)	0.653 (164)	0.454 (164)	0.577 (162)
Total, Roche, S			0.822 (178)	0.869 (134)	0.785 (139)	0.827 (162)	0.808 (161)	0.823 (162)	0.661 (116)	0.546 (146)	0.795 (164)	0.539 (130)	0.774 (164)	0.561 (164	0.670 (162)
IgG, Abbott, N				0.782 (134	0.905 (139)	0.772 (162)	0.693 (161)	0.679 (162)	0.695 (116)	0.424 (146)	0.722 (164)	0.677 (130)	0.691 (164)	0.428 (164)	0.553 (162)
IgG, Euroimmun, S					0.802 (134)	0.814 (134)	0.847 (134)	0.847 (134)	0.779 (116)	0.469 (134)	0.767 (134)	0.551 (130)	0.752 (134)	0.533 (134)	0.604 (134)
IgG, Aesku, N						0.808 (139)	0.728 (139)	0.762 (139)	0.599 (116)	0.759 (139)	0.771 (139)	0.590 (130)	0.761 (139)	0.501 (139)	0.637 (139)
IgG, Aesku, S							0.745 (161)	0.783 (161)	0.648 (116)	0.506 (146)	0.829 (162)	0.569 (130	0.713 (162)	0.592 (162)	0.691 (162)
IgG, Vircell , ELISA, N+S								0.888 (161)	0.605 (116)	0.479 (146)	0.748 (161)	0.482 (130)	0.728 (161)	0.594 (161)	0.632 (161)
IgG, Vircell, CLIA, N+S									0.586 (116)	0.517 (146	0.791 (161)	0.517 (130)	0.759 (161)	0.599 (161)	0.674 (161)
IgA, Euroimmun S										0.465 (116)	0.689 (116)	0.473 (116)	0.611 (116)	0.432 (116)	0.528 (116)
IgA, Aesku, N											0.579 (146)	0.308 (130)	0.626 (146)	0.480 (146)	0.547 (146)
IgA, Aesku, S												0.482 (130)	0.751 (164)	0.582 (164)	0.689 (162)
IgM, Aesku, N													0.571 (130)	0.454 (130)	0.488 (130)
IgM, Aesku, S														0.584 (164)	0.615 (162)
IgM-IgA, Vircell, ELISA, N+S															0.757 (162)

Abbreviation: r: coefficient of correlation, n: number of samples, S: Spike protein, N: Nucleocapsid protein, ELISA: enzyme linked immunosorbent assay, CLIA: chemiluminescent immunoassay.

Table 5 - *ROC analysis of SARS-CoV-2 serology assays.*

	Cut Off	*AUC*	*AUC 95% CI*	*Sensitivity*	*Specificity*
Total, Siemens, S	0.75	0.971	0.957 – 0.986	0.735	0.989
Total, Roche, N	0.30	0.899	0.855 – 0.944	0.738	0.979
Total, Roche, S	0.40	0.853	0.807 - 0.900	0.722	0.975
IgG, Abbott, N	0.72	0.904	0.858 – 0.950	0.685	0.971
IgG, Euroimmun, S	0.52	0.854	0.793-0.915	0.659	1.000
IgG, Aesku, N	0.63	0.897	0.844 – 0.950	1.000	0.688
IgG, Aesku, S	4.91	0.899	0.852 - 0.946	0.753	0.954
IgG, Vircell, ELISA, N+S	4.59	0.858	0.801 - 0.916	0.719	0.969
IgG, Vircell, CLIA, N+S	1.41	0.843	0.791 - 0.904	0.701	0.969
IgA, Euroimmun, S	0.54	0.821	0.746 – 0.896	0.722	0.973
IgA, Aesku, N	2.00	0.539	0.443 – 0.635	0.589	0.732
IgA, Aesku, S	1.47	0.877	0.824 - 0.929	0.727	0.923
IgM, Aesku, N	3.29	0.816	0.745 – 0.888	0.652	0.878
IgM, Aesku, S	1.14	0.818	0.752- 0.883	0.747	0.877
IgM-IgA, Vircell, ELISA, N+S	5.92	0.720	0.642 - 0.798	0.566	0.877
IgM-IgA, Vircell, CLIA, N+S	0.17	0.809	0.743 - 0.875	0.753	0.769

Abbreviation: S: Spike protein, N: Nucleocapsid protein, ROC: Receiver Operating Characteristics, AUC: Area Under the Curve, CI: Confidence Interval, ELISA: enzyme linked immunosorbent assay, CLIA: chemiluminescent immunoassay.

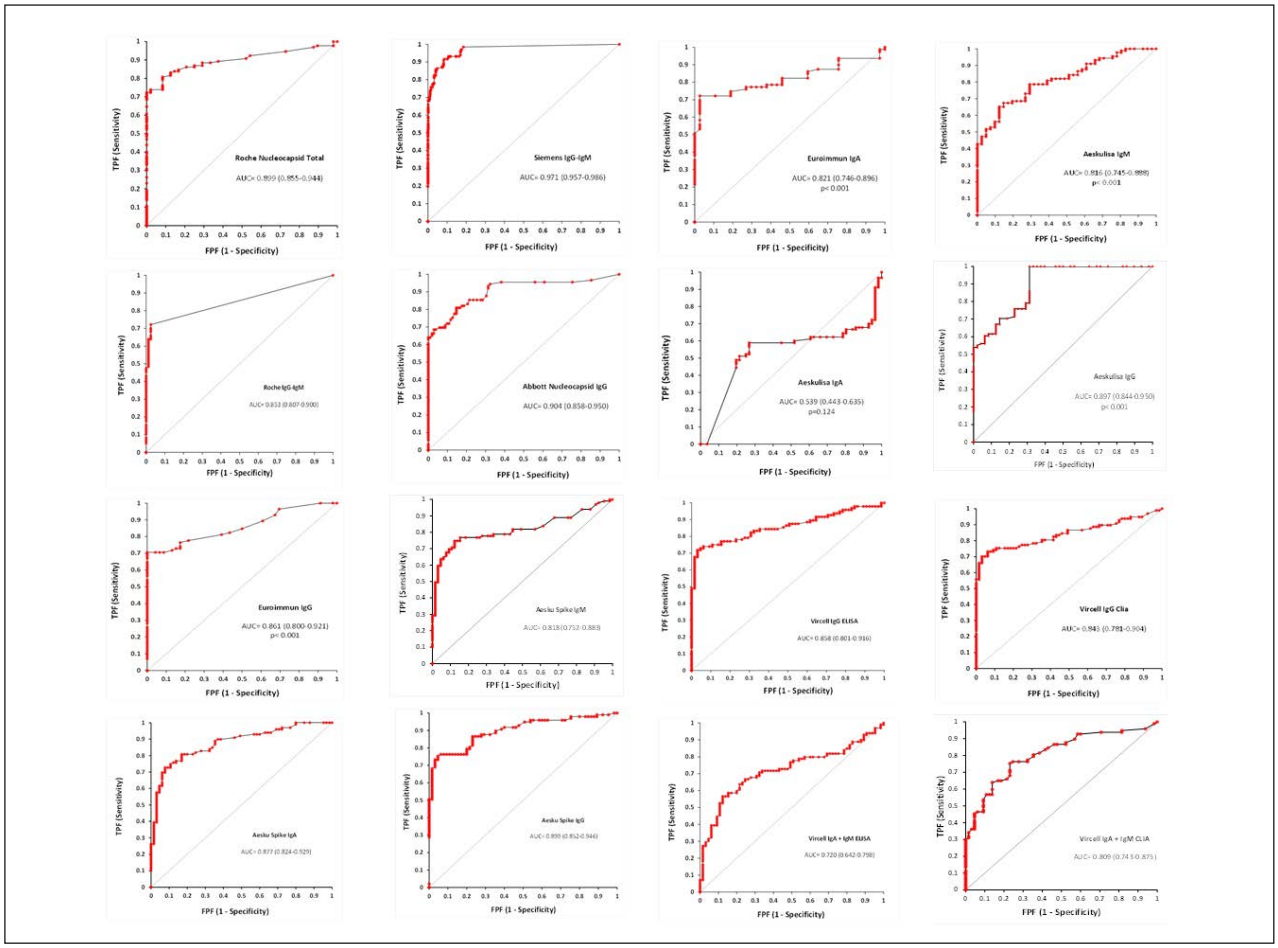

Figure 1 - *ROC curve graphics of antibody detection kits. Abbreviations: S: Spike protein, N: Nucleocapsid protein, ROC: Receiver Operating Characteristics, AUC: Area under curve, CI: confidence interval, TPF: True positive fraction (=sensitivity), FPF: False positive fraction (=1-specificity).*

to detect infected people causes delayed treatment and risks further spread of infection to others. The diagnosis of infected people is important in measuring disease spread, assessing the success of public health interventions (such as isolation), and potentially identifying individuals naturally immune to SARS-CoV-2. It has been shown that antibody tests could have a useful role in the diagnosis of COVID-19 in unvaccinated patients whose symptoms persist for >2 weeks and show negative RT-PCR test results (Corman *et al*., 2020; Opota *et al*., 2020). For patients previously vaccinated with either the mRNA or inactivated type vaccine, the diagnostic role of antibodies, neither S nor N type, was limited.

The measurement of the production and persistence of SARS-CoV-2 antibodies depends on many factors, including (1) the targeted antigens used (protein N, protein S, or domains of protein S), (2) the methodology (ELISA, CLIA, LFIA), and (3) time of serum collection after symptom onset (Fenwick *et al*., 2021; Seow *et al*., 2020). In this study, we evaluated 18 SARS-CoV-2 serological tests based on the above factors with positive sera collected from confirmed SARS-CoV-2 infected patients and negative sera from patients with lupus before the pandemic to address possible immunological cross-reactions.

As in previous studies, the assays showed high specificity but low sensitivity (Nicholson *et al*., 2016). The sensitivity and specificity of the results obtained in this study were slightly different from those reported by the manufacturers. However, the main results in terms of sensitivity and specificity were similar: IgG and total antibody assays performed better than IgM and IgA assays.

Sensitivity evaluations were stratified by days after the onset of symptoms to determine the best time interval for late acute/past diagnosis. The sensitivity of all antibody tests was too low in the first 10 days, as shown in previous kinetic antibody studies where IgA, IgM, and IgG antibodies appear simultaneously and are detected approximately 5-7 days after symptom onset. In our study, the sensitivity of all antibody tests progressed after 10 days. Serological tests are likely to have a complementary role when RT-PCR tests are negative and can be used in diagnosing late acute SARS-CoV-2 infection if seropositivity can be differentiated from post-vaccine positivity by 10 or more days from the onset of symptoms (Fernández-Barat *et al*., 2020; Lou *et al*., 2020). The sensitivity of IgM, IgA, and IgM-IgA assays was highest at 10-15 days but was clearly lower than that of the IgG and total antibody assays. At >15 days post-symptom onset, the sensitivity of the IgM, IgA, and IgM-IgA assays decreased sharply. The complementary role of IgM, IgA, and IgM-IgA assays was minor in the diagnosis of late acute SARS-CoV-2 infection, and these assays were restricted to 10-15 days post-symptom onset; hence, they could not be used for detecting past infections and could not be differentiated from mRNA vaccine positivity. The IgG and total antibody assays can be used for diagnosing past infections owing to high sensitivity levels at >15 days (15th day to 5 months) post-symptom onset in patients non-protected through vaccinations and for checking the post-vaccination seropositivity. However, the sensitivity of the >15 days group was lower than that of the 10-15 days group, probably because of the decline in IgG and total antibody levels over time (Fenwick *et al*., 2021; Seow *et al*., 2020; Espejo *et al*., 2020; Bastos *et al*., 2020; Whitman *et al*., 2020).

In our study, notable differences were not found in the technical performances between the methods or between the selected antigen types. According to previous studies based on methodology or antigen type, the sensitivity ratios were diverse, probably owing to bias of the selected study group or population and the technical performance of the selected kits. In some studies, antibodies against the S-protein were shown to be more specific and potentially neutralizing; the S-protein is under more selective pressure in terms of mutation due to its immunodominant role. In some studies, S-protein-based ELISA was more sensitive than N-protein-based ELISA, although some other studies showed N-protein to be more sensitive (Nicola *et al*., 2020; IDSA 2020; Liu *et al*., 2020; Van Elslande *et al*., 2020; Kohmera *et al*., 2020). We found strong correlations within the same antigen types, similar to those reported in the literature (Pflüger *et al*., 2020). Using ROC analysis, we found that changing the cut-off values recommended by the manufacturers increases the diagnostic value of tests by increasing AUC and sensitivity, especially in IgA, IgM, and IgM-IgA assays, which could have a crucial role in the late acute diagnosis despite vaccination status.

As shown by Fenwick *et al.* (Fenwick *et al*., 2021), additional studies should be performed to evaluate and standardize the technical performance of different sera obtained from different populations and for longer time periods, since all SARS-CoV-2 antibody assays were produced in a short time to meet urgent needs. Additionally, the affinity, avidity, and neutralization potential having differential production kinetics and post-infection studies are crucial to increase the diagnostic values of SARS-CoV-2 antibody assays by using standardized common calibrators and controls.

CONCLUSION

This independent evaluation of clinical specimens showed that serological tests have a complementary role with RT-PCR tests for diagnosing late acute SARS-CoV-2 infection if the latter is negative and used ≥10 days after the onset of symptoms in patients non-protected through vaccinations. SARS-

CoV-2 IgG and total antibodies were better diagnostic markers than IgM, IgA, IgM, and IgA. The role of IgM, IgA, and IgM+IgA assays was limited in diagnosis of late acute SARS-CoV-2 infection, being restricted to 10-15 days post-symptom onset, and could not be used for past infection. IgG and total antibody assays can be used for the diagnosis of past infections, but it should be noted that the levels of IgG and total antibody decline over time ((Bangaru *et al*., 2020; Fernández-Barat *et al*., 2020; Lou *et al*., 2020; Espejo *et al*., 2020; Bastos *et al*., 2020).

There were no statistical differences in technical performance among the methods and among the selected antigen types. Revised cutoff values should be used to increase the diagnostic value of antibody assays. This is a fast-moving field; thus, kit standardization studies should be accelerated to eventually eliminate the limitation of comparison between kits.

Conflict of Interest

Prof. Dr. Mustafa Serteser is a member of the Advisory Board of the Siemens Healthineers Company. The other authors have no conflicts of interest to declare.

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References

Bangaru S., et al. (2020). Structural analysis of full-length SARS-CoV-2 spike protein from an advanced vaccine candidate. *Science.* **370** (6520): 1089-1094.

Bastos M. L., et al. Diagnostic accuracy of serological tests for covid-19: systematic review and meta-analysis. *BMJ.* **370**: m2516.

Cantuti-Castelvetri L., et al. (2020). Neuropilin-1 facilitates SARS-CoV-2 cell entry and infectivity. *Science.* **370** (6518): 856-860.

Carter L.J. et al. (2020). Assay techniques and test development for COVID-19 diagnosis. *ACS Cent Sci.* **6** (5): 591-605. https://doi.org/10.1021/acscentsci.0c00501.

Chen C.Y., et al. (2007). Structure of the SARS coronavirus nucleocapsid protein RNA-binding dimerization domain suggests a mechanism for helical packaging of viral RNA, *J. Mol*. Biol. **368** (4): 1075-1086.

Cinquanta L., Fontana D.E., Bizzaro N. (2017). Chemiluminescent immunoassay technology: what does it change in autoantibody detection? *Auto Immun Highlights.* **8** (1): 9. https://doi.org/10.1007/s13317-017-0097-2.

Corman V.M., et al. (2020). Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill.* **25** (30).

Daly J.L., et al. (2020): Neuropilin-1 is a host factor for SARS-CoV-2 infection. *Science.* **370** (6518): 861-865.

Espejo A.P., et al. (2020). Review of current advances in serologic testing for COVID-19. *Am. J. Clin. Pathol.* **154** (3) 293-304.

Fenwick C., et al. (2021). Changes in SARS-CoV-2 spike versus nucleoprotein antibody responses impact the estimates of infections in population-based seroprevalence studies. *J. Virol.* **95** (3).

Fernández-Barat L., López-Aladid R., Torres A. (2020). The value of serology testing to manage SARS-CoV-2 infections. *Eur. Respir. J.* **56** (2): 2002411. https://doi.org/10.1183/13993003.02411-2020.

Ghaffari A., Meurant R., Ardakani A. (2020). COVID-19 serological tests: how well do they actually perform? *Diagnostics.* **10** (7) 453.

He X., et al. (2020). Temporal dynamics in viral shedding and transmissibility of COVID-19, *Nat. Med.* **26** (5) 672-675.

Infectious Diseases Society of America, IDSA COVID-19 antibody primer, https://www.idsociety.org/globalassets/idsa/publichealth/covid-19/idsa-covid-19-antibody-testing-primer.pd (2020) (Accessed 10 June 2020),

Kohmera N. et al. (2020). Brief clinical evaluation of six high-throughput SARS-CoV-2 IgG antibody assays, *J. Clin Virol.* **129**: 104480.

Krammer F., Simon V. (2020). Serology assays to manage COVID-19. *Science.* **368** (6495) 1060-1061.

Lee C.Y., et al. (2020). Serological approaches for COVID-19: epidemiologic perspective on surveillance and control. *Front. Immunol.* **11**: 879.

Liu Y., et al. (2020). Viral dynamics in mild and severe cases of COVID-19. *Lancet Infect. Dis.* **20** (6): 656-657.

Liu W., et al. (2020). Evaluation of nucleocapsid and spike protein-based enzyme-linked immunosorbent assays for detecting antibodies against SARS-CoV-2. *J. Clin. Microbiol.* **58**. https://doi.org/10.1128/JCM.00461-20.

Long Q.X., et al. (2020). Antibody responses to SARS-CoV-2 in patients with COVID-19. *Nat. Med.* **26** (6): 845-848.

Longchamp A., et al. (2020). Serum antibody response in critically ill patients with COVID-19. *Intensive Care Med.* **46** (10).

Lou B. et al. (2020). Serology characteristics of SARS-CoV-2 infection after exposure and post-symptom onset. *Eur. Respir. J.* **56** (2): 2000763. doi: 10.1016/S2213-2600(20)30084-9.

Maxim L.D., Niebo R., Utell M.J. (2019). Screening tests: a review with examples [published correction appears in Inhal Toxicol. **31** (7): 298. Inhal. Toxicol. **26** (13); (2014): 811-828. doi: 10.3109/08958378.2014.955932.

Mekonnen D., et al. (2021). Diagnostic accuracy of serological tests and kinetics of severe acute respiratory syndrome coronavirus 2 antibody: a systematic review and meta-analysis. *Rev Med Virol.* 2181. https://doi.org/10.1002/rmv.2181.

Nicola T., et al. (2020). Assessment of SARS-CoV-2 serological tests for the diagnosis of COVID-19 through the evaluation of three immunoassays: Two automated immunoassays (Euroimmun and Abbott) and one rapid lateral flow immunoassay (NG Biotech). *Journal of Clinical Virology.* **129**: 104511.

Nicholson L.B. (2016). The immune system. *Essays Biochem.* **60** (3): 275-301. https://doi.org/10.1042/EBC20160017.

Opota O., et al. (2020). Comparison of SARS-CoV-2 RT-PCR on a high-throughput molecular diagnostic platform and the cobas SARS-CoV-2 test for the diagnostic of COVID-19 on various clinical samples. *Pathog. Dis.* **78** (8).

Peeling R.W., et al. (2020). Serology testing in the COVID-19 pandemic response. *Lancet Infect. Dis.* **20** (9): e245-e249.

Pflüger L.S., et al. (2020). Clinical evaluation of five different automated SARS-CoV-2 serology assays in a cohort of hospitalized COVID-19 patients. *J Clin Virol.* **130**: 104549. https://doi.org/10.1016/j.jcv.2020.104549. Epub 17 Jul 2020.

Poland G.A., Ovsyannikova I.G., Kennedy R.B. (2020). SARS-CoV-2 immunity: review and applications to phase 3 vaccine candidates. *Lancet.* **396** (10262): 1595-1606.

Seow J., et al. (2020). Longitudinal observation and decline of neutralizing antibody responses in the three months following SARS-CoV-2 infection in humans. *Nat. Microbiol.* **5** (12): 1598-1607.

Sethuraman N., Jeremiah S.S., Ryo A. (2020). Interpreting diagnostic tests for SARS-CoV-2. *JAMA.* **323** (22): 2249-2251.

Van Elslande J. et al. (2020). Diagnostic performance of 7 rapid IgG/ IgM antibody tests and the Euroimmun IgA/IgG ELISA in COVID-19 patients. *Clin. Microbiol. Infect.* 1082-1087 https://doi.org/10.1016/j.cmi.2020.05.023.

Whitman J.D., et al. (2020). Evaluation of SARS-CoV-2 serology assays reveals a range of test performance. *Nat. Biotechnol.* **38** (10): 1174-1183.

WHO, WHO Coronavirus Disease (COVID-19) Dashboard, WHO, (2021). https://covid19.who.int/ Accessed 13/07/2022.

Wong R., Tse H. (2008). Lateral flow immunoassay. Berlin, Germany: Human Press part of Springer science & business Media.

Zhao J., et al. (2020). Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. *Clin. Infect. Dis.* **71** (16): 2027-2034.