Original Article

Sensitivity Affected by Disease Severity and Serum Sampling Time: a Performance Evaluation of Six SARS-CoV-2 Antibody Immunoassays

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ABSTRACT: Comparative validation and clinical performance data are essential for the reliable interpretation of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) antibody test results. This study aimed to assess the performance of six SARS-CoV-2 IgG immunoassays in the context of different disease severities. Four automated chemiluminescence immunoassays (Access [Beckman Coulter], Architect [Abbott], Atellica-IM [Siemens], and Elecsys [Roche]) as well as two ELISA assays (SARS-CoV-2 IgG-S1-based and NCP IgG [Euroimmun]) were evaluated using samples from 143 patients as well as 50 pre-pandemic control serum samples. Accuracy and precision tests were performed for validation purposes. Overall sensitivity ranged between 73.38–88.65% and was higher in spike protein-based assays, while the specificity was ≥98% in all immunoassays. The clinical performance of the immunoassays differed depending on disease severity and target antigen. For instance, the IgG response was lower for samples taken <20 days post-symptom onset (87.30%) compared with those taken ≥20 days post-symptom onset (94.80%). Moreover, moderate disease levels led to the highest levels of IgG. Higher levels of antibodies were detected in the clinically moderate disease group. In asymptomatic and mild groups, more antibody positivity was detected with spike protein-based assays. All the assays tested could be used to detect SARS-CoV-2 IgG. However, spike-based assays revealed relatively higher sensitivity rates than nucleoprotein-based assays, particularly in cases of asymptomatic and mild disease.

INTRODUCTION

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) emerged in late 2019 and has been a pandemic since 2020. Detection of viral RNA by real-time reverse transcriptase polymerase chain reaction testing (RT-PCR) is considered the gold standard for diagnosis and screening (1,2).

Due to the nature of the pandemic, several manufacturers rapidly designed assays to detect IgM, IgG, or total antibodies, with limited validation procedures in place. The sensitivity, specificity, and accuracy rates of the assays differ according to the test method, and can be categorized as lateral flow immunoassays (LFA), enzyme-linked immunosorbent assays (ELISA), and chemiluminescent immunoassays (CLIA). The sensitivity and specificity also vary according to the immunoglobulin class detected (IgM, IgG, or both), the SARS-CoV-2 antigen used (spike [S] or nucleoprotein [NP]), and the timing of sample collection following a positive RT-PCR and/or development of specific symptoms (3–7). Limited data exists on the clinical evaluation and accuracy of the

Table 1. Characteristics of the immunoassays evaluated

	Architect SARS-CoV-2	Access anti-SARS-CoV-2	Atellica-IM SARS-CoV-2	Elecsys anti-SARS-CoV-2	Euroimmun SARS-CoV-2	Euroimmun SARS-CoV-2 NCP
Antibody type	IgG	IgG	Total	Total	IgG	IgG
Target antigen	NP	S1-RBD	S1-RBD	NP	S1	NP
Assay principle	CMIA	CMIA	CMIA	ECLIA	ELISA	ELISA
Result calculation	Index (S/Co)	Index (S/Co)		Index (S/Co)	Index (S/Co)	Index (S/Co)
Manufacturer	Abbott diagnostics	Beckman coulter	Siemens healthcare	Roche diagnostics	Euroimmun	Euroimmun

NP, nucleoprotein; S, spike protein; RBD, receptor binding domain; CMIA, chemiluminescent microparticle immunoassay; ECLIA, electro chemiluminescent immunoassay; S/Co, signal to cut-off ratio.

results of the SARS-CoV-2 immunoassays, particularly in relation to disease severity and in use with RT-PCR-negative patients who have classic symptoms and chest CT findings. A comparative assessment of different high throughput immunoassays may provide valuable validation data to guide the interpretation of antibody test results.

This study was conducted under the guidance of the Society for the Clinical Microbiologists of Turkey and aimed to evaluate the performance of different SARS-CoV-2 antibody assays. Variable parameters considered determinants of diagnostic accuracy, particularly with regard to disease severity, were evaluated.

MATERIALS AND METHODS

Study design: This prospective study was approved by the Standing Committee on Ethics of Scientific Research of Alaaddin Keykubat University, Turkey (August 14, 2020; 22-34) and permission was obtained from the Scientific Research Council of the Ministry of Health of the Republic of Turkey (Permission no: 2020-07-28T11_45_09).

Serum samples were obtained from SARS-CoV-2 infected patients admitted to three university hospitals (Hacettepe, Ankara, and Ege Universities) and Ankara Bilkent City Hospital between June 24 and November 27, 2020. The patient group consisted of 143 patients with positive RT-PCR results, classic COVID-19 symptoms, and/or chest CT findings. Testing was performed using Bio-Speedy® SARS-CoV-2 N RT-qPCR (Bioeksen, Istanbul, Turkey), a one-step RT-PCR assay for qualitative detection of SARS-CoV-2.

A subset of 50 serum samples that has been obtained in 2019 from anti-CMV, anti-HSV1, and anti-EBNA IgG-positive patients and stored at −80°C, were used as pre-pandemic control samples. All serum samples were aliquoted and stored at −80°C until assayed.

Clinical data: For the 143 COVID-19 patients, basic demographic information, date of symptom onset, RT-PCR positivity, chest CT findings, and date of serum collection were recorded by an infectious disease physician using a standardized questionnaire. Informed written consent was obtained from each patient prior to sample collection and analysis.

Instrumentation and immunoassays: All samples were analyzed according to the manufacturer instructions of the six immunoassays, the characteristics of which are given in Table 1. The Abbott (Abbott Park, IL, USA), Beckman Coulter (Brea, CA, USA), and Euroimmun (Lübeck Germany) assays were performed at Hacettepe University Hospital; while the Roche (Basel, Switzerland) and Siemens (Berlin, Germany) assays were conducted at Ankara University Hospital and Bilkent City Hospital, respectively.

Validation tests: Accuracy was tested based on sensitivity, specificity, and positive and negative predictive values (PPV and NPV). Sensitivity was assessed by the inclusion of i) only RT-PCR positive individuals and ii) patients with either or a combination of a positive RT-PCR test, positive chest CT, or classic COVID-19 symptoms. Specificity was assessed using the pre-pandemic samples. Finally, a performance analysis was conducted in terms of serum sampling time and disease severity.

Precision studies were performed by analyzing the intra-assay and inter-assay reproducibility. For intra-assay reproducibility, five serum samples were analyzed in triplicate on one day. Inter-assay reproducibility was evaluated in duplicate over four sequential days for each assay, except for Euroimmun assays, which were assessed over three days due to limited availability of reagents.

Statistical analysis: All statistical analyses were performed using SPSS 23.0 version (IBM Corporation, Armonk, NY, USA). PPV and NPV with 95% confidence intervals (CI) were calculated based on an assumed prevalence of 10%, 15%, and 20%.

Concordance between assays was evaluated using Cohen's kappa coefficient and percent agreement. Differences between groups were calculated using the Mann–Whitney U test, Fisher's exact test, and chi-square test, as appropriate.

Normal distributions were evaluated using histograms and the Kolmogorov-Smirnov test. To evaluate precision, the coefficient of variation (CV) was calculated for intra-assay and inter-assay reproducibility.

RESULTS

Patient characteristics: Patients were grouped according to RT-PCR results and clinical symptoms. Classic symptoms, such as anosmia, fever, and dyspnea, in addition to abnormal chest CT findings, were noted
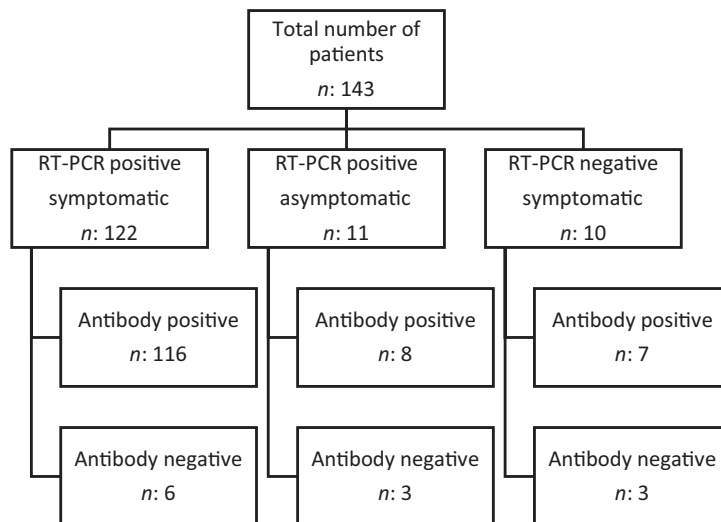
Fig. 1. Characteristics of the patients and distribution of the antibody results according to the patient groups.

for clinical suspicion. Patient characteristics and distribution of antibody results according to the groups are presented in Fig. 1. The majority of the patients were female (52.4%; $n = 75$), and the median age was 43 years (range: 20–87 years; interquartile range [IQR]: 19).

Patients were further classified into asymptomatic ($n = 11$), mild ($n = 97$), moderate ($n = 24$), and severe ($n = 11$) disease groups according to classifications by the World Health Organization (WHO) (1).

Accuracy: The total rate of antibody positivity in the study group was 131/143 (91.60%) when at least one positive immunoassay result was considered, and twelve patients were antibody negative using all assays. The Abbott, Beckman Coulter, Euroimmun S and NCP, Roche, and Siemens assays was performed in 139, 135, 138, 142, and 141 patients, respectively.

The median age of the antibody-positive patients with at least one test (median 42 years, IQR: 20) was significantly lower than that of the antibody-negative patients (median 52 years, IQR: 11) ($P = 0.034$).

Sensitivity ranged from 73.64–90.08% for the 133 RT-PCR-positive patients (Table 2). Rates of antibody positivity were 95/129, 112/125, 108/128, 98/128, 105/132, and 118/131 in the Abbott, Beckman, Euroimmun S-Ag, Euroimmun NCP-Ag, Roche, and Siemens assays, respectively. In addition to the low variation in sensitivity, the overall agreement was 67.17%. Moreover, the overall percentage agreement between the S-based and N-based assays were 83.93 and 75.13, respectively (95% CI) (Table 3).

When the RT-PCR negative cases were included, no change was detected in the sensitivity ranking of the assays. The sensitivity (95%CI) was noted as 88.15%, 83.33%, 88.65%, 73.38%, 76.09%, 78.87% for Beckman, Euroimmun S, Siemens, Abbott, Euroimmun NCP, and Roche assays, respectively.

Ten RT-PCR-negative cases had typical COVID-19 symptoms, and all except one had typical chest CT findings. Seven of the RT-PCR-negative cases were antibody positive in all assays (Table 4). Among the 12 patients who were antibody negative in all six immunoassays, serum samples had been obtained on the 15th post-symptomatic day. Three of those patients were RT-PCR negative, with symptoms, and positive chest CT findings. The remaining nine antibody-negative patients had mild disease, and the median serum sampling time was 21.75 days (range: 15–49 days). Among the 12 patients who were antibody negative by all six immunoassays, three were RT-PCR negative with symptoms and positive chest CT findings, and their serum samples were obtained on the 15. post-symptomatic day. The remaining nine antibody-negative, RT-PCR-positive patients had mild disease, and the median serum sampling time was 21.75 days (range: 15–49 days).

The overall specificity of the six immunoassays ranged from 98–100%, with both Euroimmun assays detecting one CMV IgG-positive serum sample as a false positive.

Analysis according to the sampling time: Overall evaluation revealed that antibody positivity was lower (87.30%) for the samples obtained <20 days post-symptom onset than for those obtained ≥20 days post-symptom onset (94.80%) ($P = 0.1137$). Furthermore, no significant differences were observed for antibody positivity (81% in <20 days versus 88.3% in ≥20 days) between NP antigen-based assays and S antigen-based assays (84.1% in <20 days versus 93.5% in ≥20 days) when sampling time was considered ($P = 0.225$ and $P = 0.100$, respectively), although differences were more pronounced in S antigen-based assays.

Analysis according to disease severity: The highest antibody positivity rate was found in the moderate group, followed by the mild, severe, and asymptomatic groups. In the asymptomatic and mild groups, more antibody positivity was detected using the S antigen-based assays. Higher rates of antibodies were detected in the moderate group compared with other clinical groups by any of the immunoassays or S or N antigen-based assays (95.8%) ($P = 0.002$, 0.004, and 0.018, respectively) (Fig. 2).

Precision study: Intra-assay % CV ranged from 0.48–5.20, while inter-assay % CV ranged from

Table 2. Performance characteristics of six SARS-CoV-2 immunoassays with the data of 133 RT-PCR positive patients

Immunoassay		% Sensitivity (95% CI)	% Specificity (95% CI)	PPV (95% CI)			NPV (95% CI)		
				10%	15%	20%	10%	15%	20%
S-based assays	Beckman Coulter (IgG)	89.60 (82.87–94.35%)	100.00 (92.89–100.00%)	100.00	100.00	100.00	98.86 (98.10–99.31%)	98.20 (97.02–98.91%)	97.47 (95.83–98.47%)
	Euroimmun S (IgG)	84.38 (76.91–90.19%)	98.00 (89.35–99.95%)	82.42 (40.21–97.03%)	88.16 (51.64–98.11%)	91.34 (60.21–98.66%)	98.26 (97.41–98.33%)	97.26 (95.95–98.16%)	96.17 (94.36–97.41%)
	Siemens (Total)	90.08 (83.63–94.61%)	100 (92.89–100.00%)	100.00	100.00	100.00	98.91 (98.19–99.35%)	98.28 (97.15–98.97%)	97.58 (96.01–98.54%)
NP-based assays	Abbott (IgG)	73.64 (65.16–81.01%)	100.00 (92.89–100.00%)	100.00	100.00	100.00	97.15 (96.24–97.85%)	95.56 (94.16–96.63%)	93.82 (91.92–95.29%)
	Euroimmun NCP (IgG)	76.56 (68.26–83.59%)	98.00 (89.35–99.95%)	80.96 (37.87–96.74%)	87.11 (49.19–97.92%)	90.54 (57.83–98.52%)	97.41 (96.48–98.10%)	95.95 (94.53–97.01%)	94.36 (92.42–95.82%)
	Roche (Total)	79.55 (71.65–86.07%)	100.00 (92.89–100.00%)	100.00	100.00	100.00	97.78 (96.92–98.40%)	96.52 (95.19–97.49%)	95.14 (93.32–96.48%)

Table 3. Percentage agreement between the six immunoassays evaluated (for the 143 samples tested for sensitivity)

% agreement (95% CI)	Beckman Coulter	Euroimmun S	Euroimmun NCP	Roche	Siemens
Abbott	56.6%	62.8%	85%	74%	50.5%
Beckman coulter		80.4%	55.9%	57.7%	88.1%
Euroimmun S			57.9%	70.8%	83.3%
Euroimmun NCP				66.4%	55.7%
Roche					62.5%

Table 4. The reactivity results of the RT-PCR negative patients

Patient	Abbott	Beckman	Euroimmun		Roche	Siemens
			S	NCP		
S1	7.84	19.24	9.5	5.3	26.9	>10.00
S2	5.69	37.38	10.9	3.4	1.06	>10.00
S3	8.03	62.20	10.5	5	54.27	>10.00
S4	4.08	26.02	6.6	4.4	18.92	>10.00
S5	8.17	42.45	10.5	6.2	37.01	>10.00
S6	7.29	45.65	9	5.7	31.51	>10.00
S7	5.90	2.75	2.2	3.3	15.84	3.93
S8[1]	0.02	0.02	0.1	0.02	0.096	0.10
S9[1]	0.04	0.05	0.5	0.03	0.081	0.59
S10[1]	0.05	0.03	0.1	0.08	0.086	0.55

[1]: Negative result.

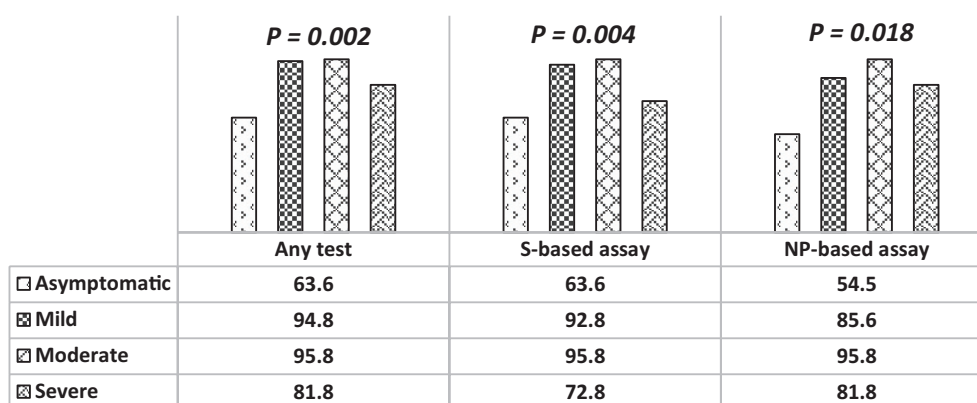|	Any test	S-based assay	NP-based assay
	P = 0.002	*P = 0.004*	*P = 0.018*
☐ Asymptomatic	63.6	63.6	54.5
⊠ Mild	94.8	92.8	85.6
⊠ Moderate	95.8	95.8	95.8
⊠ Severe	81.8	72.8	81.8

Fig. 2. Antibody positivity rates of S and NP antigen-based assays according to disease severity.

0.76–10.24 for the positive samples. Additionally, the categorical agreement between the qualitative results was 100%.

DISCUSSION

Immunoassays targeting the detection of specific antibodies to SARS-CoV-2 aid in evaluating the immune response as well as in supplementing diagnoses in asymptomatic and RT-PCR-negative patients. Each country and diagnostic laboratory has its own prerequisites and risks of shortages in the implementation of antibody testing during the COVID-19 pandemic. This study was conducted under the guidance of the Society for the Clinical Microbiologists of Turkey, with the aim of meeting the informational needs of clinical practice and the validation of the SARS-CoV-2 immunoassays available in Turkey.

In the present study, we evaluated the performance of four automated CLIA and two ELISA SARS-CoV-2 immunoassays. The manufacturers of the included assays declared sensitivities ranging from 90–100%, for samples taken on day 14 or later after symptom onset, and specificities ranging from 99.6–100% (3). Our study found mean sensitivities ranging from 73.64–90.08%. Additionally, the sensitivities of the assays differed

widely based on the clinical status of the patients, sampling time, and the target antigen used. All samples collected in this prospective study were obtained on day 14 or later after symptom onset or positive RT-PCR test. The relatively low sensitivity in our study may be partly due to patient characteristics, lack of antibody response, or disease severity. Moreover, the lower sensitivity may also be attributed to the high number of patients with mild disease, which may have yielded a moderate antibody response. A literature review revealed sensitivity results between 77.8–100% for the Abbott assay, between 76.5–81.5% for the Beckman Coulter assay, between 70.7–93.8% for the Euroimmun S IgG, between 88.89–100% for the Euroimmun NCP IgG, between 75.60–97.20% for the Roche assay, and between 85.9–98.1% for the Siemens assay (5–13).

Although RT-PCR testing is the gold standard for definitive SARS-CoV-2 diagnosis, RT-PCR tests may yield false negative results, and additional tools such as chest CTs and/or radiography may aid in diagnosing such cases (14–16). Therefore, we also analyzed the data of a small group of RT-PCR-negative patients with typical symptoms and/or positive chest CTs, and an antibody response was detected in seven of the ten RT-PCR-negative patients, indicating past infection.

The Infectious Diseases Society of America (IDSA) and European Centre for Disease Prevention and

Control (ECDC) recommend SARS-CoV-2 antibody testing to aid in the diagnosis of RT-PCR-negative, symptomatic patients (2,17). Antibody assays may support RT-PCR testing, particularly in later stage of COVID-19 infection, where it is typically difficult to obtain positive nasopharyngeal swabs. For instance, in a cohort study performed in Shenzhen, China, RT-PCR-negative contacts of SARS-CoV-2 RT-PCR-confirmed cases were tested for specific antibodies, and 4.5% of the 880 RT-PCR-negative close contacts were positive with total antibody ELISA testing (18). Long et al. also reported that serial antibody testing may be helpful in the diagnosis of RT-PCR-negative COVID-19 patients (19). Although the sample size was small, the findings in the RT-PCR-negative group in that study provided evidence for the importance of antibody testing as an additional tool for the definite diagnosis of RT-PCR-negative individuals. Gaps in the literature related to the rates of seropositivity in RT-PCR-negative individuals remain, however. Large-scale seroepidemiological studies may help to determine the exact number of people that have been in contact with SARS-CoV-2.

In addition, our study found that the sensitivity of the immunoassays varied based on the target SARS-CoV-2 antigen, with higher sensitivities in assays targeting spike antigens. The variation in sensitivity were not pronounced in assays using the same antigen as the antibody target. While several studies have revealed higher sensitivity rates for spike antigen-based assays (10,20,21), data from other studies have also indicated higher sensitivity rates with NP antigen-based assays (7,9). Turbett et al. reported that the two nucleocapsid antibody tests (Abbott and Roche) were more sensitive than the spike protein antibody test (Diasorin), with pronounced differences observed in samples collected 7–14 days after symptom onset. However, these three assays had comparable sensitivities when using samples collected >14 days post-symptom onset (22). This is in accordance with our results, as we only included samples collected after 14 days of symptom onset. Additionally, Andrey et al. reported that S-assays tended to display slightly better sensitivity and NPV than Roche S and Roche N (21). These discrepancies are not surprising as a large range of serologic assay comparisons exist, particularly in terms of patient selection, age, severity of disease, type of antibodies detected, chemical structures of the targeted antigen, and sampling time. It is not surprising to observe higher sensitivity with immunoassays detecting total antibodies in samples obtained <14 days, as IgM type of antibodies have also been detected. This was also observed in our study using the Siemens assay, which detected total antibodies targeting the S antigen.

Concordance between the assays was highest for Siemens and Beckman Coulter assays, which targeted the S antigen. These were followed by Abbott and Euroimmun NCP, which targeted the NP antigen. This result supported the approach of performing immunoassays based on the type of antigen targeted.

Our results indicated that all assays achieved high specificity, as declared by the manufacturers. High specificity results for SARS-CoV-2 antibody assays have also been reported by several other groups (5,6). With COVID-19, high specificity is more important than high sensitivity, as anti-SARS-CoV-2 antibody tests are not intended for screening purposes, and false positive results may lead to a false sense of security. High specificity is essential for achieving a high PPV. Although sensitivity is important for test performance, PPV and NPV are more useful for estimating the probability of the disease and interpreting test results. The sensitivity, specificity, and prevalence of COVID-19 in a specific population also impacts the determination of test validity. In the current study, the PPV generated for an assumed 10%, 15%, and 20% prevalence revealed that PPV was highest with the Siemens assay, followed by the Beckman Coulter, Euroimmun S, Roche, and Euroimmun NCP assays. In a multi-center comparison of seven immunoassays, Oved et al. reported that narrow differences in the specificity of the assays had profound effects on PPV in cases of low prevalence (8). With the progression of the pandemic and increasing number of vaccinations, higher prevalence settings will become more likely. The NPV with 20% prevalence in this study showed that any of the evaluated tests could reliably be used in cases of high prevalence.

The timing of sample collection in relation to the onset of specific symptoms has also been noted to affect positivity rates for SARS-CoV-2 specific antibodies (6,9). The rate of antibody positivity was relatively low (87.30%) in sample collected <20 days post-symptom onset compared with those collected ≥20 days (94.80%). In accordance with the literature, the current study revealed that sensitivity increased in line with time-dependent production of specific antibodies (5,10). To reach maximum sensitivity rates, testing for IgG after 20 days of infection remains ideal.

Limited data related to the effect of disease severity on the rate of antibody production exist. In the present study, the lowest antibody response was observed in the asymptomatic group. Although the sample size was small, this result is in accordance with previous results (5). Notably, S-based assays detected significantly fewer antibodies than NP-based assays in the severe group ($P < 0.005$), which may explain severe disease development. This may primarily be due to the lack of neutralizing anti-S antibodies, even though N antibodies are still produced. Further analysis regarding the relationship between disease severity and the type of antibody developed may be needed, however. Nevertheless, the severity of COVID-19 infection should also be considered when interpreting emerging seroprevalence and disease outcome data.

The higher rates of antibody positivity in relatively younger age groups detected in the current study may be attributed to the failure of the humoral immune response to new antigens in an aging immune system. In a comprehensive review, Bajaj et al. related the relatively decreased antibody response to SARS-CoV-2 to the limited capability of binding to a new antigen in the elderly, leading to the production of lower-affinity antibodies over a longer period of time (23).

Antibody tests were negative in all assays in nine RT-PCR-positive patients. This may have been attributed to antibody test performance, early-stage infection, or mild disease in this group. However, early stage infections were not included in our study, as all samples were obtained at least 15 days post-symptom onset

or RT-PCR positivity. Although rates of antibody-negative samples were higher in N antigen-based assays than in S antigen-based assays, the difference was not significant. This is in line with previous studies (24–26), indicating that differences in sensitivity among immunoassays during acute SARS-CoV-2 infection are only partly associated with the type of target antigen. The observation of negative antibody results in RT-PCR-confirmed cases further confirmed that negative antibody results do not rule out a prior COVID-19 infection.

The strengths of this study include the head-to-head comparison of identical well-characterized samples, which allowed for unbiased performance comparison of commonly used SARS-CoV-2 immunoassays.

Our data revealed that the clinical performance of different SARS-CoV-2 immunoassays was influenced by disease severity and the target antigen. Mild disease courses led to moderate IgG responses, whereas asymptomatic disease was related to a weak immune response. Further evidence on the performance of antibody tests may be needed, as a risk of heterogeneity may exist, particularly regarding the population included, severity of infection, serum sampling time, and target antigen of the assay. Our findings emphasized the importance of validating exams in real-life settings. Moreover, understanding antibody test limitations is vital for the appropriate use and interpretation of serological tests.

REFERENCES

1. World Health Organisation (WHO). COVID-19 Clinical Management, Living Guidance, 21 January 2021. Available at <https://www.who.int/publications/i/item/WHO-2019-nCoV-clinical-2021-2>. Accessed June 25, 2021.
2. Infectious Diseases Society of America (IDSA). COVID-19 Diagnostic Testing Policy Recommendations. 2020. Available at <www.idsociety.org/globalassets/idsa/public-health/covid-19-diagnostic-testing-policy-recommendations.pdf>. Accessed June 25, 2021.
3. Food and Drug Administration, USA. EUA authorized serology test performance. Available at <https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/eua-authorized-serology-test-performance>. Accessed June 25, 2021.
4. FIND. SARS-CoV-2 diagnostic pipeline. Available at <www.finddx.org/covid-19/pipeline/>. Accessed June 25, 2021.
5. Deeks JJ, Dinnes J, Takwoingi Y, et al. Antibody tests for identification of current and past infection with SARS-CoV-2 (Review). Available at <https://doi.org/10.1002/14651858.CD013652>. Accessed July 5, 2021.
6. Bastos ML, Tavaziva G, Abidi SK, et al. Diagnostic accuracy of serological tests for covid-19: systematic review and meta-analysis. BMJ. 2020;370:m2516.
7. Kohmer N, Westhaus S, Rühl C, et al. Brief clinical evaluation of six high-throughput SARS-CoV-2 IgG antibody assays. J Clin Virol. 2020; A129: 104480.
8. Oved K, Olmer L, Shemer-Avni Y, et al. Multi-center nationwide comparison of seven serology assays reveals a SARS-CoV-2 non-responding seronegative subpopulation. EClinicalMedicine. 2020; 29:100651.
9. Tanis J, Vancutsem E, Piérard D, et al. Evaluation of four laboratory-based SARS-CoV-2 IgG antibody immunoassays. Diagn Microbiol Infect Dis. 2021; 100: 115313.
10. 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: 1390-1400.
11. Irsara C, Egger AE, Prokop W, et al. Evaluation of four commercial, fully automated SARS-CoV-2 antibody tests suggests a revision of the Siemens SARS-CoV-2 IgG assay. Clin Chem Lab Med. 2021; 59: 1143-1154.
12. Government UK. COVID-19: laboratory evaluations of serological assays. Available at <https://www.gov.uk/government/publications/covid-19-laboratory-evaluations-of-serological-assays> Accessed June 25, 2021.
13. Jääskeläinen AJ, Kuivanen S, Kekäläinen E, et al. Performance of six SARS-CoV-2 immunoassays in comparison with microneutralisation. J Clin Virol. 2020; 129: 104512.
14. Weissleder R, Lee H, Ko J, et al. COVID-19 diagnostics in context. Sci Transl Med. 2020;12:eabc1931.
15. Wang L, Zhan D, Liu X, et al. Initial PCR testing negative, but chest CT suggesting for viral pneumonia urges for repeated testing for COVID-19 diagnosis. Front Mol Biosci. 2021; 8:640788.
16. He JL, Luo L, Luo ZD, et al. Diagnostic performance between CT and initial real-time RT-PCR for clinically suspected 2019 coronavirus disease (COVID-19) patients outside Wuhan, China. Respir Med. 2020; 168:105980.
17. European Centre for Disease Prevention and Control. Coronavirus disease 2019 (COVID-19) pandemic: increased transmission in the EU/EEA and the UK – seventh update. Available at <https://www.ecdc.europa.eu/sites/default/files/documents/RRA-seventh-update-Outbreak-of-coronavirus-disease-COVID-19.pdf>. Accessed June 25, 2021.
18. Zhang Z, Bi Q, Fang S, et al. Insight into the practical performance of RT-PCR testing for SARS-CoV-2 using serological data: a cohort study. Lancet Microbe. 2021; 2: e79-e87.
19. Long QX, Liu BZ, Deng HJ, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. Nat Med. 2020; 26: 845-848.
20. Haselmann V, Kittel M, Gerhards C, et al. Comparison of test performance of commercial anti-SARS-CoV-2 immunoassays in serum and plasma samples. Clin Chim Acta. 2020; 510: 73-78.
21. Andrey DO, Yerly S, Meyer B, et al. Head-to-head evaluation of five automated SARS-CoV-2 serology immunoassays in various prevalence settings. J Clin Med. 2021; 10: 1605.
22. Turbett SE, Anahtar M, Dighe AS, et al. Evaluation of three commercial SARS-CoV-2 serologic assays and Tteir performance in two-test algorithms. J Clin Microbiol. 2020; 59:e01892-20.
23. Bajaj V, Gadi N, Spihlman AP, et al. Aging, immunity, and COVID-19: how age influences the host immune response to coronavirus infections? Front Physiol. 2021;11:571416.
24. Harritshøj LH, Gybel-Brask M, Afzal S, et al. Comparison of 16 serological SARS-CoV-2 immunoassays in 16 clinical laboratories. J Clin Microbiol. 2021; 59:e02596-20.
25. Coste AT, Jaton K, Papadimitriou-Olivgeris M, et al. Comparison of SARS-CoV-2 serological tests with different antigen targets. J Clin Virol. 2021;134:104690.
26. Cheng MP, Yansouni CP, Basta NE, et al. Serodiagnostics for severe acute respiratory syndrome-related coronavirus 2: a narrative review. Ann Intern Med. 2020; 173: 450-460.