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Comparative Performance of Five Commercially Available Serologic Assays To Detect Antibodies to SARS-CoV-2 and Identify Individuals with High Neutralizing Titers

Eshan U. Patel,[a,b] Evan M. Bloch,[a] William Clarke,[a] Yu-Hsiang Hsieh,[c] Denali Boon,[d] Yolanda Eby,[a] Reinaldo E. Fernandez,[e] Owen R. Baker,[f] Morgan Keruly,[e] Charles S. Kirby,[a] Ethan Klock,[e] Kirsten Littlefield,[g] Jernelle Miller,[a] Haley A. Schmidt,[a] Philip Sullivan,[a] Estelle Piwowar-Manning,[a] Ruchee Shrestha,[a] Andrew D. Redd,[e,f] Richard E. Rothman,[c] David Sullivan,[g] Shmuel Shoham,[e] Arturo Casadevall,[g] Thomas C. Quinn,[e,f] Andrew Pekosz,[g] Aaron A. R. Tobian,[a,e] Oliver Laeyendecker[e,f]

[a]Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA
[b]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA
[c]Department of Emergency Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA
[d]Department of Mental Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA
[e]Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA
[f]Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Baltimore, Maryland, USA
[g]Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA

Aaron A. R. Tobian and Oliver Laeyendecker contributed equally to this article and are co-senior authors.

ABSTRACT Accurate serological assays to detect antibodies to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are needed to characterize the epidemiology of SARS-CoV-2 infection and identify potential candidates for coronavirus disease 2019 (COVID-19) convalescent plasma (CCP) donation. This study compared the performances of commercial enzyme immunoassays (EIAs) with respect to detection of IgG or total antibodies to SARS-CoV-2 and neutralizing antibodies (nAbs). The diagnostic accuracy of five commercially available EIAs (Abbott, Euroimmun, EDI, ImmunoDiagnostics, and Roche) for detection of IgG or total antibodies to SARS-CoV-2 was evaluated using cross-sectional samples from potential CCP donors who had prior molecular confirmation of SARS-CoV-2 infection ($n = 214$) and samples from prepandemic emergency department patients without SARS-CoV-2 infection ($n = 1,099$). Of the 214 potential CCP donors, all were sampled >14 days since symptom onset and only a minority ($n = 16$ [7.5%]) had been hospitalized due to COVID-19; 140 potential CCP donors were tested by all five EIAs and a microneutralization assay. Performed according to the protocols of the manufacturers to detect IgG or total antibodies to SARS-CoV-2, the sensitivity of each EIA ranged from 76.4% to 93.9%, and the specificity of each EIA ranged from 87.0% to 99.6%. Using a nAb titer cutoff value of ≥160 as the reference representing a positive test result ($n = 140$ CCP donors), the empirical area under the receiver operating curve for each EIA ranged from 0.66 (Roche) to 0.90 (Euroimmun). Commercial EIAs with high diagnostic accuracy to detect SARS-CoV-2 antibodies did not necessarily have high diagnostic accuracy to detect high nAb titers. Some but not all commercial EIAs may be useful in the identification of individuals with high nAb titers among convalescent individuals.

KEYWORDS COVID-19, SARS-CoV-2, serologic assays, neutralizing titers, convalescent plasma

Globally, as of October 2020, there were over 38.5 million reported cases of infection by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes coronavirus disease 2019 (COVID-19) disease (1). Surveillance based on case reporting

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Address correspondence to Oliver Laeyendecker, olaeyen1@jhmi.edu.

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is informative, but it significantly underestimates the true burden of infection and can lead to biased epidemiological inferences. Accurate and reliable serological assays to detect SARS-CoV-2 antibodies can be used to better understand the epidemiology of SARS-CoV-2 infection at the population level, as the presence of antibodies to SARS-CoV-2 indicates recent or prior exposure to the virus (2). Serological assays can also be useful for screening blood donations, qualifying individuals for convalescent plasma donation, clinically managing patients, and studying the immune response to infection (2–4). It remains unknown whether the presence of antibodies against SARS-CoV-2 confers immunity against reinfection or how long those antibodies persist following infection.

As of October 2020, >50 commercially available serological assays had received an individual emergency use authorization (EUA) by the U.S. Food and Drug Administration (FDA) for the detection of antibodies to SARS-CoV-2 (https://www.fda.gov/medical-devices/emergency-use-authorizations-medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices). These assays generally detect IgM, IgG, or total antibodies to epitopes of SARS-CoV-2, including antibodies to subunit 1 of the spike glycoprotein (S1), subunit 2 of the spike glycoprotein (S2), the spike glycoprotein receptor binding domain (RBD), or the recombinant nucleocapsid protein (N). The assays can also be categorized, broadly, as (i) lateral flow immunoassays (LFAs), (ii) enzyme-linked immunosorbent assays (ELISAs), and (iii) chemiluminescent immunoassays (CLIAs). ELISAs and CLIAs (collectively known as enzyme immunoassays [EIAs]) provide continuous output that is often used as a semiquantitative surrogate for antibody titers, whereas LFAs are strictly qualitative. Recent systematic reviews of the literature have noted the need for additional data on the performance of commercially available SARS-CoV-2 serologic assays, as most previous studies have been deemed to have a high risk of bias, particularly due to the use of small sample sizes and/or exclusion of specimens from asymptomatic SARS-CoV-2 infections and mild or moderate cases of COVID-19 (5–8).

Commercial SARS-CoV-2 EIAs may have an additional role in the implementation of COVID-19 convalescent plasma (CCP) therapy programs (2, 9, 10). The FDA recently issued an EUA for CCP therapy (https://www.fda.gov/media/141477/download). Indeed, observational evidence suggests that CCP is likely safe and efficacious, particularly when administered early in the disease process (11–15). Higher IgG antibody titers to the S1 protein, as measured by the Ortho-Clinical Diagnostics Vitros anti-SARS-CoV-2 IgG EIA, in CCP transfused to COVID-19 patients have been associated with decreased mortality (14). Higher anti-SARS-CoV-2 IgG or total antibody titers to the spike (S) and nucleocapsid (N) protein of SARS-CoV-2 have also been shown to correlate with titers of SARS-CoV-2 neutralizing antibodies (nAbs) (16–19), which are presumed to be critical for viral clearance. Current *in vitro* assays to detect nAbs are resource and time intensive and are not typically conducted in clinical laboratories. Accordingly, the Ortho Vitros anti-SARS-CoV-2 IgG EIA has received an EUA by the FDA to discriminate CCP donation products with "high" and "low" titers. Other commercial SARS-CoV-2 EIAs, including those targeting the S1 protein like the Ortho assay (e.g., the Euroimmun anti-SARS-CoV-2 ELISA), may also be useful to identify CCP donors with high nAb titers. However, data on the comparative performances of commercial SARS-CoV-2 EIAs to discriminate between CCP donors with high and low nAb titers are limited (3).

This report compares the performances of five commercially available EIAs to detect IgG or total antibodies to SARS-CoV-2 and to discriminate between high and low SARS-CoV-2 nAb titers.

MATERIALS AND METHODS

Ethics statement. This study used stored samples and data from two parent studies that were approved by The Johns Hopkins University School of Medicine Institutional Review Board. All samples were deidentified prior to laboratory testing. Both studies were conducted according to the ethical standards of the Helsinki Declaration of the World Medical Association.

Study specimens. To test the clinical sensitivity of SARS-CoV-2 EIAs, we included stored plasma specimens from a convenience sample of potential CCP donors that were recruited in the Baltimore, MD,

TABLE 1 Characteristics of commercial SARS-CoV-2 enzyme immunoassays evaluated[a]

Manufacturer	Assay name	Target antigen (recombinant)	Platform	Manufacturer's interpretation	No. of samples evaluated
Euroimmun, Lubeck, Germany	Anti-SARS-CoV-2 ELISA (IgG)[b]	Spike-1 protein	Manual ELISA	Negative, S/C ratio <0.8 Borderline, S/C ratio ≥0.8 & <1.1 Positive, S/C ratio ≥1.1	CCP donors, 146 Prepandemic, 561
Epitope Diagnostics, Inc. (EDI), San Diego, CA	EDI novel coronavirus COVID-19 IgG ELISA kit	Nucleocapsid protein	Manual ELISA	Negative, OD-n ≤0.18 Borderline, OD-n >0.18 & <0.22 Positive, OD-n ≥0.22	CCP donors, 146 Prepandemic, 578
ImmunoDiagnostics Limited, Sha Tin, Hong Kong	SARS-CoV-2 NP IgG ELISA kit[c]	Nucleocapsid protein	Manual ELISA	Negative, OD-n <0.15 Borderline, OD-n ≥0.25 & ≤0.50 Positive, OD-n > 0.50	CCP donors, 140 Prepandemic, 306
Abbott Laboratories Inc., Abbott Park, IL	Abbott-Architect SARS-CoV-2 IgG assay[b]	Nucleocapsid protein	Abbott Architect i2000 (CMIA)[d]	Negative, index (S/C) <1.40 Positive, index (S/C) ≥1.40	CCP donors, 146 Prepandemic, 498
Roche Diagnostics, Indianapolis, IN	Elecsys anti-SARS-CoV-2[b]	Nucleocapsid protein	Roche cobas c 422 analyzer (ECLIA)	Nonreactive, index <1.0 Reactive, index ≥1.0	CCP donors, 214 Prepandemic, 498

[a]OD-n, normalized optical density; S/C, signal/cutoff.
[b]This assay had received emergency use authorization by the US Food and Drug Administration prior to 19 October 2020.
[c]This ImmunoDiagnostics kit is marked for research use only (RUO) and recommends each lab create its own cutoff values for qualitative interpretation.
[d]This study utilized the Abbott Architect i1000sr platform.

and Washington, DC, areas from April 2020 to July 2020 ($n = 214$) (16). Individuals were eligible for enrollment if they had a documented history of a positive molecular assay test result for SARS-CoV-2 infection (confirmed by medical chart review or shared clinical documentation) and met standard self-reported eligibility criteria for blood donation. To test the clinical specificity of SARS-CoV-2 EIAs, we included stored serum specimens from an identity-unlinked HIV serosurvey conducted in 2016 among adult patients attending the Johns Hopkins Hospital Emergency Department ($n = 1,099$). Prepandemic specimens were excess (i.e., discarded) serum samples from patients who had blood drawn for clinical purposes in the emergency department (20). Both parent studies were cross-sectional, and no individual contributed multiple specimens. All plasma/serum samples were stored at −80°C until assays were performed.

SARS-CoV-2 EIAs. Plasma/serum specimens were analyzed using five commercially available EIAs: the Euroimmun anti-SARS-CoV-2 ELISA, the Epitope Diagnostics, Inc. (EDI), Novel Coronavirus COVID-19 IgG ELISA kit, the ImmunoDiagnostics SARS-CoV-2 NP IgG ELISA kit, the Abbott-Architect SARS-CoV-2 IgG chemiluminescent microparticle immunoassay (CMIA), and the Roche Diagnostics Elecsys anti-SARS-CoV-2 E-CLIA (Table 1). These EIAs were selected because data on their performance characteristics in diverse samples are limited, because of the feasibility (with respect to the supply chain) of obtaining the assay kits, and because of the availability of the necessary equipment (e.g., platform). In addition to the inclusion of EIAs that have received an EUA by the FDA for the qualitative detection of SARS-CoV-2 antibodies (Euroimmun, Abbott, Roche), EIAs that have not received an EUA were included as they may still be used for research purposes or may be approved later by the FDA (EDI and ImmunoDiagnostics) (21). The target antigen for each EIA is the nucleocapsid protein, with the exception of the Euroimmun assay, for which the target antigen is the S1 protein. The Roche assay measures total antibodies to SARS-CoV-2, whereas the others measure only anti-SARS-CoV-2 IgG. Specimens were tested by each EIA based on sample volume availability and assay kit availability at the time of testing. All EIAs were purchased from the manufacturer.

EIAs were conducted according to the manufacturers' instructions, unless specified otherwise. The intended use of each EIA per the manufacturers' instructions is the qualitative detection of antibodies; however, each EIA provides continuous output normalized by a calibrator. Indeed, interpretation of the continuous output as a semiquantitative measure is in contradiction with some of the manufacturers' instructions (i.e., Roche). For simplicity, we refer to the normalized continuous output of each EIA as a "ratio" value. The manufacturers' cutoff values to indicate positive, indeterminate/borderline, or negative serostatus for SARS-CoV-2 antibodies are listed in Table 1.

Microneutralization assay. Plasma nAb titers were quantified against 100 50% tissue culture infectious doses ($TCID_{50}$) using a microneutralization (NT) assay in VeroE6-TMPRSS2 cells, which has been previously described (16, 22). In brief, 2-fold dilutions of plasma were made starting at a 1:20 dilution. Infectious virus was added to the plasma dilutions at a final concentration of 1×10^4 $TCID_{50}$/ml. After a 1-h incubation at room temperature (19°C), 100 μl of each sample dilution was added to 6 wells in a 96-well plate of VeroE6-TMPRSS2 cells (23) and incubated for 6 h at 37°C. The inocula were removed from the plate, fresh medium was added, and the plate was incubated at 37°C for 48 h. The cells were fixed with 4% formaldehyde (in each well) (Fisher Chemical), incubated for 4 h at room temperature (19°C), and stained with Napthol Blue Black (Sigma-Aldrich). We calculated a nAb titer area under the curve (AUC) value for each sample using the exact number of wells protected from infection at every dilution. Samples with no neutralizing activity were assigned a value of one-half the lowest measured AUC.

Statistical analysis. The diagnostic accuracy of each EIA to detect IgG or total antibodies to SARS-CoV-2 was examined using CCP donor specimens as the reference positive standard and prepandemic specimens as the reference negative standard. For each EIA, nonparametric, empirical receiver

TABLE 2 Diagnostic accuracy of various enzyme immunoassays to detect IgG or total antibodies to SARS-CoV-2[a]

	Empirical analysis		Manufacturer's cutoff analysis				
				Sensitivity		Specificity	
Serologic assay	*n*	AUROC (95% CI)	AUROC (95% CI)	*n/N*	% (95% CI)	*n/N*	% (95% CI)
Euroimmun[b]	707	0.97 (0.96–0.98)	0.92 (0.90–0.94)	127/146	87.0 (80.4–92.0)	547/561	97.5 (95.8–98.6)
EDI[b]	724	0.89 (0.87–0.91)	0.83 (0.80–0.86)	115/146	78.8 (71.2–85.1)	503/578	87.0 (84.0–89.7)
ImmunoDiagnostics[b]	446	0.96 (0.93–0.97)	0.88 (0.84–0.91)	107/140	76.4 (68.5–83.2)	302/306	98.7 (96.7–99.6)
Abbott	644	0.98 (0.96–0.99)	0.96 (0.94–0.97)	135/146	92.5 (86.9–96.2)	496/498	99.6 (98.6–100.0)
Roche	712	0.97 (0.96–0.98)	0.97 (0.95–0.98)	201/214	93.9 (89.8–96.7)	496/498	99.6 (98.6–100.0)

[a]Exact binomial (Clopper-Pearson) 95% confidence intervals are shown for all estimates.
[b]Borderline/indeterminate specimens were considered negative in the manufacturer's cutoff analysis per manufacturers' cutoff values.

operating curve (ROC) analysis was performed to calculate the area under the receiver operating curve (AUROC). This analysis was also done using the manufacturers' cutoff values. Percent sensitivity was calculated as 100 × [positive/(positive + false negative)]. Percent specificity was calculated as 100 × [negative/(negative + false positive)]. For primary analyses, indeterminate/borderline results, based on the manufacturers' cutoff values, were considered to be seronegative (i.e., for Euroimmun, EDI, and ImmunoDiagnostics). The primary analyses used an available-case approach. Three separate sensitivity analyses were conducted: (i) we performed head-to-head comparisons where allowed; (ii) we considered indeterminate/borderline specimens to represent positive results; (iii) we excluded indeterminate/borderline specimens from the analysis. A subgroup analysis by HIV status was conducted for assay specificity. Exact binomial (Clopper-Pearson) 95% confidence intervals (CI) were calculated for sensitivity, specificity, and AUROC estimates.

The remaining analyses were conducted in CCP donors that had data available for all five EIAs and nAb titers ($n = 140$). The correlations of EIA ratios and nAb AUC values were examined using Spearman's correlation coefficients (ρ) with 95% CIs estimated over 1,000 bootstrap iterations. We additionally evaluated the following four binary cutoff values of the nAb AUC value to indicate "high" titers of nAbs: ≥20, ≥40, ≥80, and ≥160. For each nAb AUC threshold, we evaluated the performance of each EIA to discriminate between low and high nAb titers using empirical ROC analysis.

Under the current EUA for CCP therapy, all CCP donors are required to be identified as antibody positive for SARS-CoV-2. Thus, in the CCP donor population, we also calculated the positive percent agreement and negative percent agreement between each binary nAb threshold (reference) and each EIA using the manufacturer's cutoff values originally recommended for SARS-CoV-2 serostatus. For this analysis, indeterminate/borderline specimens were considered seronegative.

Statistical analyses were performed using Stata/MP, version 15.2 (StataCorp, College Station, TX), and R statistical software.

RESULTS

Specimen characteristics. Of the 214 specimens from potential CCP donors, 146 were tested by the Euroimmun, EDI, and Abbott assays; 140 were tested by the ImmunoDiagnostics assay; and all 214 were tested by the Roche assay (140 were assayed by all five EIAs). Of the 1,099 prepandemic control specimens included, 561 were tested by the Euroimmun assay, 578 were tested by the EDI assay, 306 were tested by the ImmunoDiagnostics assay, and 498 were tested by the Abbott and Roche assays. Due to lack of sample availability, there was no prepandemic sample tested by all five EIAs. Demographic information on the included specimens is shown in Table S1 in the supplemental material. Among CCP donors ($n = 214$), there was a median of 44 days from diagnosis until sample collection (interquartile range [IQR] = 38 to 50 days). Although all included CCP donors were symptomatic at the time of SARS-CoV-2 infection, less than 10% had a history of a COVID-19 hospitalization. Among prepandemic specimens, HIV seroprevalence was less than 7%.

Diagnostic accuracy of EIAs to detect antibodies to SARS-CoV-2. In empirical ROC analyses, all assays—with the exception of EDI—had an AUROC value that exceeded 0.95, suggesting that each assay has the capacity to accurately detect antibodies to SARS-CoV-2 (Table 2). For the ELISAs (Euroimmun, EDI, and ImmunoDiagnostics), the AUROCs were greater by ≥5 absolute percentage points in the empirical ROC analysis than in the analysis using the manufacturers' cutoff values. For the Abbott and Roche assays, the AUROCs were similar in the empirical analysis and the analysis using the manufacturers' cutoff values.

Using the manufacturers' cutoff values, the sensitivity of each EIA to detect SARS-CoV-2 antibodies ranged from 76.4% to 93.9%, whereas the specificity of each EIA

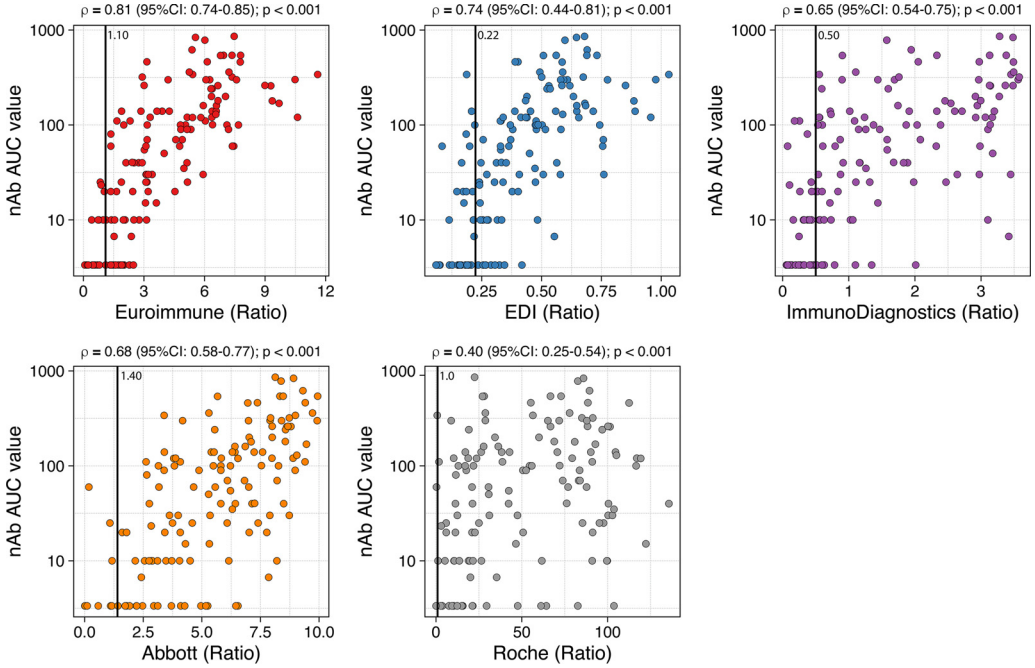
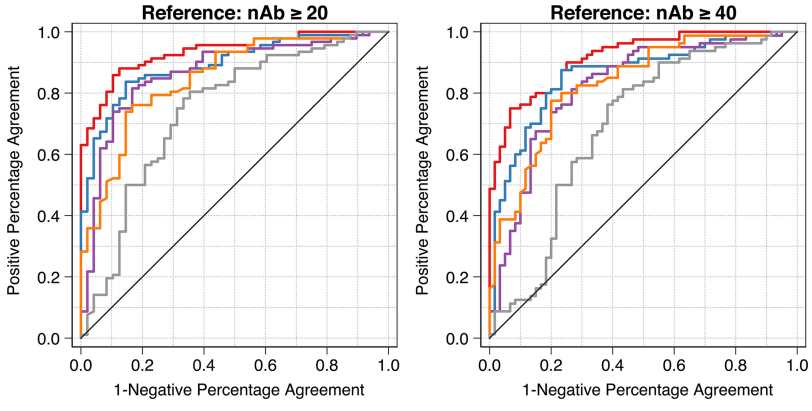


FIG 1 Correlations between SARS-CoV-2 enzyme immunoassay ratio results and neutralizing antibody titer AUC values in COVID-19 convalescent individuals ($n = 140$). Spearman correlation coefficients (ρ) were calculated with 95% confidence intervals (CI) estimated over 1,000 bootstrap iterations. The straight vertical black line indicates the cutoff for SARS-CoV-2 seropositivity.

ranged from 87.0% to 99.6%. The Abbott and Roche assays had comparable characteristics, with higher point estimates for sensitivity and specificity than the ELISAs. Considering indeterminate/borderline specimens to be seropositive rather than seronegative decreased the specificity of the ELISAs, but particularly that of the EDI (Table S2). Excluding indeterminate/borderline specimens slightly increased the sensitivity of the ELISAs (Table S2). We also performed direct head-to-head comparisons and obtained similar comparative inferences (Tables S3 and S4). Among the 140 CCP donor specimens that were tested by all five EIAs, there were 6 (4.3%) specimens that were seronegative (or indeterminate/borderline) for SARS-CoV-2 by all five EIAs. The median time from COVID-19 diagnosis for these 6 individuals was 46 days (range, 33 to 54). Interestingly, there were 2 false-positive specimens among the 498 prepandemic specimens tested by both the Abbott and Roche assays (one of which gave a false-positive result with both assays). Neither of the false-positive specimens analyzed by the Abbott or Roche assays was HIV positive (Table S5).

Among all prepandemic samples, there was greater variation in the distribution of ratio values for ELISAs than for the Abbott and Roche assays (see Fig. S1 in the supplemental material), consistent with the higher specificity observed for the Abbott and Roche assays. For the Abbott, Roche, and ImmunoDiagnostics assays, the value corresponding to three times the standard deviation above the mean value from all the prepandemic samples was below the cutoff used to define a positive sample.

Performance of EIAs to detect high SARS-CoV-2 neutralizing antibody titers. Among the 140 CCP donor specimens, the median nAb AUC value was 60 (interquartile range, 10 to 150). The prevalence of nAb AUC values of ≥20 was 65.7% ($n = 92$), the prevalence of nAb AUC values of ≥40 was 57.1% ($n = 80$), the prevalence of nAb AUC values of ≥80 was 45.7% ($n = 64$), and the prevalence of nAb AUC values of ≥160 was 25.0% ($n = 35$). There were significant positive correlations between nAb AUC values and EIA ratio values for all EIAs examined (Fig. 1), but the strongest correlation was observed for the Euroimmun assay ($\rho = 0.81$ [95% CI, 0.74 to 0.85]) and weakest correlation was observed for the Roche assay ($\rho = 0.40$ [95% CI, 0.25 to 0.54]).



Serologic Assay	AUROC (95%CI)			
	nAb ≥ 20	nAb ≥ 40	nAb ≥ 80	nAb ≥ 160
Euroimmun	0.93 (0.88-0.97)	0.92 (0.86-0.95)	0.90 (0.85-0.95)	0.90 (0.84-0.94)
EDI	0.89 (0.83-0.94)	0.86 (0.79-0.91)	0.88 (0.81-0.93)	0.87 (0.80-0.92)
ImmunoDiagnostics	0.87 (0.80-0.93)	0.82 (0.75-0.88)	0.79 (0.72-0.86)	0.83 (0.76-0.89)
Abbott	0.85 (0.78-0.90)	0.83 (0.76-0.89)	0.81 (0.74-0.87)	0.87 (0.80-0.92)
Roche	0.74 (0.66-0.81)	0.69 (0.61-0.77)	0.66 (0.57-0.74)	0.66 (0.58-0.74)

FIG 2 Empirical receiver operating curve analysis for various SARS-CoV-2 enzyme immunoassays to detect high neutralizing antibody (nAb) titers at various thresholds ($n = 140$). Four thresholds for a high nAb AUC value were examined as the reference positive test.

With high nAb titers as the reference positive, there was substantial between-assay variability in the empirical AUROCs for each EIA, but changing the threshold used to define a high nAb titer did not substantially impact the AUROCs of a given EIA (Fig. 2). For instance, for all four nAb thresholds evaluated, all empirical AUROC point estimates for the Euroimmun assay were ≥90, whereas all AUROC point estimates for the Roche assay were <0.75. For the Euroimmun assay and nAb test at a threshold of ≥160, the EIA ratio cutoff with the highest overall percent agreement (86%) was 6.0 (positive percent agreement was 77% and negative percent agreement was 89%).

Table 3 shows the positive percent agreement (sensitivity) and negative percent agreement (specificity) of each assay with the four nAb test thresholds by the use of the EIA manufacturers' cutoff values for seropositivity. All EIAs had positive percent agreement with high nAbs exceeding 90%, regardless of the threshold for high nAb titers. However, there was poor negative percent agreement between each EIA and high nAb titers. For all EIAs, the negative percent agreement decreased with increasing threshold for high nAb titers.

DISCUSSION

There was substantial variability in the performance characteristics of five commercially available EIAs for the detection of antibodies to SARS-CoV-2 and the ability to discriminate between low and high nAb titers in COVID-19 convalescent individuals. The Roche and Abbott assays had high diagnostic accuracy for the detection of

TABLE 3 Concordance between manufacturer enzyme immunoassay cutoff values for SARS-CoV-2 seropositivity and high nAb titers at various thresholds ($n = 140$)[a]

Serologic assay	Positive percent agreement, no. (%)				Negative percent agreement, no. (%)			
	nAb ≥20 ($n = 92$)	nAb ≥40 ($n = 80$)	nAb ≥80 ($n = 64$)	nAb ≥160 ($n = 35$)	nAb <20 ($n = 48$)	nAb <40 ($n = 60$)	nAb <80 ($n = 76$)	nAb <160 ($n = 105$)
Euroimmun	90 (97.8)	80 (100)	64 (100)	35 (100)	16 (33.3)	18 (30.0)	18 (23.7)	18 (17.1)
EDI	86 (93.5)	74 (92.5)	61 (95.3)	34 (97.1)	25 (52.1)	25 (41.7)	28 (36.8)	30 (28.6)
ImmunoDiagnostics	86 (93.5)	76 (95.0)	61 (95.3)	35 (100)	27 (56.3)	29 (48.3)	30 (39.5)	33 (31.4)
Abbott	90 (97.8)	79 (98.8)	64 (100)	35 (100)	9 (18.8)	10 (16.7)	11 (14.5)	11 (10.5)
Roche	90 (98.4)	78 (97.5)	63 (98.4)	34 (97.4)	6 (12.5)	6 (10.0)	7 (9.2)	7 (6.7)

[a]Four thresholds for a high neutralizing antibody (nAb) AUC value were examined as the reference positive test. Borderline/indeterminate specimens were considered negative for the EIAs per manufacturers' cutoff values.

antibodies against SARS-CoV-2. However, the Roche assay ratios weakly correlated with nAb titers and poorly identified convalescent individuals with high nAb titers. In contrast, the Euroimmun assay ratios had the highest correlations with nAb titers and high discriminative capacity for detecting high nAbs. This variability in assay performance should be considered when selecting an EIA to detect antibodies against SARS-CoV-2 and/or discriminate between high and low nAb titers.

We observed comparable performances of the Abbott and Roche assays to detect IgG or total antibodies against SARS-CoV-2. Although we found similar sensitivity estimates for the Roche and Abbott assays at the manufacturer's cutoff (94% versus 93%), a recent head-to-head comparison found that the Roche assay had slightly better sensitivity than the Abbott assay (97% versus 93%) (24). However, consistent with several previous studies, both the Abbott and Roche assays had high specificity using the manufacturers' cutoff values (>99%) (24–27). In this study, the Abbott and Roche assays appeared to have better specificity than the ELISAs evaluated, including the Euroimmun and EDI assays, a result which had also been previously observed (28, 29). Although the use of "challenge" specimens for examination of potential cross-reactivity of antibodies to other pathogens was not included in this study, prepandemic specimens from patients in an inner-city emergency department who required clinical laboratory testing were evaluated; thus, several samples were from patients likely seeking care for viral respiratory illnesses. Previous studies have shown limited evidence of cross-reactivity with other pathogens for the Euroimmun, EDI, Roche, and Abbott assays (26, 27, 30–35). There are no comparable data for the ImmunoDiagnostics assay.

Similarly to prior reports, we observed differing degrees of positive correlations between commercial EIA ratios and nAb titers (19, 36, 37). It is unsurprising that the Euroimmun ratios correlated best with nAb titers since that assay detects S1-specific antibodies—a subset of which are responsible for virus neutralization—whereas the other assays that we assessed detect N-specific antibodies, which lack virus neutralization activity. Accordingly, our empirical ROC analysis also indicates that the Euroimmun assay may have better performance in discriminating high nAb titers than the Abbott and Roche assays. It should be noted that our analysis used CCP donors whose samples had had prior molecular confirmation for SARS-CoV-2 infection as the denominator in assessing the discriminative capacity of various EIAs for high SARS-COV-2 nAb titers. Evaluations of implementation of an EIA such as the Euroimmun assay to determine SARS-CoV-2 serostatus and to predict high titers should include the consideration that the assay has an inherent false-positive rate of 2.5% for the detection of S1-specific antibodies (i.e., determining serostatus). Depending on the purpose, it may be advantageous to use a highly specific EIA to qualitatively determine SARS-CoV-2 serostatus and to reflex to a different EIA to predict high nAbs among seropositive individuals.

Interestingly, using the manufacturer's cutoff, Jääskeläinen et al. found that the Abbott assay had greater positive and negative percent agreement with nAb activity than the Euroimmun assay (38). In our study, the Abbott assay was also better able to discriminate high nAbs than the Roche assay, which is in contrast to a study by Tang et al. that showed similar performances of the Abbott and Roche assays (39). However,

similarly to the results reported by Tang et al., we found that applying the manufacturer's cutoff values for the commercial EIAs (including Euroimmun) led to suboptimal negative percent agreement with high nAb thresholds. Indeed, in a real-world setting—the investigation of a fishery vessel outbreak that had a high SARS-CoV-2 attack rate—the detection of anti-N SARS-CoV-2 antibodies by the Abbott assay had poor positive predictive value for detecting nAbs (50%) (40). Taking the data together, the discriminative capacity of commercial SARS-CoV-2 EIAs to detect high nAbs requires further investigation. Large comparative studies are needed to determine the optimal EIA and cutoff to discriminate nAb levels in convalescent individuals; such studies should consider evaluating the Ortho Vitros anti-SARS-CoV-2 IgG EIA as well as other promising EIAs that were not included in this evaluation (41).

This study had limitations. First, the data were cross-sectional, so we did not capture the influence of longitudinal antibody dynamics on diagnostic accuracy. Second, several types of specimens were not included in the evaluation, such as samples from early in SARS-CoV-2 infection (<14 days after symptom onset), samples from SARS-CoV-2-infected asymptomatic individuals, and samples from convalescent individuals who were infected >6 months ago—all of which could potentially influence assay sensitivity. Third, the samples evaluated were primarily from the Baltimore-Washington, DC, region, and results may not be generalizable elsewhere. Finally, this study included a limited number of EIAs and did not include the Ortho Vitros anti-SARS-CoV-2 IgG EIA, which is the only EIA that is currently included in the EUA for determining low and high titers for convalescent plasma donation in the United States.

Implementation of the appropriate EIAs to detect SARS-CoV-2 antibodies will require careful consideration of the inferential purpose (e.g., individual-level versus population-level inference), context (e.g., prevalence in the target population), and operational feasibility (e.g., high-throughput platform versus manual ELISA, supply chain availability, etc.) and of the underlying test performance characteristics of the assays. Although the output ratio results for some commercially available EIAs correlate with nAb titers, EIA ratios should not be universally considered a surrogate for nAb titers. This is particularly relevant for programs that are currently scaling CCP therapy per new FDA guidelines (https://www.fda.gov/media/141477/download). In combination with other predictors, ratios from some commercial EIAs may help inform models designed to predict high nAb titers. These prediction models could prove useful in the identification of optimal CCP donors in the absence of accurate and reliable high-throughput tests for nAb titers.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.5 MB.

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REFERENCES

1. Dong E, Du H, Gardner L. 2020. An interactive Web-based dashboard to track COVID-19 in real time. Lancet Infect Dis 20:533–534. https://doi.org/10.1016/S1473-3099(20)30120-1.
2. Theel ES, Slev P, Wheeler S, Couturier MR, Wong SJ, Kadkhoda K. 2020. The role of antibody testing for SARS-CoV-2: is there one? J Clin Microbiol 58:e00797-20. https://doi.org/10.1128/JCM.00797-20.
3. Theel ES, Couturier MR, Filkins L, Palavecino E, Mitchell S, Campbell S, Pentella M, Butler-Wu S, Jerke K, Dharmarha V, McNult P, Schuetz AN, American Society for Microbiology Clinical and Public Health Microbiology Committee, Subcommittee on Laboratory Practices. 5 October 2020, posting date. Application, verification and implementation of SARS-CoV-2 serologic assays with emergency use authorization. J Clin Microbiol https://doi.org/10.1128/JCM.02148-20.
4. Hanson KE, Caliendo AM, Arias CA, Englund JA, Hayden MK, Lee MJ, Loeb M, Patel R, Altayar O, El Alayli A, Sultan S, Falck-Ytter Y, Lavergne V, Morgan RL, Murad MH, Bhimraj A, Mustafa R1. 12 September 2020, posting date. Infectious Diseases Society of America guidelines on the diagnosis of COVID-19: serologic testing. Clin Infect Dis https://doi.org/10.1093/cid/ciaa1343.
5. Lisboa Bastos M, Tavaziva G, Abidi SK, Campbell JR, Haraoui L-P, Johnston JC, Lan Z, Law S, MacLean E, Trajman A, Menzies D, Benedetti A, Ahmad Khan F. 2020. Diagnostic accuracy of serological tests for covid-19: systematic review and meta-analysis. BMJ 370:m2516. https://doi.org/10.1136/bmj.m2516.
6. Deeks JJ, Dinnes J, Takwoingi Y, Davenport C, Spijker R, Taylor-Phillips S, Adriano A, Beese S, Dretzke J, Ferrante di Ruffano L, Harris IM, Price MJ, Dittrich S, Emperador D, Hooft L, Leeflang MM, Van den Bruel A, Cochrane COVID-19 Diagnostic Test Accuracy Group. 2020. Antibody tests for identification of current and past infection with SARS-CoV-2. Cochrane Database Syst Rev 6:CD013652. https://doi.org/10.1002/14651858.CD013652.
7. Caini S, Bellerba F, Corso F, Diaz-Basabe A, Natoli G, Paget J, Facciotti F, Angelis Pietro DS, Raimondi S, Palli D, Mazzarella L, Pelicci PG, Vineis P, Gandini S. 2020. Meta-analysis of diagnostic performance of serological tests for SARS-CoV-2 antibodies up to 25 April 2020 and public health implications. Euro Surveill 25:2000980. https://doi.org/10.2807/1560-7917.ES.2020.25.23.2000980.
8. Wang H, Ai J, Loeffelholz MJ, Tang Y-W, Zhang W. 2020. Meta-analysis of diagnostic performance of serology tests for COVID-19: impact of assay design and post-symptom-onset intervals. Emerg Microbes Infect 9:2200–2211. https://doi.org/10.1080/22221751.2020.1826362.
9. Bloch EM, Shoham S, Casadevall A, Sachais BS, Shaz B, Winters JL, van Buskirk C, Grossman BJ, Joyner M, Henderson JP, Pekosz A, Lau B, Wesolowski A, Katz L, Shan H, Auwaerter PG, Thomas D, Sullivan DJ, Paneth N, Gehrie E, Spitalnik S, Hod EA, Pollack L, Nicholson WT, Pirofski LA, Bailey JA, Tobian AA. 2020. Deployment of convalescent plasma for the prevention and treatment of COVID-19. J Clin Invest 130:2757–2765. https://doi.org/10.1172/JCI138745.
10. Bloch EM, Goel R, Montemayor C, Cohn C, Tobian AAR. 20 September 2020, posting date. Promoting access to COVID-19 convalescent plasma in low- and middle-income countries. Transfus Apher Sci https://doi.org/10.1016/j.transci.2020.102957.
11. Joyner MJ, Wright RS, Fairweather D, Senefeld JW, Bruno KA, Klassen SA, Carter RE, Klompas AM, Wiggins CC, Shepherd JR, Rea RF, Whelan ER, Clayburn AJ, Spiegel MR, Johnson PW, Lesser ER, Baker SE, Larson KF, Ripoll JG, Andersen KJ, Hodge DO, Kunze KL, Buras MR, Vogt MN, Herasevich V, Dennis JJ, Regimbal RJ, Bauer PR, Blair JE, Van Buskirk CM, Winters JL, Stubbs JR, Paneth NS, Verdun NC, Marks P, Casadevall A. 2020. Early safety indicators of COVID-19 convalescent plasma in 5,000 patients. J Clin Invest 130:4791–4797. https://doi.org/10.1172/JCI140200.
12. Joyner MJ, Bruno KA, Klassen SA, Kunze KL, Johnson PW, Lesser ER, Wiggins CC, Senefeld JW, Klompas AM, Hodge DO, Shepherd JRA, Rea RF, Whelan ER, Clayburn AJ, Spiegel MR, Baker SE, Larson KF, Ripoll JG, Andersen KJ, Buras MR, Vogt MNP, Herasevich V, Dennis JJ, Regimbal RJ, Bauer PR, Blair JE, van Buskirk CM, Winters JL, Stubbs JR, van Helmond N, Butterfield BP, Sexton MA, Diaz Soto JC, Paneth NS, Verdun NC, Marks P, Casadevall A, Fairweather D, Carter RE, Wright RS. 2020. Safety update: COVID-19 convalescent plasma in 20,000 hospitalized patients. Mayo Clin Proc 95:1888–1897. https://doi.org/10.1016/j.mayocp.2020.06.028.
13. Klassen SA, Senefeld J, Johnson PW, Carter RE, Wiggins CC, Shoham S, Grossman BJ, Henderson JP, Musser JM, Salazar E, Hartman WR, Bouvier NM, Liu STH, Pirofski L, Baker SE, van Helmond N, Wright RS, Fairweather D, Bruno KA, Wang Z, Paneth NS, Casadevall A, Joyner MJ. 2020. Evidence favoring the efficacy of convalescent plasma for COVID-19 therapy. medRxiv https://doi.org/10.1101/2020.07.29.20162917.
14. Joyner MJ, Senefeld JW, Klassen SA, Mills JR, Johnson PW, Theel ES, Wiggins CC, Bruno KA, Klompas AM, Lesser ER, Kunze KL, Sexton MA, Diaz Soto JC, Baker SE, Shepherd JRA, van Helmond N, van Buskirk CM, Winters JL, Stubbs JR, Rea RF, Hodge DO, Herasevich V, Whelan ER, Clayburn AJ, Larson KF, Ripoll JG, Andersen KJ, Buras MR, Vogt MNP, Dennis JJ, Regimbal RJ, Bauer PR, Blair JE, Paneth NS, Fairweather D, Wright RS, Carter RE, Casadevall A. 12 Aug 2020. Effect of convalescent plasma on mortality among hospitalized patients with COVID-19: initial three-month experience. medRxiv https://doi.org/10.1101/2020.08.12.20169359.
15. Tobian AAR, Shaz BH. 2020. Earlier the better: convalescent plasma. Blood 136:652–654. https://doi.org/10.1182/blood.2020007638.
16. Klein SL, Pekosz A, Park HS, Ursin RL, Shapiro JR, Benner SE, Littlefield K, Kumar S, Naik HM, Betenbaugh MJ, Shrestha R, Wu AA, Hughes RM, Burgess I, Caturegli P, Laeyendecker O, Quinn TC, Sullivan D, Shoham S, Redd AD, Bloch EM, Casadevall A, Tobian AA. 19 October 2020, posting date. Sex, age, and hospitalization drive antibody responses in a COVID-19 convalescent plasma donor population. J Clin Invest https://doi.org/10.1172/JCI142004.
17. Meschi S, Colavita F, Bordi L, Matusali G, Lapa D, Amendola A, Vairo F, Ippolito G, Capobianchi MR, Castilletti C, INMICovid-19 laboratory team. 2020. Performance evaluation of Abbott ARCHITECT SARS-CoV-2 IgG immunoassay in comparison with indirect immunofluorescence and virus microneutralization test. J Clin Virol 129:104539. https://doi.org/10.1016/j.jcv.2020.104539.
18. Ng DL, Goldgof GM, Shy BR, Levine AG, Balcerek J, Bapat SP, Prostko J, Rodgers M, Coller K, Pearce S, Franz S, Du L, Stone M, Pillai SK, Sotomayor-Gonzalez A, Servellita V, Martin CSS, Granados A, Glasner DR, Han LM, Truong K, Akagi N, Nguyen DN, Neumann NM, Qazi D, Hsu E, Gu W, Santos YA, Custer B, Green V, Williamson P, Hills NK, Lu CM, Whitman JD, Stramer SL, Wang C, Reyes K, Hakim JMC, Sujishi K, Alazzeh F, Pham L, Thornborrow E, Oon C-Y, Miller S, Kurtz T, Simmons G, Hackett JJ, Busch MP, Chiu CY. 2020. SARS-CoV-2 seroprevalence and neutralizing activity in donor and patient blood. Nat Commun 11:4698. https://doi.org/10.1038/s41467-020-18468-8.
19. Luchsinger LL, Ransegnola B, Jin D, Muecksch F, Weisblum Y, Bao W, George PJ, Rodriguez M, Tricoche N, Schmidt F, Gao C, Jawahar S, Pal M, Schnall E, Zhang H, Strauss D, Yazdanbakhsh K, Hillyer CD, Bieniasz PD, Hatziioannou T. 11 September 2020, posting date. Serological assays estimate highly variable SARS-CoV-2 neutralizing antibody activity in recovered COVID19 patients. J Clin Microbiol https://doi.org/10.1128/JCM.02005-20.
20. Kelen GD, Hsieh Y-H, Rothman RE, Patel EU, Laeyendecker OB, Marzinke MA, Clarke W, Parsons T, Manucci JL, Quinn TC. 2016. Improvements in the continuum of HIV care in an inner-city emergency department. AIDS 30:113–120. https://doi.org/10.1097/QAD.0000000000000896.
21. Benner SE, Patel EU, Laeyendecker O, Pekosz A, Littlefield K, Eby Y, Fernandez RE, Miller J, Kirby CS, Keruly M, Klock E, Baker OR, Schmidt HA, Shrestha R, Burgess I, Bonny TS, Clarke W, Caturegli P, Sullivan D, Shoham S, Quinn TC, Bloch EM, Casadevall A, Tobian AAR, Redd AD. 10 September 2020, posting date. SARS-CoV-2 antibody avidity responses in COVID-19 patients and convalescent plasma donors. J Infect Dis https://doi.org/10.1093/infdis/jiaa581.
22. Schaecher SR, Stabenow J, Oberle C, Schriewer J, Buller RM, Sagartz JE, Pekosz A. 2008. An immunosuppressed Syrian golden hamster model for SARS-CoV infection. Virology 380:312–321. https://doi.org/10.1016/j.virol.2008.07.026.
23. Matsuyama S, Nao N, Shirato K, Kawase M, Saito S, Takayama I, Nagata N, Sekizuka T, Katoh H, Kato F, Sakata M, Tahara M, Kutsuna S, Ohmagari N, Kuroda M, Suzuki T, Kageyama T, Takeda M. 2020. Enhanced isolation of SARS-CoV-2 by TMPRSS2-expressing cells. Proc Natl Acad Sci U S A 117:7001–7003. https://doi.org/10.1073/pnas.2002589117.
24. National SARS-CoV-2 Serology Assay Evaluation Group. 2020. Performance characteristics of five immunoassays for SARS-CoV-2: a head-to-head benchmark comparison. Lancet Infect Dis https://doi.org/10.1016/S1473-3099(20)30634-4.
25. Bryan A, Pepper G, Wener MH, Fink SL, Morishima C, Chaudhary A, Jerome KR, Mathias PC, Greninger AL. 2020. Performance characteristics of the Abbott Architect SARS-CoV-2 IgG assay and seroprevalence in

Boise, Idaho. J Clin Microbiol 58:e00941-20. https://doi.org/10.1128/JCM.00941-20.
26. Perkmann T, Perkmann-Nagele N, Breyer M-K, Breyer-Kohansal R, Burghuber OC, Hartl S, Aletaha D, Sieghart D, Quehenberger P, Marculescu R, Mucher P, Strassl R, Wagner OF, Binder CJ, Haslacher H. 10 August 2020, posting date. Side by side comparison of three fully automated SARS-CoV-2 antibody assays with a focus on specificity. Clin Chem https://doi.org/10.1093/clinchem/hvaa198.
27. Muench P, Jochum S, Wenderoth V, Ofenloch-Haehnle B, Hombach M, Strobl M, Sadlowski H, Sachse C, Torriani G, Eckerle I, Riedel A. 2020. Development and validation of the Elecsys anti-SARS-CoV-2 immunoassay as a highly specific tool for determining past exposure to SARS-CoV-2. J Clin Microbiol 58:e01694-20. https://doi.org/10.1128/JCM.01694-20.
28. Theel ES, Harring J, Hilgart H, Granger D. 2020. Performance characteristics of four high-throughput immunoassays for detection of IgG antibodies against SARS-CoV-2. J Clin Microbiol 58:e01243-20. https://doi.org/10.1128/JCM.01243-20.
29. Manthei DM, Whalen JF, Schroeder LF, Sinay AM, Li S-H, Valdez R, Giacherio DA, Gherasim C. 9 October 2020, posting date. 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 https://doi.org/10.1093/ajcp/aqaa200.
30. Lau CS, Hoo SP, Yew SF, Ong SK, Lum LT, Heng PY, Tan JG, Wong MS, Aw T2. 5 July 2020, posting date. Evaluation of an electrochemiluminescent SARS-CoV-2 antibody assay. J Appl Lab Med https://doi.org/10.1093/jalm/jfaa134.
31. Merrill AE, Jackson JB, Ehlers A, Voss D, Krasowski MD. 27 July 2020, posting date. Head-to-head comparison of two SARS-CoV-2 serology assays. J Appl Lab Med https://doi.org/10.1093/jalm/jfaa125.
32. Charlton CL, Kanji JN, Johal K, Bailey A, Plitt SS, MacDonald C, Kunst A, Buss E, Burnes LE, Fonseca K, Berenger BM, Schnabl K, Hu J, Stokes W, Zelyas N, Tipples G. 2020. Evaluation of six commercial mid- to high-volume antibody and six point-of-care lateral flow assays for detection of SARS-CoV-2 antibodies. J Clin Microbiol 58:e01361-20. https://doi.org/10.1128/JCM.01361-20.
33. Whitman JD, Hiatt J, Mowery CT, Shy BR, Yu R, Yamamoto TN, Rathore U, Goldgof GM, Whitty C, Woo JM, Gallman AE, Miller TE, Levine AG, Nguyen DN, Bapat SP, Balcerek J, Bylsma SA, Lyons AM, Li S, Wong AW-Y, Gillis-Buck EM, Steinhart ZB, Lee Y, Apathy R, Lipke MJ, Smith JA, Zheng T, Boothby IC, Isaza E, Chan J, Acenas DD, Lee J, Macrae TA, Kyaw TS, Wu D, Ng DL, Gu W, York VA, Eskandarian HA, Callaway PC, Warrier L, Moreno ME, Levan J, Torres L, Farrington LA, Loudermilk RP, Koshal K, Zorn KC, Garcia-Beltran WF, Yang D, et al. 2020. Evaluation of SARS-CoV-2 serology assays reveals a range of test performance. Nat Biotechnol 38:1174–1183. https://doi.org/10.1038/s41587-020-0659-0.
34. Brochot E, Demey B, Handala L, François C, Duverlie G, Castelain S. 2020. Comparison of different serological assays for SARS-CoV-2 in real life. J Clin Virol 130:104569. https://doi.org/10.1016/j.jcv.2020.104569.
35. Brecher SM, Dryjowicz-Burek J, Yu H, Campbell S, Ratcliffe N, Gupta K. 2020. Patients with common cold coronaviruses tested negative for IgG antibody to SARS-CoV-2. J Clin Microbiol 58:e01029-20. https://doi.org/10.1128/JCM.01029-20.
36. Weidner L, Gansdorfer S, Unterweger S, Weseslindtner L, Drexler C, Farcet M, Witt V, Schistal E, Schlenke P, Kreil TR, Jungbauer C. 2020. Quantification of SARS-CoV-2 antibodies with eight commercially available immunoassays. J Clin Virol 129:104540. https://doi.org/10.1016/j.jcv.2020.104540.
37. Gniadek TJ, Thiede JM, Matchett WE, Gress AR, Pape KA, Fiege JK, Jenkins MK, Menachery VD, Langlois RA, Bold TD. 16 September 2020, posting date. SARS-CoV-2 neutralization and serology testing of COVID-19 convalescent plasma from donors with nonsevere disease. Transfusion https://doi.org/10.1111/trf.16101.
38. Jääskeläinen AJ, Kuivanen S, Kekäläinen E, Ahava MJ, Loginov R, Kallio-Kokko H, Vapalahti O, Jarva H, Kurkela S, Lappalainen M. 2020. Performance of six SARS-CoV-2 immunoassays in comparison with microneutralisation. J Clin Virol 129:104512. https://doi.org/10.1016/j.jcv.2020.104512.
39. Tang MS, Case JB, Franks CE, Chen RE, Anderson NW, Henderson JP, Diamond MS, Gronowski AM, Farnsworth CW. 7 September 2020, posting date. Association between SARS-CoV-2 neutralizing antibodies and commercial serological assays. Clin Chem https://doi.org/10.1093/clinchem/hvaa211.
40. Addetia A, Crawford KHD, Dingens A, Zhu H, Roychoudhury P, Huang M-L, Jerome KR, Bloom JD, Greninger AL. 2020. Neutralizing antibodies correlate with protection from SARS-CoV-2 in humans during a fishery vessel outbreak with a high attack rate. J Clin Microbiol 58:e02107-20. https://doi.org/10.1128/JCM.02107-20.
41. Bonelli F, Sarasini A, Zierold C, Calleri M, Bonetti A, Vismara C, Blocki FA, Pallavicini L, Chinali A, Campisi D, Percivalle E, DiNapoli AP, Perno CF, Baldanti F. 2020. Clinical and analytical performance of an automated serological test that identifies S1/S2-neutralizing IgG in COVID-19 patients semiquantitatively. J Clin Microbiol 58:e01224-20. https://doi.org/10.1128/JCM.01224-20.