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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

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34 **ABSTRACT**

35

36 Accurate serological assays to detect antibodies to SARS-CoV-2 are needed to characterize the
37 epidemiology of SARS-CoV-2 infection and identify potential candidates for COVID-19
38 convalescent plasma (CCP) donation. This study compared the performance of commercial
39 enzyme immunoassays (EIAs) to detect IgG or total antibodies to SARS-CoV-2 and neutralizing
40 antibodies (nAb). The diagnostic accuracy of five commercially available EIAs (Abbott,
41 Euroimmun, EDI, ImmunoDiagnostics, and Roche) to detect IgG or total antibodies to SARS-
42 CoV-2 was evaluated from cross-sectional samples of potential CCP donors that had prior
43 molecular confirmation of SARS-CoV-2 infection for sensitivity (n=214) and pre-pandemic
44 emergency department patients for specificity (n=1,102). Of the 214 potential CCP donors, all
45 were sampled >14 days since symptom onset and only a minority had been hospitalized due to
46 COVID-19 (n=16 [7.5%]); 140 potential CCP donors were tested by all five EIAs and a
47 microneutralization assay. When performed according to the manufacturers' protocol to detect
48 IgG or total antibodies to SARS-CoV-2, the sensitivity of each EIA ranged from 76.4% to
49 93.9%, and the specificity of each EIA ranged from 87.0% to 99.6%. Using a nAb titer cutoff
50 of ≥160 as the reference positive test (n=140 CCP donors), the *empirical* area under receiver
51 operating curve of each EIA ranged from 0.66 (Roche) to 0.90 (Euroimmun). Commercial EIAs
52 with high diagnostic accuracy to detect SARS-CoV-2 antibodies did not necessarily have high
53 diagnostic accuracy to detect high nAbs. Some but not all commercial EIAs may be useful in the
54 identification of individuals with high nAbs in convalescent individuals.

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62 **INTRODUCTION**

63 Globally, as of August 2020, there have been over 23.5 million reported cases of Severe Acute
64 Respiratory Syndrome Associated Coronavirus 2 (SARS-CoV-2) infection, which causes
65 Coronavirus-19 (COVID-19) disease.[1] Surveillance based on case-reporting is informative but it
66 significantly underestimates the true burden of infection and can lead to biased epidemiological
67 inferences. Accurate and reliable serological assays to detect SARS-CoV-2 antibodies can be
68 used to better understand the epidemiology of SARS-CoV-2 infection at the population-level, as
69 the presence of antibodies to SARS-CoV-2 indicates recent or prior exposure to the virus.[2]
70 Serological assays can also be useful for screening blood donations, qualifying individuals for
71 convalescent plasma donation, monitoring immune responses to vaccine candidates, clinically
72 managing patients, and studying the natural history of infection.[2] It is still unknown whether the
73 presence of antibodies against SARS-CoV-2 confers immunity against reinfection with the virus,
74 or how long those antibodies persist following infection.

75 As of August 2020, 39 commercially available serological assays have received an individual
76 emergency use authorization (EUA) by the US Food and Drug Administration (FDA) for the
77 detection of antibodies to SARS-CoV-2.[3] These assays detect IgA, IgM, IgG or total antibodies
78 to the subunit 1 of the spike glycoprotein (S1), the spike glycoprotein receptor binding domain
79 (RBD), or the recombinant nucleocapsid protein (N) of the virus. The assays can also be
80 categorized, broadly, as (1) lateral flow immunoassays (LFAs); (2) enzyme-linked
81 immunosorbent assays (ELISAs); and (3) chemiluminescent immunoassays (CLIAs). ELISAs
82 and CLIAs (collectively known as enzyme immunoassays [EIAs]) often provide semiquantitative
83 output that can be interpreted as antibody titers, whereas current LFAs are strictly qualitative.
84 Recent systematic reviews of the literature have noted the need for additional data on the

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85 performance of commercially available SARS-CoV-2 serologic assays, as most previous studies
86 have been deemed to have a high risk of bias, particularly due to the use of small sample sizes
87 and/or exclusion of specimens from asymptomatic SARS-CoV-2 infections and mild or
88 moderate cases of COVID-19.[4–6]

89 Commercial SARS-CoV-2 EIAs may have an additional role in the implementation of COVID-
90 19 convalescent plasma (CCP) therapy programs.[2,7] The FDA recently issued an EUA for CCP
91 therapy.[8] Indeed, observational evidence suggests CCP is likely safe and efficacious, particularly
92 when administered early in the disease process.[9–12] Higher IgG antibody titers to the S1 protein
93 in CCP transfused to COVID-19 patients have been associated with decreased mortality.[12]
94 Higher IgG antibody titers to the spike (S) and nucleocapsid (N) protein of SARS-CoV-2 have
95 also been shown to correlate with SARS-CoV-2 neutralizing antibody (nAb) titers[13–16], which are
96 presumed to be critical for viral clearance. Current in vitro assays to detect nAbs are resource-
97 and time-intensive, and are not typically conducted in clinical laboratories. Commercial SARS-
98 CoV-2 EIAs that are already in use to qualify CCP donors could also potentially be applied to
99 identify those with high nAbs. However, data on the comparative performance of commercial
100 SARS-CoV-2 EIAs to discriminate between CCP donors with high and low nAbs are limited.

101 This study was designed to compare the performance of five commercially available EIAs to
102 detect IgG or total antibodies to SARS-CoV-2 and to discriminate between high and low nAbs.

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104 **MATERIALS AND METHODS**

105 **Ethics statement**

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

110 **Study specimens**

111 To test the clinical sensitivity of SARS-CoV-2 EIAs, we included stored plasma specimens from
112 a convenience sample of potential CCP donors that were recruited in the Baltimore, MD and
113 Washington DC area (n=214).[13] Individuals were eligible for enrollment if they had a
114 documented history of a positive molecular assay test result for SARS-CoV-2 infection
115 (confirmed by medical chart review or shared clinical documentation) and met standard self-
116 reported eligibility criteria for blood donation. Demographic information of included CCP
117 donors is shown in ***Supplemental Table 1***. Among included CCP donors, there was a median of
118 44 days from diagnosis until sample collection (interquartile range, 38-50 days). Although all
119 included CCP donors were symptomatic at the time of SARS-CoV-2 infection, less than 10%
120 had a history of hospitalization due to COVID-19. To test the clinical specificity of SARS-CoV-
121 2 EIAs, we included stored serum specimens from an identity-unlinked serosurvey conducted in
122 2016 among adult patients attending the Johns Hopkins Hospital Emergency Department
123 (n=1,102). Both parent studies were cross-sectional and no individual contributed multiple
124 specimens. All plasma/serum samples were stored at -80°C until assays were performed.

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125 **SARS-CoV-2 EIAs**

126 Plasma/serum specimens were analyzed using five commercially available EIAs: the Euroimmun
127 Anti-SARS-CoV-2 ELISA, the Epitope Diagnostics, Inc. (EDI) Novel Coronavirus COVID-19
128 IgG ELISA Kit, the ImmunoDiagnostics SARS-CoV-2 NP IgG ELISA kit, the Abbott-Architect
129 SARS-CoV-2 IgG chemiluminescent microparticle immunoassay (CMIA) and the Roche
130 Diagnostics Elecsys®Anti-SARS-CoV-2 E-CLIA (**Table 1**). These commercially available EIAs
131 were selected either because data on performance characteristics for the assay are limited and/or
132 the assay has received an EUA by the FDA. The target antigen for each EIA is the nucleocapsid
133 protein with the exception of the Euroimmun ELISA for which the target antigen is the S1
134 protein. The Roche assay measures total antibodies to SARS-CoV-2, whereas the others measure
135 only IgG to SARS-CoV-2. EIAs were conducted according to the manufacturers' instructions.
136 The intended use of each EIA is the qualitative detection of antibodies; however, each EIA
137 provides semi-quantitative output normalized by a calibrator. For simplicity, we refer to the
138 normalized continuous output of each EIA as a "ratio" value. The manufacturers' ratio cutoffs to
139 qualitatively indicate seropositivity, indeterminate serostatus, or seronegativity for SARS-CoV-2
140 antibodies are provided (**Table 1**). Specimens were tested by each EIA based on sample volume
141 availability and assay kit availability at the time of testing.

142 **Microneutralization assay**

143 Plasma nAb titers were quantified against 100 fifty percent tissue culture infectious doses
144 (TCID50) using a microneutralization (NT) assay in VeroE6-TMPRSS2 cells, which has been
145 previously described.[13,17] In brief, plasma was diluted 1:20 and subsequent two-fold dilutions.
146 Infectious virus was added to the plasma dilutions at a final concentration of $1x10^4$ $TCID_{50}$/ml.

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147 After a 1-hour incubation at room temperature, 100μL of each sample dilution was added to 6
148 wells in a 96-well plate of VeroE6-TMPRSS2 cells,[18] and incubated for 6 hours at 37˚C. The
149 inocula were removed from the plate, fresh media was added, and the plate was incubated at
150 37˚C for 48 hours. The cells were fixed with 4% formaldehyde (in each well), incubated for 4
151 hours at room temperature, and stained with Napthol Blue Black (Sigma-Aldrich). We calculated
152 a nAb titer area under the curve (AUC) value for each sample using the exact number of wells
153 protected from infection at every dilution. Samples with no neutralizing activity were assigned a
154 value of one-half the lowest measured AUC.

155 **Statistical analysis**

156 The diagnostic accuracy of each EIA to detect IgG or total antibodies to SARS-CoV-2 was
157 examined using CCP donor specimens as reference standard positive and pre-pandemic
158 specimens as reference standard negative. For each EIA, non-parametric, empirical receiver
159 operating curve analysis (ROC) was performed to calculate the area under the receiver operating
160 curve (AUROC). This analysis was also done using the manufacturers' cut-offs. Sensitivity (%)
161 was calculated as 100 x (Positive/[Positive + False-Negative]). Specificity (%) was calculated as
162 100 x (Negative/[Negative + False-Positive]). For these analyses, an available-case approach was
163 used for each EIA and indeterminate results were considered to be seronegative. Three separate
164 sensitivity analyses were conducted: (1) we performed head-to-head comparisons, (2) we
165 considered indeterminate specimens as positive, and (3) we excluded indeterminate specimens.
166 Exact binomial (Clopper-Pearson) 95% confidence intervals (CI) were calculated for estimates.

167 The remaining analyses were conducted in CCP donors that had data for all five EIAs and nAb
168 titers (n=140). The correlation of EIA ratios and nAb AUC values were examined using

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169 spearman's correlation coefficients (ρ) with 95% CIs estimated over 1000 bootstrap iterations.
170 We evaluated four binary cut-offs of the nAb AUC value to indicate "high" nAbs titers: ≥20,
171 ≥40, ≥80, and ≥160. For each nAb AUC cut-off, we evaluated the performance of each EIA to
172 discriminate between low and high nAb titers using empirical ROC analysis.

173 According to the recent EUA for CCP therapy, all CCP donors will be required to be antibody
174 positive for SARS-CoV-2. Thus, we also calculated the positive percentage agreement and
175 negative percentage agreement between each binary nAb threshold and each EIA using the
176 manufacturer's cut-offs originally recommended for SARS-CoV-2 serostatus in the CCP donor
177 population (indeterminates were considered as seronegative).

178 Statistical analyses were performed in Stata/MP, version 15.2 (StataCorp, CollegeStation, TX)
179 and R statistical software.

180 RESULTS

181 Of the 214 specimens from potential CCP donors, 146 were tested by the Euroimmun, EDI, and
182 Abbott assays; 140 were tested by the ImmunoDiagnostics assay, and all 214 were tested by the
183 Roche assay (140 were assayed by all five EIAs). Of the 1,102 pre-pandemic specimens
184 included, 562 were tested by the Euroimmun assay, 579 were tested by the EDI assay, 306 were
185 tested by the ImmunoDiagnostics assay, and 500 were tested by the Abbott and Roche assays.

186 In empirical ROC analyses, all assays —with the exception of EDI— had an AUROC value that
187 exceeded 0.95, suggesting each assay has the capacity to accurately detect antibodies to SARS-
188 CoV-2 (**Table 2**). For the ELISAs (Euroimmun, EDI, and ImmunoDiagnostics) the AUROCs
189 were greater by 5 absolute percentage points in the empirical ROC analysis compared to the

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190 analysis using the manufacturers' cutoffs. For the Abbott and Roche assays, the AUROCs were
191 similar in the empirical analysis and the analysis using the manufacturers' cut-offs.

192 Using the manufacturers' cut-offs, the sensitivity of each EIA to detect SARS-CoV-2 antibodies
193 ranged from 76.4% to 93.9%, whereas the specificity of each EIA ranged from 87.0% to 99.6%.
194 Both the Abbott and Roche assays had comparable characteristics as each other with higher point
195 estimates for sensitivity and specificity compared to the ELISAs. Considering
196 indeterminate/borderline specimens as seropositive as opposed to seronegative decreased the
197 specificity of EDI; however, excluding indeterminate/borderline specimens had minimal impact
198 on estimates (***Supplemental Table 2***). Similar estimates were also obtained in direct comparisons
199 (***Supplemental Tables 3, 4***). It is also notable that among the 140 CCP donor specimens that
200 were tested by all five EIAs, there were 6 (4.3%) specimens that were seronegative (or
201 indeterminate) for SARS-CoV-2 by all five EIAs. The median time from COVID-19 diagnosis
202 for these 6 individuals was 46 days (range, 33-54). Interestingly, there were 2 false-positive
203 specimens of the 500 pre-pandemic specimens tested by both Abbott and Roche (one of which
204 was false-positive on both assays).

205 Among pre-pandemic samples, there was greater variation in the distribution of ratio values for
206 ELISAs than for the Abbott and Roche assays (***Supplemental Figure 1***), consistent with the
207 higher specificity observed for the Abbott and Roche assays. For the Abbott, Roche and
208 ImmunoDiagnostics assays, the value of three times the standard deviation above the mean value
209 from all the pre-pandemic samples was below the cutoff used to define a positive sample.

210 Among the 140 CCP donor specimens, the median nAb AUC value was 60 (interquartile range:
211 10, 150). The prevalence of nAb AUC ≥20 was 65.7% (n=92), the prevalence of nAb AUC ≥40

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212 was 57.1% (n=80), the prevalence of nAb AUC ≥80 was 45.7% (n=64), and the prevalence of
213 nAb AUC ≥160 was 25.0% (n=35). There were significant positive correlations between nAb
214 AUC values and EIA ratio values for all EIAs examined (**Figure 1**), but the strongest correlation
215 was observed for the Euroimmun assay (ρ=0.81 [95%CI: 0.74-0.85]) and weakest correlation
216 was observed for the Roche assay (ρ=0.40 [95%CI: 0.25-0.54]). With “high” nAb titers as the
217 reference positive, there was substantial between-assay variability in the empirical AUROCs of
218 each EIA, but changing the threshold used to define a “high” nAb titer did not substantially
219 impact the AUROCs of a given EIA (**Figure 2**). For instance, for all four nAbs thresholds
220 evaluated, all empirical AUROC point estimates for the Euroimmun assay were ≥90, whereas all
221 AUROC point estimates for the Roche assay were <0.75. For the Euroimmun assay and nAB
222 test at a threshold of ≥160, the EIA ratio cut-off with the highest overall percent agreement
223 (86%) was 6.0 (positive percent agreement was 77% and negative percent agreement was 89%).

224 **Table 3** shows the positive percentage agreement (sensitivity) and negative percentage
225 agreement (specificity) of each assay with the four nAb test thresholds when using the EIA
226 manufacturers cut-offs for seropositivity. All EIAs had a positive percent agreement with “high”
227 nAbs exceeding 90%, regardless of the threshold for high nAbs. However, there was poor
228 negative percentage agreement between each EIA and nAbs. For all EIAs, the negative
229 percentage agreement decreased with increasing threshold for high nAbs.

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231 **DISCUSSION**

232 We observed substantial variability in the performance characteristics of five commercially
233 available EIAs for the detection of antibodies to SARS-CoV-2 and detection of high nAb titers in
234 convalescent individuals. The Roche and Abbott assays had high diagnostic accuracy for the
235 detection of antibodies against SARS-CoV-2. However, the Roche assay ratios weakly correlated
236 with nAb titers and poorly identified persons with high nAb titers. In contrast, the Euroimmun
237 assay ratios had the highest correlations with nAb titers and high discriminative capacity for
238 detecting high nAbs. This variability in assay performance should be considered when selecting
239 an EIA to detect antibodies against SARS-CoV-2 and/or high nAbs among recovered persons.

240 Consistent with our findings, there is growing evidence that both the Abbott and Roche assays
241 have comparable performance characteristics that are often superior to many other commercially
242 available ELISAs to detect IgG or total antibodies against SARS-CoV-2 in convalescent
243 individuals.[19,20] Although we did not include "challenge" specimens to examine potential cross-
244 reactivity of antibodies to other pathogens, others have shown limited evidence of cross-
245 reactivity for the Euroimmun, EDI, Roche, and Abbott assays.[19,21–25] Data on the performance of
246 the ImmunoDiagnostics ELISA to detect SARS-CoV-2 antibodies are limited.

247 Large public health laboratories and large blood collection centers often rely on automated
248 serological platforms—like those by Roche and Abbott—for screening of multiple pathogens
249 including SARS-CoV-2. While our data support the use of Roche and Abbott to detect SARS-
250 CoV-2 antibodies, their utility to detect high nAbs in CCP donors is less clear. Similar to prior
251 reports, we observed varying degrees of positive correlations between commercial EIA ratios
252 and neutralizing titers.[14,26] It is perhaps unsurprising that the Euroimmun ELISA ratios

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253 correlated best with nAb titers since it detects S1-specific antibodies—a subset of which are
254 responsible for virus neutralization—while the other assays we assessed detect N-specific
255 antibodies which lack virus neutralization activity. Accordingly, our empirical ROC analysis also
256 indicates Euroimmun may have better performance in discriminating high nAb titers, as
257 compared to the Abbott and Roche assays. Interestingly, using the manufacturer's cut-off,
258 Jaaskelainen et al. found the Abbott assay had greater positive and negative percent agreement
259 with nAb activity than the Euroimmun assay.[27] In our study, the Abbott assay was also better
260 able to discriminate high nAbs than the Roche assay, which is in contrast to a study by Tang et
261 al. that found similar performance between the Abbott and Roche assays.[28] However, similar to
262 Tang et al., we found that applying the manufacturer's cutoffs for the commercial EIAs
263 (including Euroimmun) led to suboptimal negative percentage agreement with high nAbs near
264 the FDA recommended nAb titer cut-off of ≥1:160.[28] Larger comparative studies are needed to
265 determine the optimal EIA and cut-off to discriminate nAb levels in convalescent donors,
266 including other promising EIAs that were not included in these evaluations.[29]

267 This study has limitations. First, the data were cross-sectional, so we were unable to capture the
268 influence of longitudinal antibody dynamics on diagnostic accuracy. Second, there were several
269 types of specimens that were not included in the evaluation, such as samples from early in
270 SARS-CoV-2 infection (e.g., <14 days post-symptom onset), samples from individuals who were
271 asymptomatic when infected with SARS-CoV-2, and samples from convalescent individuals
272 who were infected >6 months ago—all of which could potentially influence our estimates of
273 assay sensitivity. Third, the samples used to examine assay specificity were not well-
274 characterized due to the identity-unlinked design of the JHHED serosurvey. However, given that
275 we used samples from patients in an inner-city emergency department that delivers primary care

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276 to the local underserved community, several included patients who were likely seeking care for
277 viral respiratory illnesses. Finally, the samples evaluated were primarily from the Baltimore-
278 Washington D.C. region, and results may not be generalizable elsewhere.

279 Implementation of the appropriate EIAs to detect SARS-CoV-2 antibodies will require careful
280 consideration of the inferential purpose (e.g., individual- vs. population-level inference), context
281 (e.g., prevalence in target population), operational feasibility (e.g., high-throughput platform vs.
282 manual ELISA) and the underlying test performance characteristics of the assays. Although the
283 output ratio results for commercially available EIAs correlate with nAb titers, EIA ratios should
284 not be universally considered a surrogate for nAb titers. This is particularly relevant for
285 programs that are currently scaling CCP therapy per new FDA guidelines. Ratios from some
286 commercial EIAs, however, may help inform prediction models that can also incorporate other
287 predictors of high nAb titers. These models could prove useful in the identification of optimal
288 CCP donors in the absence of accurate and reliable high-throughput tests for nAb titers.

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380 **FIGURE LEGENDS**

381

382 **Figure 1. Correlations between SARS-CoV-2 enzyme immunoassay antibody titers and neutralizing**
383 **antibody titer AUC values in COVID-19 convalescent individuals (n=140).** Spearman correlation
384 coefficients (ρ) were calculated with 95% confidence intervals (CI) estimated over 1000 bootstrap iterations.

385

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

389 **MAIN TABLES**

390

391 **Table 1. Characteristics of commercial SARS-CoV-2 enzyme immunoassays evaluated.**

392

Manufacturer	Assay Name	Target antigen (recombinant)	Platform	Manufacturer's interpretation	No. Samples Evaluated
Euroimmun, Lubeck, Germany	Anti-SARS-CoV-2 ELISA (IgG) [a]	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 Pre-pandemic: 562
Epitope Diagnostics, Inc., 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 Pre-pandemic: 579
ImmunoDiagnostics Limited, Sha Tin, Hong Kong [b]	SARS-CoV-2 NP IgG ELISA kit	Nucleocapsid protein	Manual ELISA	Negative: OD-n < 0.15 Borderline: OD-n ≥0.25 & ≤0.50 Positive: OD-n > 0.50	CCP donors: 140 Pre-pandemic: 306
Abbott Laboratories Inc., Abbott Park, IL	Abbott-Architect SARS-CoV-2 IgG assay [a]	Nucleocapsid protein	Abbott Architect™ i2000 (CMIA) [c]	Negative: index (S/C) <1.40 Positive: index (S/C) ≥1.40	CCP donors: 146 Pre-pandemic: 500
Roche Diagnostics	Elecsys® Anti-SARS-CoV-2 [a]	Nucleocapsid protein	Roche cobas™ c 422 analyzer (ECLIA)	Non-reactive: index <1.0 Reactive: index ≥1.0	CCP donors: 214 Pre-pandemic: 500

393

394 [a] This assay had received emergency use authorization by the US Food and Drug Administration prior to August 20, 2020.

395 [b] ImmunoDiagnostics recommends each lab create its own cut-offs for qualitative interpretation.

396 [c] This study utilized the Abbott Architect i1000sr platform.

397

Table 2. Diagnostic accuracy of various enzyme immunoassays to detect IgG or total antibodies to SARS-CoV-2.

Serologic assay	Empirical analysis		Manufacturer's cutoff				
	N	AUROC (95% CI)	AUROC (95% CI)	Sensitivity		Specificity	
				n/N	% (95% CI)	n/N	% (95% CI)
Euroimmun*	708	0.97 (0.96-0.99)	0.92 (0.90-0.94)	127/146	87.0 (80.4-92.0)	548/562	97.5 (95.9-98.6)
EDI*	725	0.89 (0.87-0.91)	0.83 (0.80-0.86)	115/146	78.8 (71.2-85.1)	504/579	87.0 (84.0-89.7)
ImmunoDiagnostics*	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	646	0.98 (0.96-0.99)	0.96 (0.94-0.97)	135/146	92.5 (86.9-96.2)	498/500	99.6 (98.6-100.0)
Roche	714	0.97 (0.96-0.98)	0.97 (0.95-0.98)	201/214	93.9 (89.8-96.7)	498/500	99.6 (98.6-100.0)

Note: Exact binomial (Clopper-Pearson) 95% confidence intervals are shown for all estimates.

* Borderline/indeterminate specimens per manufacturer's cutoffs were considered negative in the manufacturer's cutoff analysis.

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Table 3. Concordance between manufacturer enzyme immunoassay cut-offs for SARS-CoV-2 seropositivity and high neutralizing antibody titers at various thresholds.

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

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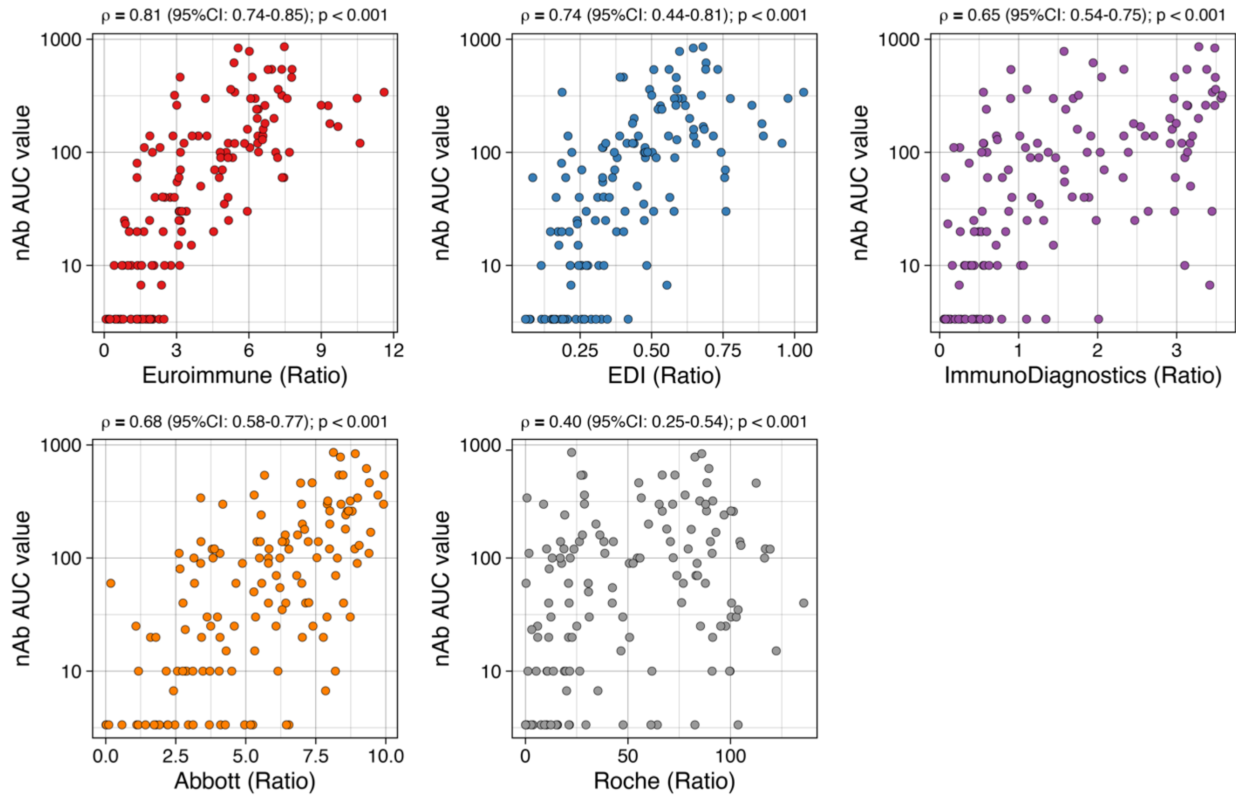

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445 **Figure 2. Empirical receiver operating curve analysis for various SARS-CoV-2 enzyme**
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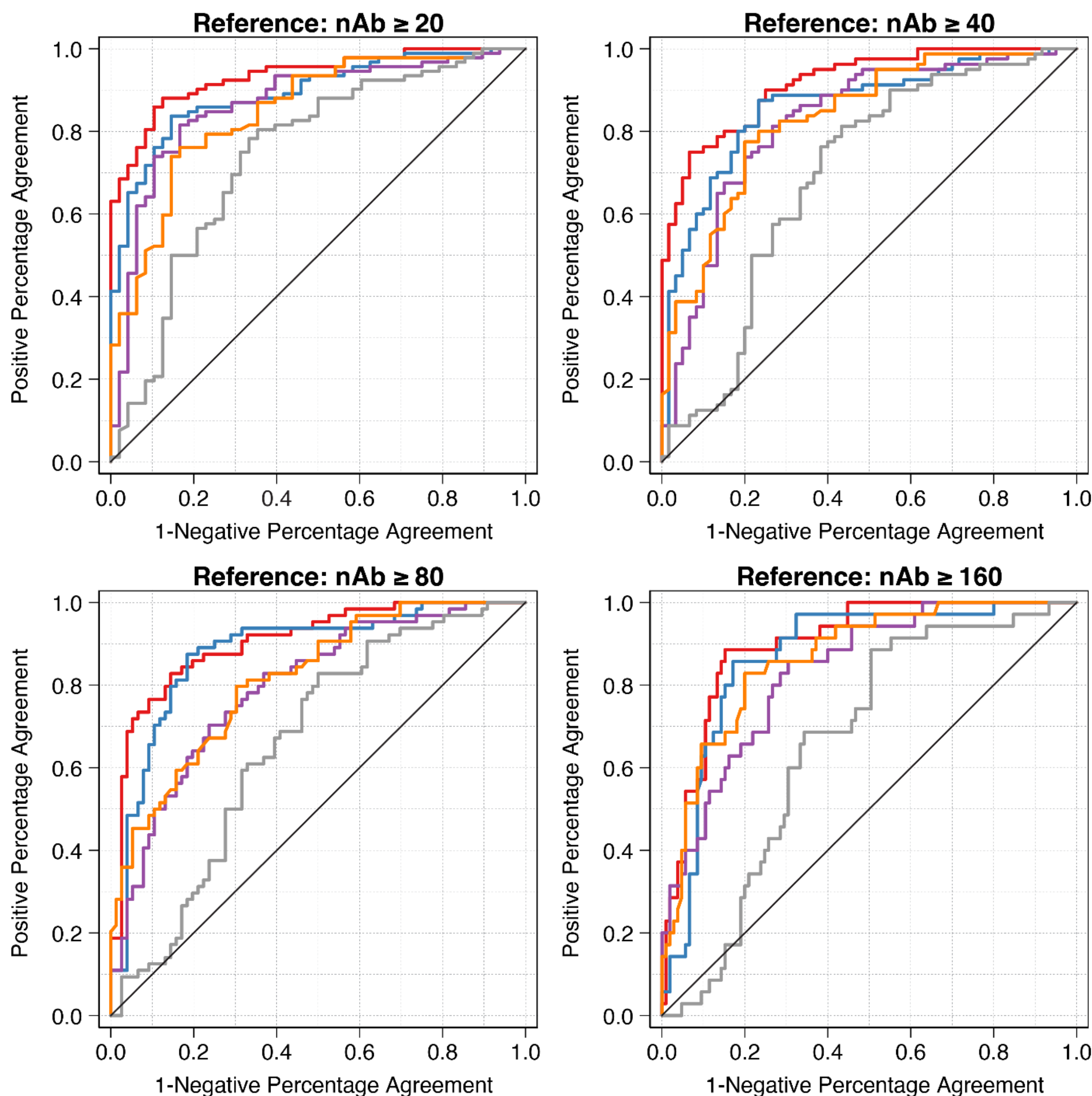

Serologic Assay	AUROC (95%CI)			
	nAb ≥ 20	nAb ≥ 40	nAb ≥ 80	nAb ≥ 160
Euroimmun	0.93 (0.88-0.97)	0.92 (0.86-0.95)	0.90 (0.85-0.95)	0.90 (0.84-0.94)
EDI	0.89 (0.83-0.94)	0.86 (0.79-0.91)	0.88 (0.81-0.93)	0.87 (0.80-0.92)
ImmunoDiagnostics	0.87 (0.80-0.93)	0.82 (0.75-0.88)	0.79 (0.72-0.86)	0.83 (0.76-0.89)
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)

449