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1   **Comparative performance of five commercially available serologic assays to detect**

2   **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

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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4

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.

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 commercially available EIAs

were selected either because data on performance characteristics for the assay are limited and/or

the assay has received an EUA by the FDA. The target antigen for each EIA is the nucleocapsid

protein with the exception of the Euroimmun ELISA for which the target antigen is the S1

protein. The Roche assay measures total antibodies to SARS-CoV-2, whereas the others measure

only IgG to SARS-CoV-2. EIAs were conducted according to the manufacturers' instructions.

The intended use of each EIA is the qualitative detection of antibodies; however, each EIA

provides semi-quantitative output normalized by a calibrator. For simplicity, we refer to the

normalized continuous output of each EIA as a "ratio" value. The manufacturers' ratio cutoffs to

qualitatively indicate seropositivity, indeterminate serostatus, or seronegativity for SARS-CoV-2

antibodies are provided (**Table 1**). Specimens were tested by each EIA based on sample volume

availability and assay kit availability at the time of testing.

Microneutralization assay

Plasma nAb titers were quantified against 100 fifty percent tissue culture infectious doses

(TCID50) using a microneutralization (NT) assay in VeroE6-TMPRSS2 cells, which has been

previously described.[13,17] In brief, plasma was diluted 1:20 and subsequent two-fold dilutions.

Infectious virus was added to the plasma dilutions at a final concentration of 1×10^4 $TCID_{50}$/ml.

6

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

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

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

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.

230

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

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 $\geq$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

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[TM] 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

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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.

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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)
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)

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.

426 **Table 3. Concordance between manufacturer enzyme immunoassay cut-offs for SARS-CoV-2**
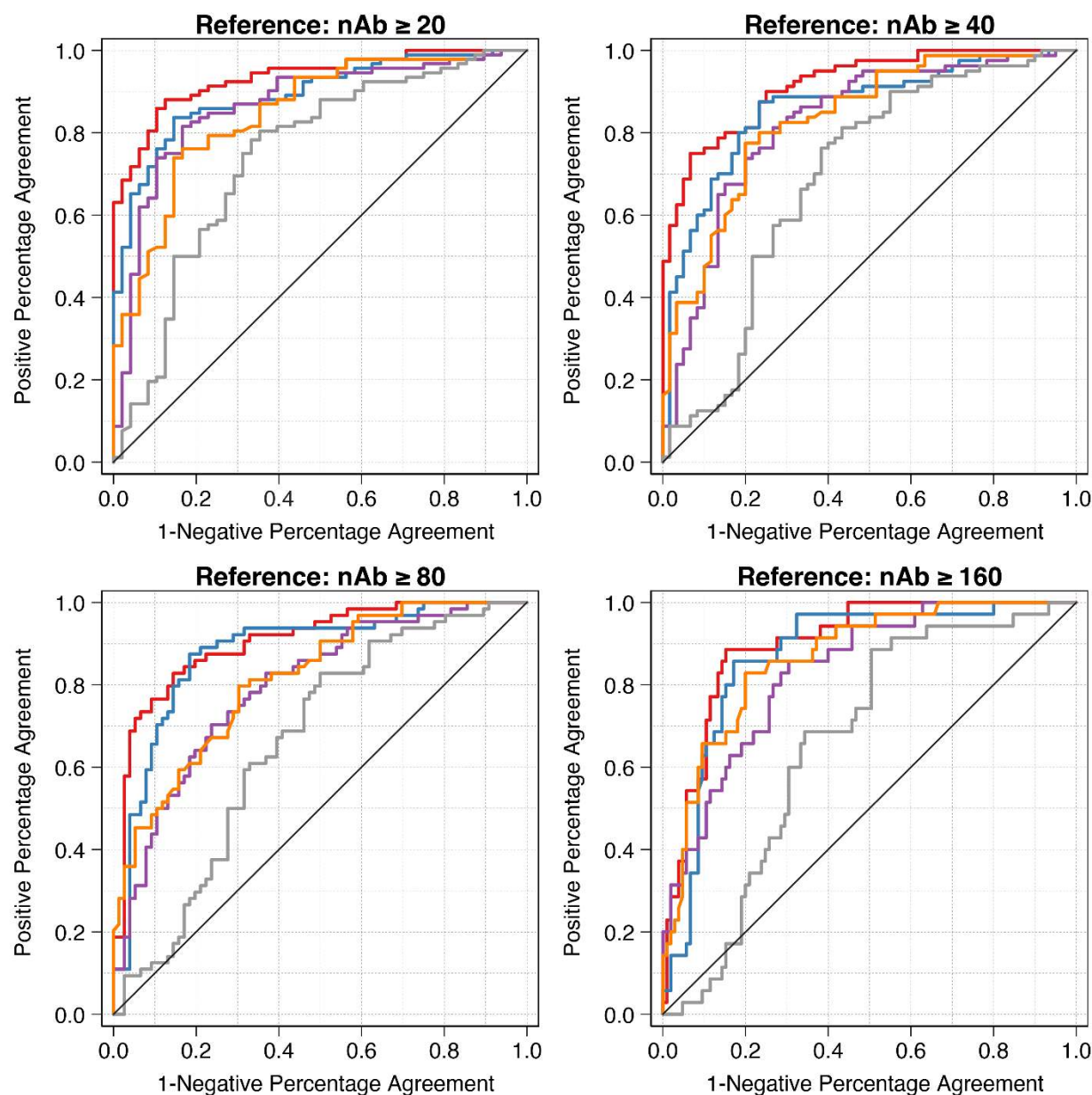427 **seropositivity and high neutralizing antibody titers at various thresholds.**
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	Positive Percentage Agreement, no. (%)			
Serologic assay	**nAb ≥20** (n=92)	**nAb ≥40** (n=80)	**nAb ≥80** (n=64)	**nAb ≥160** (n=35)
Euroimmun	90 (97.8%)	80 (100%)	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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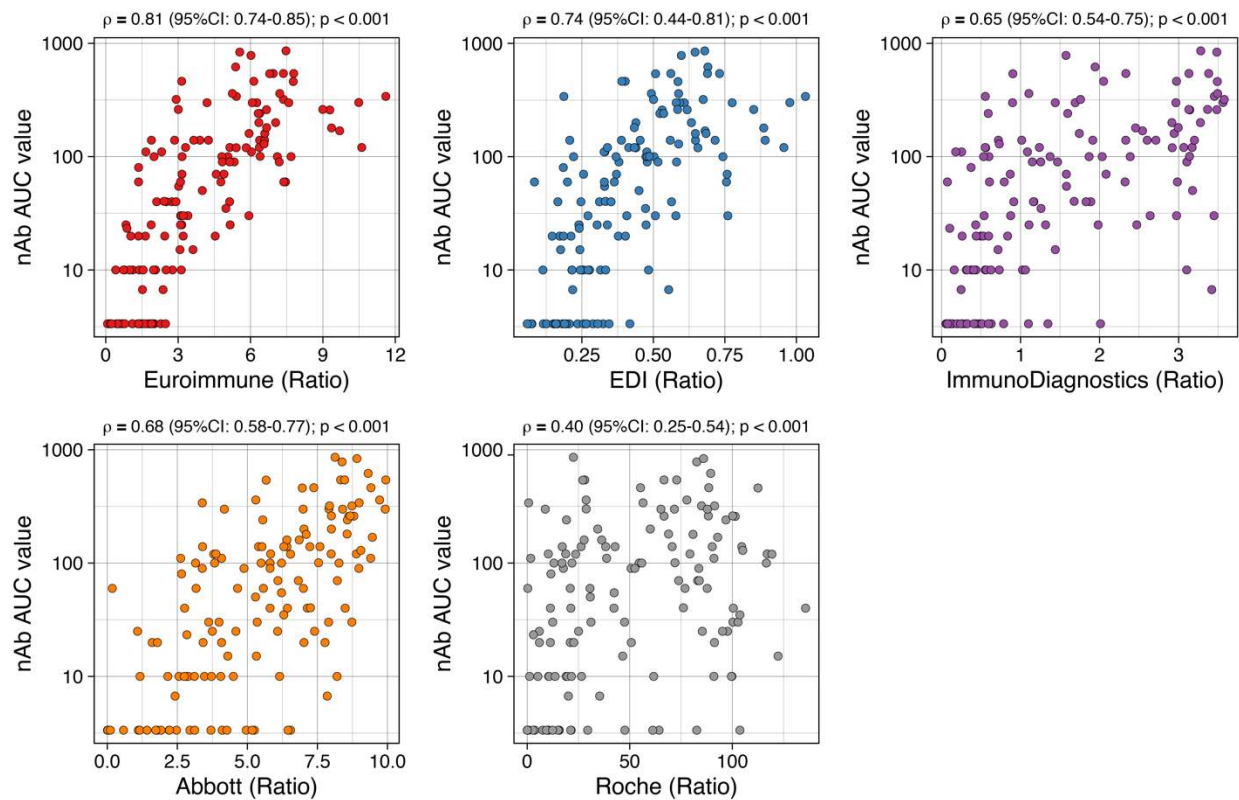
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447 Four thresholds for a high nAb AUC value were examined as the reference positive test.
448

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)

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