

1 **Association between SARS-CoV-2 neutralizing antibodies and commercial**  
2 **serological assays**

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27 **Abstract**

28 **Introduction:** Commercially available SARS-CoV-2 serological assays based on  
29 different viral antigens have been approved for the qualitative determination of anti-  
30 SARS-CoV-2 antibodies. However, there is limited published data associating the  
31 results from commercial assays with neutralizing antibodies.

32 **Methods:** 67 specimens from 48 patients with PCR-confirmed COVID-19 and a  
33 positive result by the Roche Elecsys SARS-CoV-2, Abbott SARS-CoV-2 IgG, or  
34 EUROIMMUN SARS-CoV-2 IgG assays and 5 control specimens were analyzed for  
35 the presence of neutralizing antibodies to SARS-CoV-2. Correlation, concordance,  
36 positive percent agreement (PPA), and negative percent agreement (NPA) were  
37 calculated at several cutoffs. Results were compared in patients categorized by clinical  
38 outcomes.

39 **Results:** The correlation between SARS-CoV-2 neutralizing titer ( $EC_{50}$ ) and the Roche,  
40 Abbott, and EUROIMMUN assays was 0.29, 0.47, and 0.46 respectively. At an  $EC_{50}$   
41 of 1:32, the concordance kappa with Roche was 0.49 (95% CI; 0.23-0.75), with Abbott  
42 was 0.52 (0.28-0.77), and with EUROIMMUN was 0.61 (0.4-0.82). At the same  
43 neutralizing titer, the PPA and NPA for the Roche was 100% (94-100) & 56% (30-80);  
44 Abbott was 96% (88-99) & 69% (44-86); and EUROIMMUN was 91% (80-96) & 81%  
45 (57-93) for distinguishing neutralizing antibodies. Patients who died, were intubated,  
46 or had a cardiac injury from COVID-19 infection had significantly higher neutralizing  
47 titers relative to those with mild symptoms.

48 **Conclusion:** COVID-19 patients generate an antibody response to multiple viral  
49 proteins such that the calibrator ratios on the Roche, Abbott, and EUROIMMUN assays  
50 are all associated with SARS-CoV-2 neutralization. Nevertheless, commercial

51 serological assays have poor NPA for SARS-CoV-2 neutralization, making them  
52 imperfect proxies for neutralization.

53

## 54 **INTRODUCTION**

55 Host cell infections by the recently-emerged severe acute respiratory syndrome  
56 coronavirus 2 (SARS-CoV-2) begin when the viral spike (S) protein engages the host  
57 angiotensin-converting enzyme 2 (ACE2) receptor (1). The humoral immune response  
58 can block infection through neutralizing antibodies, which bind the virus in a manner  
59 that prevents host cell infection (2). For SARS-CoV-2, this may be achieved by  
60 interfering with the spike -ACE2 receptor interaction, or by disrupting the fusion  
61 mechanisms the virus uses to enter host cell cytoplasm (2).

62

63 In the absence of a vaccine, there is considerable interest in identifying high-affinity  
64 neutralizing antibodies to SARS-CoV-2 to assess immune status and to evaluate  
65 vaccine responses. We previously demonstrated that passive transfer of monoclonal  
66 antibodies against SARS-CoV-2 S protein reduced viral titers and pathology in the  
67 lungs in a mouse model of SARS-CoV-2 (3). Monoclonal antibodies engineered from  
68 neutralizing antibodies, initially identified from convalescent COVID-19 patients, have  
69 been advanced as potential antiviral therapeutics (4-6), and early results from  
70 convalescent plasma use in patients indicate a protective effect of antibodies against  
71 SARS-CoV-2 (7-10). While early results are promising, the antibody titer conferring  
72 protection remains unclear and the role of neutralizing antibodies in protection has not  
73 been fully elucidated (11).

74

75 Despite widespread interest in neutralizing antibodies, methods for their detection and  
76 quantification are relatively low-throughput and limited to Biosafety Level 3-equipped  
77 research laboratories. While high-throughput methods have emerged, most rely on  
78 recombinant Vesicular Stomatitis Viruses (VSV) engineered to express a portion of the  
79 SARS-CoV-2 viral spike protein, and their subsequent entry into cell lines (12-14).  
80 Commercially available serological assays are high-throughput, relatively inexpensive,  
81 and use readily available instrumentation. The use of automated serological SARS-  
82 CoV-2 assays as a surrogate for neutralizing titers is therefore an attractive option. To  
83 date, limited data are available correlating commercially available assays with the  
84 presence of neutralizing antibodies.

85

86 We previously compared the clinical performance of three commercial serological  
87 assays (15, 16). Here, we further assess the ability of these assays to predict the presence  
88 of neutralizing antibodies.

89

## 90 **MATERIALS AND METHODS**

91

92 **Specimens:** This study was approved by the Institutional Review Board of Washington  
93 University in St. Louis. Residual plasma from physician-ordered complete blood count  
94 were utilized. Specimens were obtained from patients with PCR-confirmed COVID-19  
95 and at least one previously positive SARS-CoV-2 serological result. A subset of pre-  
96 pandemic samples obtained in 2015 and stored at -80 °C were used as negative controls.

97

98 **Clinical information:** Duration from symptom onset was obtained from two  
99 independent assessors by review of the electronic medical record (EMR) and inferred

100 from physician encounter notes. Symptoms included cough, fever, shortness of breath,  
101 loss of taste or smell, sore throat, and headache (17). The EMR also was used to collect  
102 data on outcomes for each patient. Mortality and intubation were determined by  
103 physician encounter notes, acute kidney injury (AKI) was defined using RIFLE criteria  
104 of 2-fold increase in serum creatinine and urine output less than 5 mL/kg/hr, cardiac  
105 injury was defined as a troponin I concentration  $> 0.03$  ng/mL (Abbott Diagnostics).

106

107 **INSTRUMENTATION:** Specimens were analyzed on three commercially available  
108 immunoassays and reported previously (15, 16). The Roche Elecsys Anti-SARS-CoV-  
109 2 assay was performed on an a Cobas e 601. The Roche assay detects total antibodies  
110 (IgG, IgA, IgM) against an epitope of the viral nucleocapsid protein. The Abbott SARS-  
111 CoV-2 IgG assay was performed on an i2000 Abbott Architect (Abbott Diagnostics)  
112 and detects IgG antibodies against the viral nucleocapsid protein. The EUROIMMUN  
113 (EI) SARS-CoV-2 IgG assay was performed on a QUANTA-Lyser 240 (Inova  
114 Diagnostics) assay and detects anti-SARS-CoV-2 IgG directed against the S1 domain  
115 of viral spike protein. All three assays use an assay-specific calibrator to report the ratio  
116 of the signal from the specimen to the signal of the calibrator. The results are interpreted  
117 as positive or negative relative to a threshold value. For the Roche assay, a positive is  
118 a cutoff index (COI)  $\geq 1$ ; for the Abbott assay, a signal to cut-off (S/CO)  $\geq 1.4$  is positive  
119 and  $< 1.4$  is negative; for the EI assay, a ratio  $\geq 1.2$  is positive 0.80-1.19 is  
120 indeterminate, and  $< 0.8$  is negative. The cutoff of 1.2 was used as a positive result for  
121 the EI. All three assays specify a positive result as the signal of the sample/the signal  
122 of a calibrator, therefore all results are reported here as a ratio.

123

124 **FOCUS REDUCTION NEUTRALIZATION ASSAYS:** Neutralization assays were  
125 performed as previously described (18). Briefly, SARS-CoV-2 strain 2019 n-  
126 CoV/USA\_WA1/2020 was obtained from the Centers of Disease Control and passaged  
127 in Vero E6 cells with DMEM (Corning) supplemented with glucose, L-glutamine,  
128 sodium pyruvate, and 10% FBS. Indicated dilutions of plasma were incubated with 10<sup>2</sup>  
129 focus forming units (FFU) of SARS-CoV-2 for 1h at 37°C before addition of the  
130 antibody virus complex to Vero E6 monolayers at 37°C for 1h. Cells were overlaid with  
131 a 1% w/v methylcellulose in MEM supplemented with 2% FBS and harvested 30h later.  
132 Methylcellulose overlays were removed and fixed with 4% paraformaldehyde in PBS  
133 at room temperature. Plates were then washed and incubated with 1 µg/mL anti-S  
134 antibody (CR3022) (19) and HRP-conjugated goat anti-Human IgG. Cells infected by  
135 SARS-CoV-2 were visualized using TrueBlue peroxidase substrate (KPL) and cell foci  
136 were quantified using an ImmunoSpot microanalyzer (Cellular Technologies). For each  
137 specimen, a minimum of 8 dilutions of human plasma were performed in duplicate and  
138 a standard curve generated. The 1/Log<sub>10</sub> plasma dilution (EC<sub>50</sub>) is the dilution at which  
139 50% of the cells were infected with virus and formed foci (**Supplemental Figure 1**).  
140

141 **STATISTICS:** Correlation between clinical assays and neutralizing titers were  
142 calculated using linear regression. Concordance between the assays was calculated  
143 using Cohen's Kappa. Area under the curve (AUC) for receiver operator characteristic  
144 (ROC) curves were calculated using the Wilson/Brown method. Kappa, positive  
145 percent agreement (PPA), and negative percent agreement (NPA) analysis were  
146 performed using multiple cutoffs for neutralizing titers owing to a lack of consensus  
147 regarding the relevant protective titer. Differences between antibody and neutralizing  
148 titers categorized by outcomes were calculated using unpaired T-tests. For outcome

149 comparisons, all specimens were >d10 post-symptom onset. All statistical analyses  
150 were performed with GraphPad Prism 8 (GraphPad).

151

## 152 **RESULTS**

153 40/42 specimens from PCR-confirmed COVID-19 patients with positive antibody  
154 results from commercial SARS-CoV-2 assays had neutralizing titers >1:20 by d14 post-  
155 symptom onset (**Figure 1A**). The mean neutralizing titer by d21 was 1:250 (95% CI;  
156 1:149- 1:436). In contrast, pre-pandemic control samples were not neutralizing at a titer  
157 of 1:20. Neutralizing titers increased subsequently with days post-symptom onset  
158 (**Supplemental Figure 2**). A subset of patients with serial measurements demonstrated  
159 a rapid rise in neutralizing titers between d5-15 that plateaued ~1:250 and remained  
160 elevated through the time course tested (**Figure 1B**).

161

162 The correlation of the SARS-CoV-2 neutralizing titer with the ratio reported by the  
163 Roche, Abbott, and EI assays was 0.29, 0.47, and 0.46 respectively (**Figure 2A-C**).  
164 Higher neutralizing titers were generally associated with a higher ratio as measured by  
165 all three assays. At a cutoff of 1:32 for the neutralizing assay, the concordance kappa  
166 with Roche was 0.61 (95% CI; 0.35-0.86), with Abbott was 0.65 (0.42-0.88), and with  
167 EI was 0.69 (0.49-0.89). For all three assays, the concordance decreased with an  
168 increased threshold for neutralizing titers.

169

170 ROC curves to determine the PPA and NPA of a positive antibody result on commercial  
171 assays for neutralizing titers  $\geq$  1:32 revealed an AUC of 0.94 (95% CI; 0.88-1.0), 0.89  
172 (0.79-0.99), and 0.93 (0.87-0.99) for the Roche, Abbott and EI assays respectively  
173 (**Figure 3A**). For both the Roche and Abbott assays, the ratio established by the

174 manufacturers produced maximum PPA with decreased NPA for neutralizing  
175 antibodies. Lowering the cutoff for EI increased the PPA without negatively impacting  
176 NPA. When evaluated for a neutralizing titer of 1:128, the AUC of the Roche assay  
177 was 0.86 (95% CI;0.77-0.95), for the Abbott was 0.82 (0.71-0.94), and for the EI was  
178 0.9 (0.83-0.97) (**Figure 3B**). At this neutralizing titer, the manufacturers' ratios for a  
179 positive result for all three assays maximized PPA while reducing NPA for anti-SARS-  
180 CoV-2 neutralizing antibodies.

181

182 At a neutralizing titer of 1:32, the PPA and NPA for the Roche assay was 100% (95%  
183 CI; 94-100) and 56% (30-80) at a ratio of 1.0 (**Table 1**). The ratio for each assay that  
184 improved the NPA while minimally affecting the PPA was assessed. The NPA  
185 improved to 81% (54-96) with the same PPA if the ratio for a positive result on the  
186 Roche was increased to 2.1. For the Abbott assay, the PPA was 96% (88-99) and the  
187 NPA was 69% (44-86) at a ratio of 1.4. The PPA and NPA for the Abbott changed to  
188 95% (85-99) and 88% (65-96) respectively if the ratio for a positive result was adjusted  
189 to 2.2. For the EI assay, the PPA was 91% (80-96) and the NPA was 81% (57-93) at a  
190 cutoff of 1.2. By decreasing the ratio for a positive result to 0.72, the PPA improved to  
191 96% (88-99) without effecting the NPA. NPA decreased for all three assays with  
192 increasing cutoff for a protective titer. To achieve an NPA >70% for all three assays at  
193 a neutralizing titer of 1:128, the ratio for a positive result would be 13.0 for the Roche,  
194 4.8 for the Abbott, and 2.4 for the EI assays. PPA remained above 80% for all assays  
195 at these cutoffs.

196

197 Patients that died as a result of COVID-19 had higher neutralizing antibody titers  
198 (mean, 1:576) compared to patients that survived (mean, 1:162) (**Figure 4A**). In



199 contrast, no significant difference in ratio was observed between patients that died from  
200 COVID-19 compared to those that survived using the Roche, Abbott, or EI assays.  
201 Increased neutralizing antibody titers were also higher in patients that were intubated,  
202 had cardiac injury, or AKI relative to those with milder COVID-19 symptoms (**Figure**  
203 **4B-D**). In contrast, no significant differences were noted between the groups regardless  
204 of outcomes when using the Roche, Abbott, and EI assays. However, similar non-  
205 significant trends (*i.e.*, increase in ratio) were observed in patients who were intubated,  
206 had cardiac injury, or AKI with the EI assay. Neutralizing titers trended higher in male  
207 patients and patients >60 years old, although this was not statistically significant.  
208 Similar trends were observed with the serology assay ratios as well (**Supplemental**  
209 **Figure 3**). If categorized by low (<1:256) or high neutralizing titers (>1:256), there  
210 were no significant differences in outcomes between patients. However, there was an  
211 increase in the ratio observed in high neutralizing titer patients (6.3, 95% CI; 5.7-6.9)  
212 compared to low titer patients (5.1, 95% CI; 4.1-6.1) on the Abbott assay and the EI  
213 assay (8.2, 7.1-9.2 vs. 6.1, 4.6-7.6) (**Supplemental Table 1**). A similar, but non-  
214 significant trend was observed with the Roche assay.

215

## 216 **DISCUSSION**

217 The emergence of commercially available serological assays for the detection of  
218 antibodies to SARS-CoV-2 has outpaced scientific understanding of their  
219 immunological meaning and their value in clinical decision making. Here, we assessed  
220 the utility of three commercially available clinical assays for correlation with  
221 neutralizing antibodies to SARS-CoV-2. We observed modest correlation, but poor  
222 concordance and NPA between the Roche, Abbott and EI SARS-CoV-2 assays for the  
223 detection of SARS-CoV-2 neutralizing antibodies. Interestingly, the three commercial

224 assays demonstrated similar performance with modest correlation but poor  
225 concordance and NPA for the detection of neutralizing antibody titers. Several studies  
226 have demonstrated that neutralizing antibodies are primarily against the S1, S2, and  
227 RBD domains of the SARS-CoV-2 spike protein (3, 4). As a result, clinical assays  
228 targeting these regions have been hypothesized to better predict neutralizing titers.  
229 However, our findings indicate that the Roche (nucleocapsid), Abbott (nucleocapsid),  
230 and EI (S1) assays have similar performance for identifying patients with neutralizing  
231 antibodies. This implies that patients infected with SARS-CoV-2 develop a broad-  
232 based antibody repertoire against multiple proteins and epitopes, with a relatively fixed  
233 proportion of those acting as neutralizing antibodies.

234

235 While the World Health Organization (WHO) and the Centers for Disease Control  
236 (CDC) have advised against associating immunity with seropositivity (20, 21), some  
237 have proposed that this warning is unnecessarily conservative (22). Our findings  
238 suggest that SARS-CoV-2 serological assays should be interpreted with caution. While  
239 the majority of patients with antibodies detected by commercial assays had neutralizing  
240 antibodies present by d14 post-onset of symptoms, ~10% of patients past d14 had titers  
241 that were <1:32. This implies that some patients with previous SARS-CoV-2 infections  
242 and positive antibody results by commercial assays may have neutralizing antibodies  
243 near the cutoff for a positive result. Although further studies are warranted, these low  
244 titers may be inadequate for protection, particularly if neutralizing antibodies are the  
245 primary therapeutic benefit of convalescent plasma. While higher reported ratios from  
246 all three commercial assays correlated with higher neutralizing titers, this was not  
247 universally true. Consistent with this, the correlation between neutralizing titers and  
248 serological results were <0.5 on all three commercial assays. These findings are

249 consistent with a previous study demonstrating modest linear correlation between  
250 neutralizing SARS-CoV-2 titers with anti-RBD IgG or anti-S IgG using laboratory  
251 developed ELISAs (23). Nonetheless, we found that higher ratios reported by all three  
252 commercial assays was associated with higher neutralizing titers. Importantly, all three  
253 serological assays used in this study currently have Emergency Use Authorization  
254 (EUA) to qualitatively determine the presence of antibodies against SARS-CoV-2.  
255 While a negative result on SARS-CoV-2 serological assays is likely to be associated  
256 with the absence of neutralizing antibody titers, a positive result is not reliable for  
257 predicting the presence of neutralizing antibodies. Furthermore, since these assays are  
258 under the EUA, they cannot be modified by the laboratory to report quantitative units.  
259 Our results argue for a potential utility in reporting the ratio calculated for commercially  
260 available assays relative to the calibrator. We, along with others, have previously  
261 suggested that commercially available serological assays for SARS-CoV-2 may have  
262 utility for identifying convalescent plasma donors (24, 25). To this end, reporting  
263 quantitative units is more likely to identify convalescent patients with higher  
264 neutralizing antibody titers than qualitative cutoffs. Furthermore, if neutralizing  
265 antibodies are shown to confer protection to SARS-CoV-2, quantitative serological  
266 assays may assist in identifying neutralizing titers in mildly symptomatic and  
267 asymptomatic populations. However, further studies are needed to demonstrate the  
268 clinical benefit of this approach, especially by characterizing this association in a more  
269 diverse patient population.

270

271 While the NPA for neutralizing antibodies was >90% for all three commercial assays,  
272 this was only when a 1:20 neutralizing titer was used as a cutoff. It is important to note  
273 that this is far below the FDA recommended neutralizing titer for convalescent plasma

274 donors ( $\geq 1:160$ ) (26). At a similar cutoff of 1:128, the NPA for neutralizing titers was  
275 below 60% for all three of the assays. Furthermore, while it is expected that neutralizing  
276 antibodies confer some protection against SARS-CoV-2, the titer required for this  
277 protective effect has not been established (11). Due to the low sensitivity of serological  
278 assays for diagnosing early SARS-CoV-2 infection (15, 27), some studies have  
279 suggested lowering the assay cutoff ratios to improve sensitivity (28, 29). However, if  
280 the intended utility of serology is to determine the presence of neutralizing antibodies,  
281 our ROC analyses suggest that the assay cutoff should be increased to improve the  
282 NPA. Interestingly, some manufacturers are now associating positive serological  
283 results with neutralizing antibodies in their validation studies. For instance, the  
284 LIAISON SARS-CoV-2 S1/S2 IgG assay claims high agreement with neutralizing  
285 antibodies. However, the cutoff titer used for the neutralizing assay was 1:40; far below  
286 that recommended by FDA for convalescent plasma therapy (13). If neutralizing  
287 antibodies  $> 1:256$  are required for protection, then commercial assays at the current  
288 cutoffs may have limited utility for identifying patients with protective antibodies; with  
289 NPA between 18-40% for the assays tested in this study.

290

291 Here, we observed that higher neutralizing titers are associated with worse clinical  
292 outcomes, a finding that was not observed with commercial serological assays. While  
293 seemingly counterintuitive, it is consistent with previous literature and may be a result  
294 of higher antigen burdens or hyperactive immune responses among other reasons (30-  
295 34). A study of service members in the US Navy with predominantly mild symptoms  
296 revealed that ~40% of those with a positive ELISA by the CDC assay had no  
297 neutralizing titers at a cutoff of 1:40 (35). Similarly, a recent study demonstrated  
298 neutralizing titers at  $< 1:50$  in 33% of recovered patients and below 1:1000 in 79% of

299 patients (23). Our findings are also consistent with a study assessing the agreement  
300 between the EI IgG result and neutralizing titers on predominantly non-hospitalized  
301 convalescent plasma donors (33). The authors demonstrated that at a neutralizing titer  
302 of 1:320, the PPA and NPA were 96% and 32% respectively and that neutralizing titers  
303 were higher in a small cohort of hospitalized patients. Similarly, we demonstrate higher  
304 neutralizing titers among patients with worse outcomes in an almost entirely  
305 hospitalized cohort. Unique to this study, we also compare commercial tests head-to-  
306 head and, by extension, compare serologies to two different protein antigens with  
307 similar results. Taken together, previous studies coupled with the findings presented  
308 here are consistent with the notion that neutralizing antibodies, while an important  
309 component of the immune response, (3, 4) are unlikely to be the only mechanism of  
310 SARS-CoV-2 clearance and protection. Other immune responses such as cellular  
311 immunity, T cells, antibody mediated cellular immunity and antibody mediated  
312 complement fixation likely play a pivotal role in protection from SARS-CoV-2.

313

314 Due to both heavy marketing and misunderstanding of their utility, patients have sought  
315 antibody testing for SARS-CoV-2 to determine if they had been previously infected  
316 and for peace-of-mind, assuming that they may have some level of protection (the  
317 concept of an “immunity passport”). At our institution, ~85% of the SARS-CoV-2  
318 serological tests are performed in the outpatient setting. This implies that the vast  
319 majority of these tests may be performed on mildly symptomatic and asymptomatic  
320 populations. Therefore, it is crucial that future studies address the correlation between  
321 neutralizing titers and commercial assays in the mildly symptomatic and the  
322 asymptomatic COVID-19 population. If symptomatic and severely ill patients have the  
323 highest titers of neutralizing antibodies, low concordance demonstrated here may be

324 exacerbated by including asymptomatic and mildly symptomatic patients. Furthermore,  
325 while neutralizing titers appear to persist in the small group of patients with longitudinal  
326 specimens, the duration of follow up in our study was too short to determine the  
327 durability of neutralizing antibodies. Nonetheless, previous studies have demonstrated  
328 a reduction in neutralizing titers after 8 weeks post-hospital discharge (31).

329

330 There are several limitations associated with this study. The true sensitivity and  
331 specificity of neutralizing titers in PCR-confirmed SARS-CoV-2 infected patients  
332 could not be accurately determined because specimens were pre-selected for serological  
333 positivity by commercially available immunoassays. This approach was chosen given  
334 the highly manual nature of testing for neutralizing antibodies and the primary goal of  
335 comparing neutralizing antibody titers to commercial assays. Furthermore, while the  
336 neutralizing assay utilized is robust and reproducible, it has not been validated for  
337 clinical use. In contrast to other studies, this assay uses an infectious strain of SARS-  
338 CoV-2 as opposed to pseudotyped rhabdoviruses or lentiviruses that heterologously  
339 express the SARS-CoV-2 spike protein. Furthermore, the relatively small number of  
340 patients tested means that potentially subtle differences in PPA, NPA, and concordance  
341 between the three assays could not be distinguished as a result of wide, overlapping  
342 confidence intervals. Finally, while others have demonstrated that neutralizing titers  
343 appear as early as d10 post-onset of symptoms, it is possible that assessing patients at  
344 later time points (*i.e.*, d28) would reveal a higher concordance. While the majority of  
345 patients tested serially had neutralizing titers that peaked by d14-15, future studies are  
346 needed at later timepoints to correlation with commercial assays at later timepoints.  
347 This includes several months after infection, when other studies have demonstrated the  
348 neutralizing response beginning to diminish.

349

350 In conclusion, our findings suggest that positive serological results by three  
351 commercially available assays that measure antibodies against the viral spike or  
352 nucleocapsid protein of SARS-CoV-2 have modest correlation with neutralizing  
353 antibody titers. COVID-19 patients generate an antibody response to multiple viral  
354 proteins such that the quantitative ratios on the Roche, Abbott, and EUROIMMUN  
355 assays have comparable associations with SARS-CoV-2 neutralization. Nevertheless,  
356 commercial serological assays have poor NPA for SARS-CoV-2 neutralization, making  
357 them imperfect proxies for neutralization.

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- 473

474 **TABLE 1. PPA and NPA of SARS-CoV-2 serological assays for neutralizing**  
 475 **antibodies at multiple neutralizing titers**

Neutralizing Titer		Roche			Abbott			EUROIMMUN		
		Ratio	PPA (95% CI)	NPA (95% CI)	Ratio	PPA (95% CI)	NPA (95% CI)	Ratio	PPA (95% CI)	NPA (95% CI)
1:20	Manufacturer Ratio	1	100 (94-100)	69 (42-87)	1.4	93 (84-97)	69 (42-87)	1.2	90 (79-85)	92 (67-100)
	Ideal Ratio	2.1	100 (94-100)	100 (77-100)	3.5	86 (73-92)	100 (77-100)	0.72	95 (86-99)	92 (67-100)
1:32	Manufacturer Ratio	1.0	100 (94-100)	56 (30-80)	1.4	96 (88-99)	69 (44-86)	1.2	91 (80-96)	81 (57-93)
	Ideal Ratio	2.1	100 (94-100)	81 (54-96)	2.2	95 (85-99)	88 (65-96)	0.72	96 (88-99)	81 (57-93)
1:64	Manufacturer Ratio	1.0	100 (93-100)	47 (27-68)	1.4	96 (87-99)	50 (30-70)	1.2	92 (82-97)	70 (48-85)
	Ideal Ratio	3.0	98 (90-100)	74 (51-88)	2.6	94 (84-98)	70 (48-85)	0.72	98 (90-100)	70 (48-85)
1:128	Manufacturer Ratio	1.0	100 (92-100)	31 (17-49)	1.4	98 (85-99)	40 (25-58)	1.2	95 (84-99)	55 (38-72)
	Ideal Ratio	13.0	83 (69-92)	72 (54-85)	4.8	86 (72-93)	73 (56-86)	2.4	93 (81-98)	72 (54-85)
1:256	Manufacturer Ratio	1.0	100 (85-100)	18 (10-31)	1.4	100 (85-100)	24 (15-38)	1.2	100 (85-100)	35 (23-49)
	Ideal Ratio	28.0	68 (47-84)	71 (58-82)	6.1	77 (57-90)	73 (60-84)	7.6	68 (47-84)	71 (58-82)

476

477

478 **FIGURE LEGENDS**

479 **Fig. 1. SARS-CoV-2 neutralizing titers in patients with and without PCR-**  
480 **confirmed COVID-19 Infection. (A)** Neutralizing titers of 5 control specimens  
481 collected in 2015 and stored at  $-80^{\circ}\text{C}$  and 67 specimens from 48 patients with PCR-  
482 positive COVID-19 relative to days from symptom onset. **(B)** Neutralizing titers  
483 relative to days of symptom onset. **(C)** Time to positive neutralizing antibodies in 12  
484 patients with serial samples. Gray dotted horizontal lines represent the limit of detection  
485 at 1:20.

486

487 **Fig. 2. Correlation between neutralizing antibody titer and three commercial anti-**  
488 **SARS-CoV-2 serology assays. (A)** Roche SARS-CoV-2 total antibody Immunoassay.  
489 Horizontal dotted line represents the cutoff off for Roche positivity (Ratio 1.0). **(B)**  
490 Abbott SARS-CoV-2 IgG Immunoassay. Horizontal dotted line represents the cutoff  
491 off for Abbott positivity (Ratio 1.4). **(C)** EUROIMMUN anti-SARS-CoV-2 IgG  
492 ELISA. Horizontal dotted line represents the cutoff off for EUROIMMUN positivity  
493 (Ratio 1.2). Specimens from 5 expected negative specimens collected in 2015 (gray  
494 triangles) and 67 specimens from 48 patients with PCR-positive COVID-19. Vertical  
495 dotted lines represented the cutoff for neutralizing antibody positivity at the indicated  
496 titer.

497

498 **Fig. 3. Receiver operating characteristic (ROC) curves for three commercial anti-**  
499 **SARS-CoV-2 serology assays to detect neutralizing anti-SARS CoV-2 antibodies.**  
500 **(A)** Titer for neutralizing antibody positivity set at  $\text{EC}_{50}=32$ . **(B)** Titer for neutralizing  
501 antibody positivity set at  $\text{EC}_{50}=128$ . Dotted line represents AUC 0.5 (random guess  
502 line). Specimens from 5 expected negative specimens collected in 2015 and 67

503 specimens from 48 patients with PCR-positive COVID-19. Arrows represents  
504 commercial assay cutoff (Roche Ratio= 1.0; Abbott Ratio = 1.4; EUROIMMUN Ratio  
505 = 1.2). AUC= area under the curve.

506

507 **Fig. 4. Association between clinical outcomes and anti-SARS CoV-2 neutralizing**  
508 **or commercial antibodies.** (A) Death. (B) Intubation. (C) Cardiac Injury. (D) Acute  
509 kidney injury. Data from 40 patients with PCR-positive COVID-19. Solid horizontal  
510 line represents the mean. \*  $p < 0.05$ .

511

Fig. 1

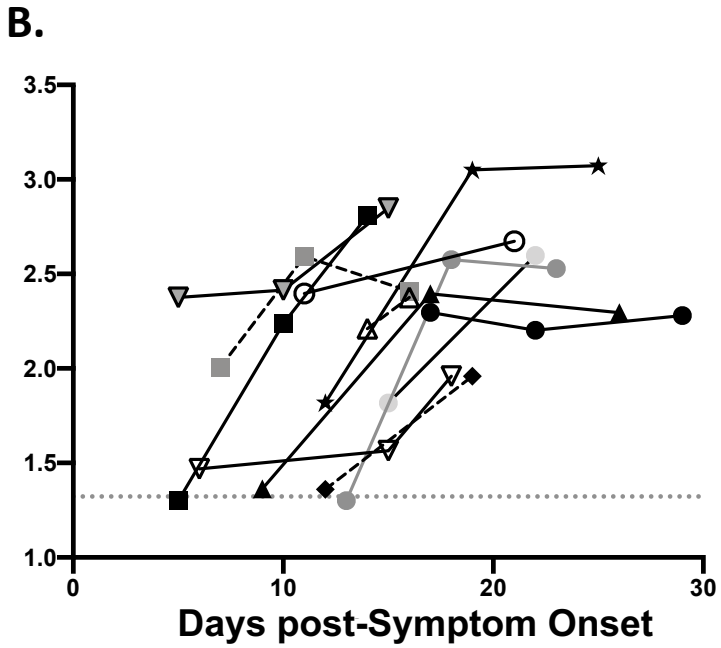
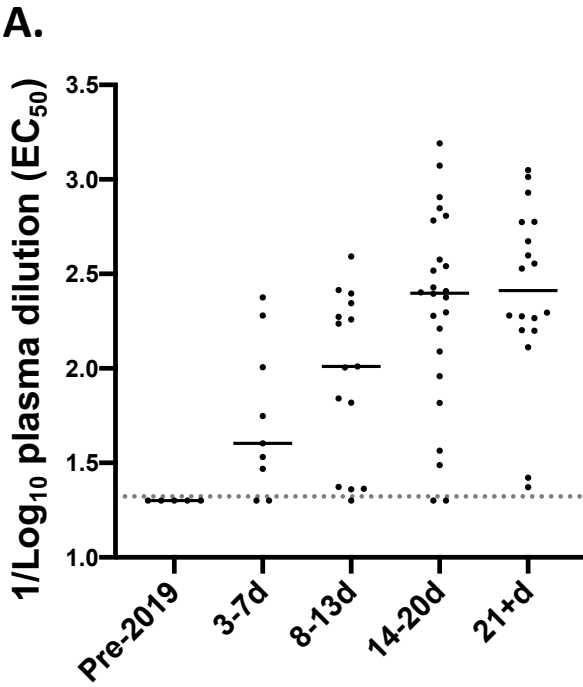




Fig 2.

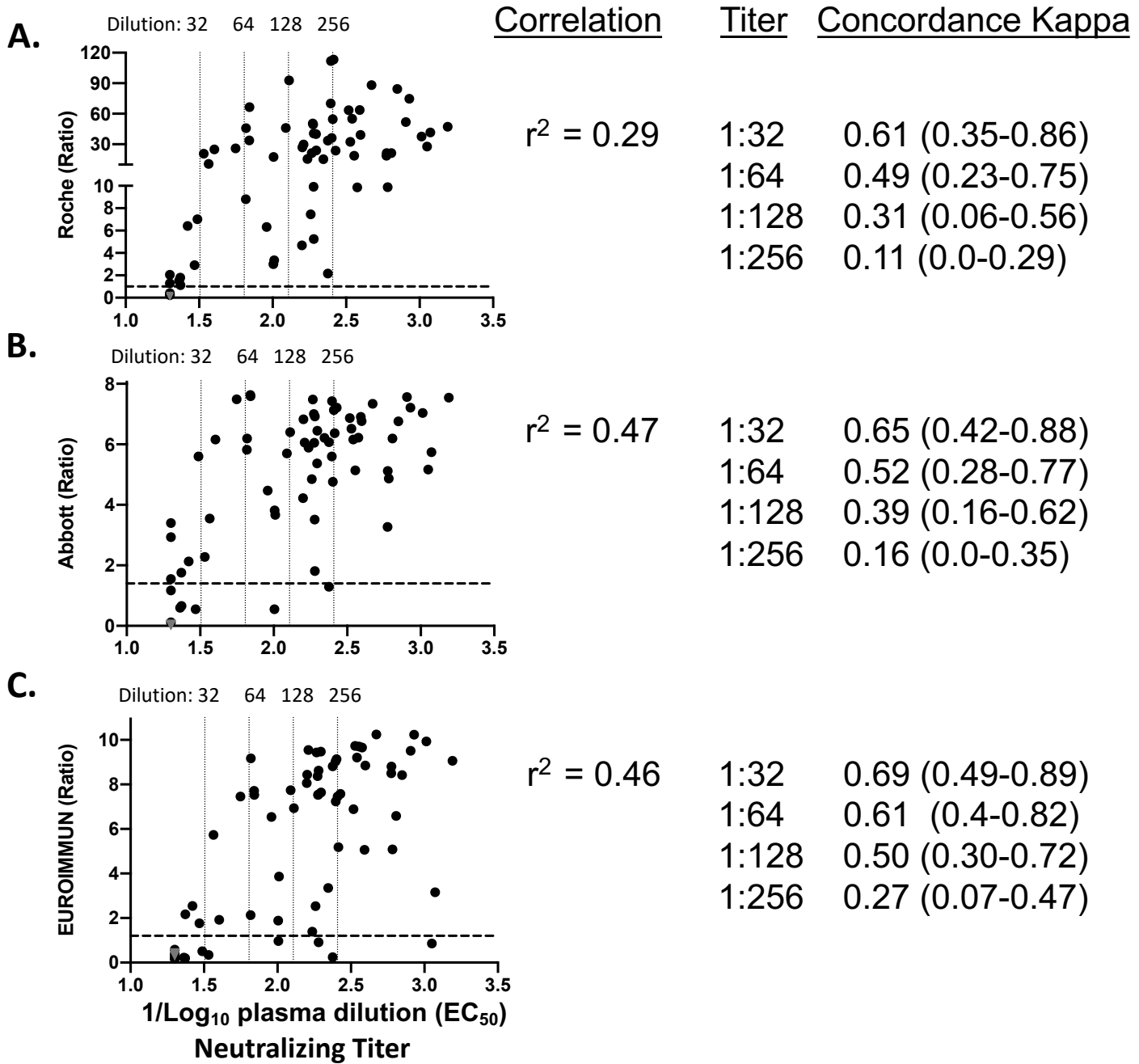
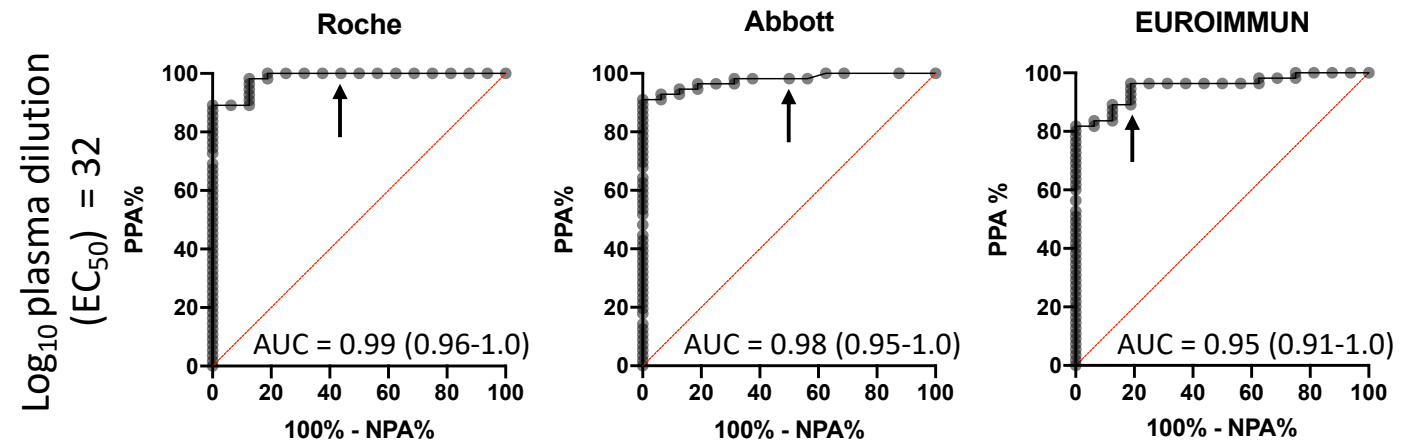


Fig 3.

**A.**



**B.**

