

1 **Performance Characteristics of Four High-Throughput Immunoassays for**
2 **Detection of IgG Antibodies against SARS-CoV-2**

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7 Running Title: SARS-CoV-2 IgG Immunoassays

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10

11 **Abstract**

12 The role of serologic testing for SARS-CoV-2, both in the clinical and public health settings, will
13 continue to evolve as we gain increasing insight into our immune response to the virus. Here, we
14 evaluated four high throughput serologic tests for detection of anti-SARS-CoV-2 IgG antibodies,
15 including assays from Abbott Laboratories (Abbott Park, IL), Epitope Diagnostics Inc. (San Diego, CA),
16 Euroimmun (Lubeck, Germany), and Ortho-Clinical Diagnostics (Rochester, NY), using a panel of serially
17 collected serum samples (N=224) from 56 patients with confirmed COVID-19, healthy donor sera from
18 2018 and a cross-reactivity serum panel collected in early 2020. Sensitivity of the Abbott, Epitope,
19 Euroimmun and Ortho-Clinical IgG assays in convalescent serum samples collected more than 14 days
20 post symptom onset or initial positive RT-PCR result was 92.9% (78/84), 88.1% (74/84), 97.6% (82/84)
21 and 98.8% (83/84), respectively. Among unique convalescent patients, sensitivity of the Abbott, Epitope,
22 Euroimmun and Ortho-Clinical anti-SARS-CoV-2 IgG assays was 97.3% (36/37), 73% (27/37), 94.6%
23 (35/37) and 97.3% (36/37), respectively. Overall assay specificity and positive predictive values based on
24 a 5% prevalence rate are 99.6%/92.8%, 99.6%/90.6%, 98.0%/71.2% and 99.6%/92.5%, respectively, for
25 the Abbott, Epitope, Euroimmun and Ortho-Clinical IgG assays. In conclusion, we show high sensitivity in
26 convalescent sera and high specificity for the Abbott, Euroimmun and Ortho-Clinical anti-SARS-CoV-2
27 IgG assays. With the unprecedented influx of commercially available serologic tests for detection of
28 antibodies against SARS-CoV-2, it remains imperative that laboratories thoroughly evaluate such assays
29 for accuracy prior to implementation.

30 Introduction

31 Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus
32 2 (SARS-CoV-2), was officially classified as a pandemic by the World Health Organization (WHO) on
33 March 11, 2020. As of the writing of this manuscript, over 4 million cases have been confirmed
34 worldwide, with over 1 million in the United States of which nearly 100,000 have resulted in death
35 (<https://coronavirus.jhu.edu/map.html>; accessed May 19, 2020). Diagnostic testing of patients who
36 present with symptoms consistent with COVID-19 relies on detection of viral RNA using molecular
37 methods, including reverse-transcriptase polymerase chain reaction (RT-PCR), which can be performed
38 on an array of different respiratory tract specimen types (1). Serologic assays to detect antibodies
39 against SARS-CoV-2 are now also available as an additional tool in our global response to the COVID-19
40 pandemic. Currently, most infectious disease and microbiology organizations generally agree that the
41 role for antibody testing is limited to use in seroprevalence and epidemiology studies, screening of
42 potential convalescent plasma donors, assessment of candidate vaccine efficacy, and in select scenarios,
43 as an aid for the diagnosis of COVID-19 in SARS-CoV-2 RT-PCR negative patients who present later in
44 disease course and for whom collection of a lower respiratory tract sample is not possible (2-4). The role
45 of serologic testing for SARS-CoV-2 will likely evolve in the future as we gain better understanding of our
46 immune response to the virus, identify correlates of immunity and determine the level and duration of
47 such immunity following infection or vaccination. Until then, the impact of an antibody result at the
48 individual patient level is limited and should not be used to guide decisions related to use of personal
49 protective equipment or adherence to social distancing policies.

50 While the Food and Drug Administration (FDA) required Emergency Use Authorization (EUA) for
51 SARS-CoV-2 molecular assays at the outset of the outbreak, until recently, serologic tests for SARS-CoV-2
52 fell under the Section IV.D of the FDA's 'Policy for Diagnostic Tests for Coronavirus Disease-2019' (*i.e.*,

53 'Pathway D'), which required commercial manufacturers to only notify the FDA of their validated
54 product, ultimately leading to the rapid influx of over 180 commercially available SARS-CoV-2 serologic
55 test kits ([https://www.fda.gov/medical-devices/emergency-situations-medical-devices/faqs-diagnostic-](https://www.fda.gov/medical-devices/emergency-situations-medical-devices/faqs-diagnostic-testing-sars-cov-2)
56 [testing-sars-cov-2](https://www.fda.gov/medical-devices/emergency-situations-medical-devices/faqs-diagnostic-testing-sars-cov-2); accessed May 1, 2020). On May 4th, 2020, the FDA amended these guidelines to
57 require all commercial manufacturers to submit their validation data for EUA, and to date, 14
58 manufacturers or laboratories have received EUA for their serologic test. The commercially available
59 serologic assays vary in the antibody detected (*i.e.*, IgM, IgA, IgG, or total antibody), the targeted SARS-
60 CoV-2 antigen (*i.e.*, subunit 1 [S1] of the spike protein, the nucleocapsid protein [NP], the receptor
61 binding domain [RBD]) and the method used (*e.g.*, lateral flow assay [LFA], enzyme-linked
62 immunosorbent assay [ELISA], chemiluminescent immunoassay [CLIA], etc.), with few peer-reviewed
63 studies currently available which independently evaluate their performance characteristics.

64 Here, we provide an assessment of four high throughput serologic tests, including anti-SARS-
65 CoV-2 IgG assays from Abbott Laboratories (Abbott Park, IL), Epitope Diagnostics Inc. (San Diego, CA),
66 Euroimmun (Lubeck, Germany), and Ortho-Clinical Diagnostics (Rochester, NY), using a panel of serially
67 collected serum samples from RT-PCR confirmed patients with COVID-19, healthy donor sera from 2018
68 and a cross-reactivity antibody panel collected in early 2020.

69

70 **Methods**

71 **Specimens Collected from COVID-19 Confirmed Patients.** A total of 224 serum samples were collected
72 between March and April 2020 from 56 adult patients confirmed positive for COVID-19 by a laboratory
73 developed or commercially available FDA EUA SARS-CoV-2 RT-PCR assay, performed on a
74 nasopharyngeal swab (5). Among the 56 COVID-19 RT-PCR confirmed patients, 33 were hospitalized (in-
75 patient group) and 23 were treated as out-patients (out-patient group). Remnant, serial serum samples

76 were collected as available throughout the hospital stay for the in-patient group until discharge,
77 whereas prospective collection of acute (≤ 7 days post first RT-PCR positive result) and convalescent (*i.e.*,
78 > 20 days post first RT-PCR positive result) sera was completed for out-patients. Medical records were
79 reviewed to determine date of symptom onset, or if unavailable, the date of first SARS-CoV-2 RT-PCR
80 positive result, and to identify whether the patient was hospitalized or treated as an out-patient. The
81 focus of this study was to assess assay performance characteristics, therefore although patient
82 demographics, presenting symptoms, disease course and outcomes were documented, they were not
83 included in this manuscript. This study was approved by the Mayo Clinic institutional review board.

84 **Serum Specimens Collected for Specificity Studies.** A total of 149 healthy adult donor serum samples
85 collected in 2018, prior to the SARS-CoV-2 outbreak (stored at -70°C) and 105 de-identified patient sera
86 submitted for testing as part of routine clinical care in January and early February 2020, were evaluated
87 by each assay to assess specificity. The 105 patient serum samples comprised the cross-reactivity panel
88 for this study, which included sera positive for IgM and/or IgG antibodies against cytomegalovirus
89 (N=15), influenza (N=10), *Mycoplasma pneumoniae* (N=15), *Chlamydia pneumoniae* (N=21), and 12
90 sera from patients positive for *Streptococcus pneumoniae* urinary antigen. Additionally, sera were
91 collected from patients positive by a respiratory pathogen RT-PCR panel for commonly circulating
92 coronaviruses (N=6), influenza (N=14), metapneumovirus (N=4), respiratory syncytial virus (N=3),
93 adenovirus (N=6) or rhinovirus/enterovirus (N=1). Among these 254 sera, antibodies to HBV surface
94 antigen, HIV and HCV were detected in 90, 20 and 6 samples, respectively.

95 **Serologic Assays**

96 **Euroimmun Anti-SARS-CoV-2 IgG ELISA (Lübeck, Germany).** The Euroimmun (EI) anti-SARS-CoV-2 IgG
97 ELISA is based on a recombinant S1 protein from the SARS-CoV-2 spike protein. Testing was performed
98 in accordance with manufacturer instructions. Patient sera was diluted 1:101 in sample buffer, added to

99 antigen-coated microtiter wells and incubated at 37°C for 60 minutes alongside undiluted calibrator,
100 negative and positive controls. The microtiter wells were washed and 100 µl of peroxidase-labelled anti-
101 human IgG antibody enzyme conjugate was added and allowed to incubate at 37°C for 30 minutes. After
102 a second wash step, 100 µl of chromogen/substrate (TMB/H₂O₂) was added and incubated at room
103 temperature for 30 minutes. Finally, 100 µl of 0.5M sulfuric acid stop solution was added and the optical
104 density (OD) was spectrophotometrically measured at 450nm with a 620nm reference filter. Patient
105 index values were calculated by dividing patient sera OD values by the mean of the duplicate calibrator
106 OD values. Index values (signal to cut-off [S/Co] ratios) of <0.8, ≥0.8 to <1.1, and ≥1.1 were interpreted
107 as negative, indeterminate, and positive, respectively, per the instructions for use. All testing was
108 performed on AGILITY® automated ELISA analyzers (DYNEX Technologies Inc., Chantilly, VA).

109 **Epitope Diagnostics Inc. Novel Coronavirus COVID-19 IgG ELISA (San Diego, CA).** The Epitope
110 Diagnostics Inc. (EDI) COVID-19 IgG ELISA employs a full length recombinant nucleocapsid protein from
111 SARS-CoV-2. All samples were tested in singlet and the assay was performed on the AGILITY® automated
112 ELISA analyzers. Testing was performed at room temperature, with 100 µl of undiluted negative and
113 positive control, and patient sera diluted 1:101 in sample diluent, added to antigen-coated microwells
114 and allowed to incubate for 30 minutes. Following a wash step, 100 µl of horseradish peroxidase-
115 conjugated anti-human IgG was added and incubated for 30 minutes. After washing, 100 µl of
116 chromogen/substrate (TMB/ H₂O₂) was added and incubated for 20 minutes, followed by addition of
117 100 µl of 0.5M sulfuric acid stop solution, and OD measurement at 450nm. Patient index values were
118 calculated by dividing patient sera OD values by the mean of the triplicate negative control OD values
119 plus 0.18. The qualitative index value cutoff thresholds used for negative, indeterminate and positive
120 results were <1.01, ≥1.01 to <1.21, and ≥1.21, respectively.

121 **Abbott Laboratories SARS-CoV-2 IgG Chemiluminescent Microparticle Immunoassay (CMIA; Abbott**
122 **Park, IL).** The Abbott Laboratories SARS-CoV-2 IgG assay is a two-step qualitative CMIA which employs
123 acridinium ester mediated chemiluminescence and is performed on the ARCHITECT i2000SR automated
124 immunoassay analyzer. Testing was performed in accordance with manufacturer instructions. A
125 combination of sample, SARS-CoV-2 nucleocapsid antigen coated paramagnetic microparticles, and
126 assay diluent are added to a reaction vessel which allows specific antibodies present in patient sample
127 bind to the antigen coated microparticles. Following an incubation step the mixture is washed and anti-
128 human IgG acridinium-labeled conjugate is added to create a reaction mixture. After incubation and a
129 wash step, H₂O₂ and NaOH are added and the resulting chemiluminescent reaction is measured by the
130 ARCHITECT i2000SR system optics. The patient sample signal is divided by the calibrator signal, with
131 calculated signal to cutoff (S/C) values of <1.4 and ≥1.4 reported as negative and positive, respectively.

132 **Ortho-Clinical Diagnostics VITROS Anti-SARS-CoV-2 IgG Chemiluminescent Immunoassay (CLIA;**
133 **Rochester, NY).** The Ortho-Clinical Diagnostics anti-SARS-CoV-2 IgG assay is a qualitative CLIA utilizing
134 luminol-horseradish peroxidase (HRP) mediated chemiluminescence that is performed on the VITROS
135 3600 automated immunoassay analyzer. Testing was performed in accordance with manufacturer
136 instructions. In the first stage, specific antibodies present in patient sample react with recombinant
137 SARS-CoV-2 spike antigen coated to reaction wells, and after an incubation period, unbound materials
138 are removed by a washing step. For the second stage, horseradish peroxidase (HRP)-labeled anti-human
139 IgG antibody conjugate is added, allowed to incubate, and followed with a wash step to remove
140 unbound conjugate. Finally, luminogenic substrate reagent and electron transfer agents are added to
141 the reaction wells. The HRP in the bound conjugate catalyzes oxidation of the luminol which produces a
142 chemiluminescent reaction measured by a luminometer. The patient sample signal is divided by the
143 calibrator signal, with the resulted signal to cutoff (S/C) values of <1.00 and ≥1.00 corresponding to non-
144 reactive and reactive results, respectively.

145 A summary of the attributes of these four assays, including assay principle, EUA status, timing to first
146 result and throughput, are summarized in Table 1.

147 **Statistics.** Sensitivity, specificity and 95% confidence intervals (CIs) were determined using GraphPad
148 QuickCalcs software (La Jolla, CA, USA; <https://www.graphpad.com/quickcalcs>). All graphs and figures
149 were created in Microsoft Excel. For statistical analysis, indeterminate results by the Euroimmun and
150 Epitope anti-SARS-CoV-2 IgG ELISAs were considered 'negative'.

151

152 **Results**

153 **COVID-19 Patient Demographics and Serial Serum Collection.** Median age of the 33 in-patients was 61
154 years (range: 24 to 90 years) and 61% (20/33) were male. A total of 190 residual sera were collected
155 from these in-patients, ranging in timeframe from 0 to 26 days post symptom onset and 26 patients had
156 two or more serial serum samples collected (range 2 to 17 serial sera) (Table 2). Among the 23 out-
157 patients, the median age was 37 years (range: 21 to 64 years) and 43% (10/23) were male. Review of
158 medical records for this group revealed that the date of symptom onset was not routinely documented;
159 therefore date of serum sample collection was compared to the date of first positive SARS-CoV-2 RT-PCR
160 result. In total, 34 sera were prospectively collected from the out-patient group: 11 patients had both
161 baseline and convalescent serum samples collected at 3 to 7 days and 20 to 31 days post-initial positive
162 SARS-CoV-2 RT-PCR result, respectively, and the remaining 12 out-patients only had a convalescent
163 sample collected (Table 2).

164 **Sensitivity of the anti-SARS-CoV-2 IgG serologic assays.** Among samples collected from hospitalized
165 patients, the Abbott, Epitope, Euroimmun and Ortho-Clinical anti-SARS-CoV-2 IgG assays were positive
166 in 10.5% (4/38), 2.6% (1/38), 0% (0/38) and 2.6% (1/38) sera collected seven days or less post reported
167 symptom onset (Table 3). Sensitivity increased for all four assays in samples collected eight to 14 days

168 post symptom onset (range: 27.5% to 49.5%), and all except the Abbott assay achieved 100% sensitivity
169 in samples collected at least 15 days following initial disease manifestation. Sensitivity of the Abbott
170 assay was 91.8% (56/61) in samples collected 15 days or longer post symptom onset, however the five
171 negative sera were all from a single patient who seroconverted at day 20 post onset. At the individual
172 patient level, all in-patients who had a serum sample available after 14 days of symptoms (N=14),
173 seroconverted by all four IgG assays. The temporal kinetics of the anti-SARS-CoV-2 IgG antibody
174 response for each of the four evaluated assays is shown in Figure 1 for in-patients who seroconverted
175 during their hospital stay and who had at least five serial draws available (N=11). The S/Co ratios
176 increased over time for all patients and assays, and while seroconversion was observed as early as days
177 six to nine by all methods, the median time to seroconversion was day 12 for the Abbott and Epitope
178 assays, day 13 for the Ortho-Clinical assay and day 14 for the Euroimmun assay.

179 Among the 23 out-patients, the Abbott, Epitope, Euroimmun and Ortho-Clinical anti-SARS-CoV-2
180 IgG assays were positive in 18.2% (2/11), 9.1% (1/11), 18.2% (2/11) and 9.1% (1/11) of samples collected
181 seven days or less post initial positive SARS-CoV-2 RT-PCR assay (Table 3). Similar to the in-patient
182 samples, sensitivity of the Abbott, Euroimmun, and Ortho-Clinical IgG assays increased significantly to
183 95.7% (22/23), 91.3% (21/23) and 95.7% (22/23), respectively, in samples collected 20 to 31 days
184 following an initial positive molecular test (Tables 3). While sensitivity of the Epitope anti-SARS-CoV-2
185 IgG ELISA also increased in this convalescent sample set to 56.5% (13/23), it was notably lower than the
186 other three assays. Combining both in-patient and out-patient sensitivity rates in convalescent serum
187 samples collected at least 15 days post symptom onset or post initial positive RT-PCR result, the overall
188 sensitivity of the Abbott, Epitope, Euroimmun and Ortho-Clinical IgG assays was 92.9% (78/84), 88.1%
189 (74/84), 97.6% (82/84) and 98.8% (83/84), respectively, with overlapping 95% confidence intervals
190 suggesting that these differences are insignificant (Table 4). At the individual patient level, among the 37
191 patients with at least one serum sample collected 15 days post symptom onset (N= 14) or following the

192 first positive SARS-CoV-2 molecular test (N=23), the sensitivity of the Abbott, Epitope, Euroimmun and
193 Ortho-Clinical anti-SARS-CoV-2 IgG assays was 97.3% (36/37), 73% (27/37), 94.6% (35/37) and 97.3%
194 (36/37), respectively (Table 4). Notably, the 95% confidence intervals for the Abbott and Ortho-Clinical
195 assays did not overlap with the Epitope ELISA. Although all four assays are qualitative in design, the
196 mean S/Co ratios for the Epitope, Euroimmun and Ortho-Clinical anti-SARS-CoV-2 IgG assays were nearly
197 double in convalescent sera from hospitalized patients versus convalescent sera from out-patients: S/Co
198 3.8 versus 1.7 for Epitope, S/Co 8.5 versus 4.0 for Euroimmun and S/Co 15.7 versus 9.0 for Ortho-
199 Clinical, respectively (Figure 2). The difference in mean S/Co ratios in convalescent samples from in-
200 patients and out-patients (S/Co 5.5 versus 4.3) for the Abbott anti-SARS-CoV-2 IgG assay was not as
201 high.

202 **Specificity of the anti-SARS-CoV-2 IgG Assays.** Among 149 sera collected from healthy adults in 2018,
203 the Abbott, Epitope, Euroimmun and Ortho-Clinical anti-SARS-CoV-2 IgG assays were negative in 100%
204 (149/149), 100% (149/149), 99.3% (148/149) and 99.3% (148/149) of samples, respectively (Table 3).
205 Using a set of 105 sera collected in late January to early February 2020, which were positive for
206 antibodies to or nucleic acid from other respiratory viral or bacterial pathogens, the Abbott, Epitope,
207 Euroimmun and Ortho-Clinical IgG assays were negative in 99% (104/105), 99% (104/105; 3
208 indeterminate), 96.2% (101/105; 1 indeterminate) and 100% (105/105) of these samples, respectively.
209 When combined with the healthy donor results, overall specificity was 99.6% (95% CI: 97.6% - 100%),
210 99.6% (95% CI: 97.6% - 100%), 98.0% (95% CI: 95.3% - 99.3%) and 99.6% (95% CI: 97.6% - 100%),
211 respectively (Table 3). For this analysis, 'indeterminate' results yielded by the Epitope and Euroimmun
212 anti-SARS-CoV-2 IgG ELISAs were considered 'negative,' however if samples with 'indeterminate' results
213 were grouped as 'positive', the overall specificity of these two assays decreases to 98% (249/254; 95%
214 CI: 95.3% - 99.3%) and 97.2% (247/254; 95% CI: 94.3% - 98.8%), respectively. The Abbott CMIA was
215 positive (S/Co: 1.77) in a single serum sample positive for IgM and IgG antibodies against *C. pneumoniae*,

216 the Epitope ELISA was positive in one serum sample with antibodies to *C. pneumoniae* (S/Co 1.67), the
217 Euroimmun ELISA was positive in one anti-*C. pneumoniae* IgG positive sera (S/Co 2.09), one serum
218 sample (S/Co 1.91) from an RSV RT-PCR positive patient, and two sera (S/Co 2.89, 2.98) from patients
219 positive for *Streptococcus pneumoniae* antigen in urine (Figure 2; data not shown). Notably, none of the
220 samples positive by one assay were positive by any of the other three assays, suggesting that these are
221 all false-positive results. Using a prevalence rate of 5%, our reported convalescent sensitivity in
222 individual patients and the overall specificity values, the positive predictive values of the Abbott,
223 Epitope, Euroimmun and Ortho-Clinical anti-SARS-CoV-2 IgG immunoassays is 92.8% (95% CI: 81.6% -
224 97.5%), 90.6% (95% CI: 79.4% - 98.1%), 71.2% (95% CI: 59.3% - 80.8%) and 92.5% (95% CI: 81.6% -
225 97.5%), respectively.

226

227 Discussion

228 This study presents a head-to-head comparison of four high-throughput, commercially available
229 anti-SARS-CoV-2 IgG serologic tests from Abbott Laboratories, Epitope Diagnostics Inc, Euroimmun, and
230 Ortho-Clinical Diagnostics, using serially collected acute and convalescent sera from both hospitalized
231 patients and out-patients with RT-PCR confirmed COVID-19. We show that less than 20% (range 0 –
232 18.2%) of serum samples collected within seven days of symptom onset or first positive SARS-CoV-2 RT-
233 PCR result as positive by any of the four evaluated methods. This is consistent with other studies
234 evaluating the Abbott, Epitope and Euroimmun anti-SARS-CoV-2 IgG assays (6-9). Seroconversion to
235 anti-SARS-CoV-2 IgG positive in most individuals occurs towards the end of week two post infection, and
236 our data continue to underscore the importance of not solely relying on such testing to diagnose
237 infection in acutely symptomatic patients (10, 11). As with other infections however, detecting
238 seroconversion between acute and convalescent samples, collected 7 to 14 days apart, may be helpful

239 to diagnose recent COVID-19 infection in certain, challenging scenarios. Sensitivity increased
240 significantly in sera collected 15 days or longer post symptom onset, and with the exception of the
241 Epitope anti-SARS-CoV-2 IgG ELISA, all three remaining assays were positive in 92% to 97% of
242 convalescent samples. At the individual patient level, the Abbott, Euroimmun and Ortho-Clinical anti-
243 SARS-CoV-2 IgG immunoassays were positive in 94% to 97% of patients tested during the convalescent
244 phase. Although neither of these tests achieved 100% sensitivity, with one to two COVID-19 confirmed
245 out-patients remaining seronegative or indeterminate at day 22 to 27 post initial diagnosis, our findings
246 indicate that the majority of infected individuals develop an immune response to SARS-CoV-2,
247 irrespective of disease severity or the viral antigen used in the immunoassay.

248 The notable exception to this is the Epitope Diagnostics anti-SARS-CoV-2 IgG ELISA, which
249 although positive in all serum samples collected from hospitalized patients with more than 15 days of
250 symptoms, yielded negative results in 43.5% of out-patients tested during the convalescent phase.
251 Additional serial serum samples to determine whether seroconversion by this assay occurred at a later
252 time point were unavailable. The overall sensitivity of the Epitope IgG assay in convalescent sera was
253 88% in our study, similar to the 90% sensitivity reported by a recent pre-print manuscript in sera
254 collected 20 days or longer post symptom onset (9). The Epitope IgG assay is based on a recombinant
255 SARS-CoV-2 nucleocapsid protein, considered the most abundant coronavirus protein. Although prior
256 studies suggest later seroconversion of anti-nucleocapsid based assays, we did not observe a notable
257 delay with the Abbott IgG CMIA, which also targets antibodies to the nucleocapsid, and the median time
258 to seroconversion between all assays was similar (12-14 days) (10, 12). Collectively, these data suggest
259 lower sensitivity of the Epitope IgG ELISA, which is potentially limited to mildly ill patients, although a
260 definitive reason for this remains unclear.

261 The specificity of the four evaluated assays was high, above 97%, for all assays using sera from
262 both healthy donors collected prior to the pandemic and samples positive for antibodies to or nucleic
263 acid from other viral or bacterial pathogens. While we identified positive results for three of these
264 assays in a number of samples positive for antibodies to bacterial pathogens, it is unlikely that they are
265 causing the anti-SARS-CoV-2 IgG positivity. Although possible that these samples, collected in January
266 and February of 2020, were from patients previously infected with SARS-CoV-2, none of them were
267 positive by more than one anti-SARS-CoV-2 IgG assay, suggesting that the observed reactivity was non-
268 specific. Specificity of the Abbott anti-SARS-CoV-2 IgG assay was recently reported to be 99.4% and
269 99.9% by two separate studies, consistent with our findings (6, 8). Two prior published studies have
270 evaluated the Euroimmun anti-SARS-CoV-2 IgG ELISA and shown specificity ranges of approximately 95%
271 (145/153) to 96% (195/203), which is lower than what we report here (97% to 98%) and may be
272 attributed to differences in the populations tested, inter-lab performance or lot-to-lot test kit variability
273 (7, 8). Specificity of the Eitope anti-SARS-CoV-2 IgG ELISA was reported to range from 85% to 90% by
274 the aforementioned pre-print study, which is significantly lower than our overall finding of 99.6% (9).
275 The specificity differences may be due to multiple factors, including our use of an automated ELISA
276 platform to perform the testing, which may provide higher processing consistency, and our use of a
277 higher S/Co threshold for positive results (≥ 1.21) compared to those recommended by the manufacturer
278 (≥ 1.01). Notably, application of the lower S/Co threshold for positive results also yielded high overall
279 specificity of 98% using our sample set. Finally, to our knowledge, this is the first study to independently
280 report on the specificity of the Ortho-Clinical anti-SARS-CoV-2 IgG immunoassay, which we found to be
281 99.6%.

282 Although we report high specificity for the four assays evaluated here, the overall accuracy of
283 these tests will be impacted significantly by the prevalence of the disease in the population tested. The
284 current prevalence of SARS-CoV-2 in the United States and in other countries remains largely unknown

285 due to the high rates of asymptomatic infection and will vary from region-to-region. For assay
286 comparison purposes, the FDA has chosen a 5% prevalence benchmark with which to evaluate the
287 positive (PPV) and negative predictive values (NPV) for EUA assays. Based on this prevalence rate, our
288 reported overall specificity and sensitivity in individual convalescent patients, we found that while the
289 PPVs did not significantly differ between the Abbott, Epitope and Ortho-Clinical anti-SARS-CoV-2 IgG
290 immunoassays (90%-92%), the Euroimmun assay was associated with a notably lower PPV (71%),
291 despite a high overall specificity of 98%. In an effort to improve on the PPV given the still low prevalence
292 of SARS-CoV-2 in many regions of the United States, the FDA has suggested that laboratories consider
293 confirming initial antibody positive results with a second serologic assay, based on an alternative viral
294 target antigen. Confirmatory testing of initially antibody positive samples is not an uncommon practice
295 to improve upon the overall diagnostic accuracy of serologic tests, and such an algorithmic approach will
296 likely be useful, particularly for assays with lower specificity profiles.

297 There are a number of limitations associated with this study. First, the number of unique COVID-
298 19 patients included in the in-patient and out-patient groups was low. However, aside from the Epitope
299 anti-SARS-CoV-2 IgG ELISA, differences in sensitivity between these two groups were not notable for the
300 other three assays. Second, specificity of these assays was not evaluated using samples which were
301 known to be positive for antibodies to the commonly circulating human coronaviruses (*i.e.*, 229E, NL63,
302 OC43, HKU1). Prior seroprevalence studies show that 65% to 75% of children have antibodies to at least
303 one of the commonly circulating coronaviruses, and over 90% of adults over the age of 50 years are
304 seropositive for antibodies to all four common coronaviruses (13, 14). Collectively, based on these
305 seroprevalence findings and the overall low false positivity rates identified in this study, we anticipate
306 that although false positive reactions due to antibodies against other common coronaviruses can occur
307 by these assays, the rate appears to be low. Additionally, samples in the cross-reactivity panel were
308 collected during January and early February of 2020, thus preventing us from definitively ruling out prior

309 infection with SARS-CoV-2, and potentially negatively biasing our overall specificity rate. However,
310 applying the FDA suggested approach of confirming positive samples with an assay based on an
311 alternative SARS-CoV-2 antigen, all samples with positive results in this panel would be considered
312 falsely positive. Third, due to lack of reporting, timing of serum collection for out-patients was compared
313 to the first positive SARS-CoV-2 RT-PCR result, which may have led to an overestimation of anti-SARS-
314 CoV-2 IgG assay sensitivity compared to true date of infection. Similarly, symptom onset in hospitalized
315 patients was extrapolated based on date reported by the patient or assumed by the provider, which
316 may be subject to recall inaccuracies. Finally, correlation of results from these assays relative to a
317 neutralizing antibody (NABs) test was not performed. Anti-SARS-CoV-2 NABs primarily develop against
318 the spike protein, specifically to the S1 receptor-binding domain, and initial studies indicate that NAB
319 titers correlate better to spike-based assays as compared to nucleocapsid assays (10, 15). While one
320 study has shown high correlation of NABs with the Euroimmun IgG assay, as NAB assays become
321 increasingly accessible, more detailed correlation studies across assays will be possible (7).

322 In conclusion, we show that the Abbott, Epitope, Euroimmun and Ortho-Clinical anti-SARS-CoV-2
323 IgG immunoassays perform similarly with respect to sensitivity in COVID-19 hospitalized patients, and
324 with the exception of the Epitope assay, also in individuals with milder forms of the infection. The
325 Abbott and Ortho-Clinical anti-SARS-CoV-2 IgG immunoassays provided the highest overall specificity at
326 over 99%. Given that one of the primary uses of serologic assays (at this time) is for the purpose of
327 monitoring local and regional seroprevalence, and that SARS-CoV-2 prevalence rates remain largely
328 unknown, it will be important to use immunoassay(s) with the highest specificity in an effort to
329 maximize the PPV of results. With the unprecedented influx of commercially available test kits for
330 detection of antibodies against SARS-CoV-2, it remains imperative that laboratories thoroughly evaluate
331 these assays for accuracy prior to implementation.

332 **Acknowledgement**

333 The authors would like to acknowledge all of the patients who consented to participate in this study and
334 the mobile phlebotomy unit at Mayo Clinic for their assistance in collecting acute and convalescent sera
335 from out-patients confirmed positive for COVID-19. We also thank all of the front line workers both at
336 the bedside and at the bench for their incredible efforts during this global pandemic.

Table. 1 Attributes for the Evaluated Anti-SARS-CoV-2 IgG Serologic Assays				
	Abbott	Epitope	Euroimmun	Ortho-Clinical
Assay principle ^a	CMIA	ELISA	ELISA	CLIA
Solid-phase antigen	Nucleocapsid	Nucleocapsid	Spike S1	Spike
Specimen type	Serum, plasma	Serum	Serum	Serum
Sample volume	25 µL	10 µL	10 µL	20 µL
EUA status	Granted	Submitted	Granted	Granted
Result calculation	Index (S/Co)	Index (S/Co)	Index (S/Co)	Index (S/Co)
Positive Cutoff Threshold	≥ 1.4	≥ 1.21 ^b	≥ 1.1	≥ 1.00
Indeterminate Cut-off Range	N/A	≥ 1.01 to < 1.21 ^b	≥ 0.8 to < 1.1	N/A
Operational type	Continuous random access	Batch	Batch	Continuous, random access
Time to first result	29 min	80 min	120 min	48 min
Test throughput per 8 hr	1600	630 ^c	630 ^c	1040 to 1200

337 Abbreviations: CMIA, chemiluminescent microparticle immunoassay; ELISA, enzyme-linked
 338 immunosorbent assay; CLIA, chemiluminescence immunoassay; EUA, emergency use authorization;
 339 S/Co, signal-to-cut-off; N/A, not applicable.

340 ^a All assays are indirect in format

341 ^b Laboratory determined cut-off threshold

342 ^c Testing performed on the Dynex Agility automated ELISA processor (Chantilly, VA)

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Table 2. COVID-19 Confirmed Patient Demographics (N=56) and Collected Serum Samples (N=224)		
	In-Patient	Out-Patient
Number of Patients	33	23
Median Age in years (range)	61 (24-90)	37 (21 – 64)
Male (%)	20 (61%)	10 (43%)
Number of serum samples collected at days post-symptom onset:		
0 to 7 days	38	NA
8-14 days	91	NA
15 to 26 days	61	NA
Number of serum samples collected at days post first positive SARS-CoV-2 RT-PCR result:		
0 to 7 days	NA	11
20 to 31 days	NA	23

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Table 3. Performance Characteristics of Four Commercially Available anti-SARS-CoV-2 IgG Assays

	% Sensitivity (No. of Samples)					% Specificity (No. of Samples)		
	Serum Samples from In-Patients, Days Post-Symptom Onset ^a			Serum Samples from Out-Patients, Days Post First RT-PCR Positive Result ^b		Healthy Donors	Cross-Reactivity Panel	Overall (95% CI)
	≤ 7	8-14	≥15	≤ 7	≥20			
Abbott	10.5% (4/38)	49.5% (45/91)	91.8% (56/61)	18.2% (2/11)	95.7% (22/23)	100% (149/149)	99% (104/105)	99.6% (97.6% - 100%)
Epitope	2.6% (1/38)	45.1% (41/91)	100% (61/61)	9.1% (1/11)	56.5% (13/23)	100% (149/149)	99% (104/105)	99.6% (97.6% - 100%) ^c
Euroimmun	0% (0/38)	27.5% (25/91)	100% (61/61)	18.2% (2/11)	91.3% (21/23)	99.3% (148/149)	96.2% (101/105)	98% (95.3% - 99.3%) ^d
Ortho-Clinical	2.6% (1/38)	38.5% (35/91)	100% (61/61)	9.1% (1/11)	95.7% (22/23)	99.3% (148/149)	100% (105/105)	99.6% (97.6% - 100%)

^aThe number of unique patients comprising samples collected ≤7, 8 to 14 and ≥15 days post symptom onset was 11, 28 and 14, respectively.

^bThe number of unique patients comprising samples collected ≤7 and ≥15 days post first positive RT-PCR result was 11 and 23, respectively.

^cOverall specificity is 98% (95% CI: 95.3% - 99.3%) if indeterminate results are counted as 'positive'.

^dOverall specificity is 97.2% (95% CI: 94.3% - 98.8%) in indeterminate results are counted as 'positive'.

350

Table 4. Anti-SARS-CoV-2 IgG assay sensitivity in convalescent sera and in individual patients tested ≥15 days post-symptom onset or first positive SARS-CoV-2 RT-PCR result

	Serum Samples (N=84) ^a		Individuals Patients (N=37) ^b	
	% Positive	95% CI	% Positive ^c	95% CI
Abbott	92.9% (78/84)	85% - 97%	97.3% (36/37)	85% - 100%
Epitope	88.1% (74/84)	79.3% - 93.6%	73% (27/37)	56.9% - 84.8%
Euroimmun	97.6% (82/84)	91.2% - 99.9%	94.6% (35/37)	81.4% - 99.4%
Ortho-Clinical	98.8% (83/84)	92.9% - 100%	97.3% (36/37)	85% - 100%

^aThe 84 serum samples included 61 sera collected at least 15 days post-symptom onset from in-patients and 23 sera collected 20 days or longer post initial positive SARS-CoV-2 RT-PCR result in outpatients (see Table 3).

^bThe 37 individual patients included all 23 out-patients with sera collected 20 days or longer post initial positive SARS-CoV-2 RT-PCR result and 14 in-patients who had a serum sample collected 15 days or longer post-symptom onset. Patients were counted as positive if at least one sample collected 15 days or longer post onset or first RT-PCR was positive.

^cOne patient was negative by the Euroimmun and Ortho-Clinical assays, and a second patient was negative by both the Epitope and Abbott assays. One and two patients respectively were indeterminate by the Euroimmun and Epitope assays, but positive by all other assays.

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412 **Figure Legends**

413 **Figure 1. Anti-SARS-CoV-2 IgG Kinetics in COVID-19 RT-PCR Confirmed In-Patients.**

414 Signal to cut-off (S/Co) values for the SARS-CoV-2 IgG assays from (A) Abbott
415 Laboratories, (B) Epitope Diagnostics Inc., (C) Euroimmun and (D) Ortho-Clinical
416 Diagnostics, were plotted against days post symptom onset for all in-patients with at
417 least five serial serum samples. Each symbol indicates an individual patient (N=11).

418

419 **Figure 2. Signal to Cut-off (Index Value) Distribution Among SARS-CoV-2 RT-PCR Confirmed Patients**

420 **and Controls.** Signal to cut-off (S/Co) ratios are shown relative to days post symptom onset for in-
421 patients (A) or days post first SARS-CoV-2 RT-PCR positive result in out-patients (B). Signal intensities are
422 also shown for healthy control samples collected in 2018 and for cross-reactivity panel (C). Grey shaded
423 bars represent the S/Co range for positive results by the four assays (range: 1.0 to 1.4 S/Co).
424 Abbreviations: AB, Abbott Laboratories; EDI, Epitope Diagnostics Inc.; EI, Euroimmun; OCD, Ortho-
425 Clinical Diagnostics.

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