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

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Abstract

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

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

While the Food and Drug Administration (FDA) required Emergency Use Authorization (EUA) for SARS-CoV-2 molecular assays at the outset of the outbreak, until recently, serologic tests for SARS-CoV-2 fell under the Section IV.D of the FDA’s “Policy for Diagnostic Tests for Coronavirus Disease-2019” (i.e.,
‘Pathway D’), which required commercial manufacturers to only notify the FDA of their validated product, ultimately leading to the rapid influx of over 180 commercially available SARS-CoV-2 serologic test kits (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 require all commercial manufacturers to submit their validation data for EUA, and to date, 14 manufacturers or laboratories have received EUA for their serologic test. The commercially available serologic assays vary in the antibody detected (i.e., IgM, IgA, IgG, or total antibody), the targeted SARS-CoV-2 antigen (i.e., subunit 1 [S1] of the spike protein, the nucleocapsid protein [NP], the receptor binding domain [RBD]) and the method used (e.g., lateral flow assay [LFA], enzyme-linked immunosorbent assay [ELISA], chemiluminescent immunoassay [CLIA], etc.), with few peer-reviewed studies currently available which independently evaluate their performance characteristics.

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

Methods

Specimens Collected from COVID-19 Confirmed Patients. A total of 224 serum samples were collected between March and April 2020 from 56 adult patients confirmed positive for COVID-19 by a laboratory developed or commercially available FDA EUA SARS-CoV-2 RT-PCR assay, performed on a nasopharyngeal swab (5). Among the 56 COVID-19 RT-PCR confirmed patients, 33 were hospitalized (in-patient group) and 23 were treated as out-patients (out-patient group). Remnant, serial serum samples
were collected as available throughout the hospital stay for the in-patient group until discharge, whereas prospective collection of acute (≤7 days post first RT-PCR positive result) and convalescent (i.e., >20 days post first RT-PCR positive result) sera was completed for out-patients. Medical records were reviewed to determine date of symptom onset, or if unavailable, the date of first SARS-CoV-2 RT-PCR positive result, and to identify whether the patient was hospitalized or treated as an out-patient. The focus of this study was to assess assay performance characteristics, therefore although patient demographics, presenting symptoms, disease course and outcomes were documented, they were not included in this manuscript. This study was approved by the Mayo Clinic institutional review board.

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

Serologic Assays

Euroimmun Anti-SARS-CoV-2 IgG ELISA (Lübeck, Germany). The Euroimmun (EI) anti-SARS-CoV-2 IgG ELISA is based on a recombinant S1 protein from the SARS-CoV-2 spike protein. Testing was performed in accordance with manufacturer instructions. Patient sera was diluted 1:101 in sample buffer, added to
antigen-coated microtiter wells and incubated at 37°C for 60 minutes alongside undiluted calibrator, negative and positive controls. The microtiter wells were washed and 100 µl of peroxidase-labelled anti-human IgG antibody enzyme conjugate was added and allowed to incubate at 37°C for 30 minutes. After a second wash step, 100 µl of chromogen/substrate (TMB/H₂O₂) was added and incubated at room temperature for 30 minutes. Finally, 100 µl of 0.5M sulfuric acid stop solution was added and the optical density (OD) was spectrophotometrically measured at 450nm with a 620nm reference filter. Patient index values were calculated by dividing patient sera OD values by the mean of the duplicate calibrator OD values. Index values (signal to cut-off [S/Co] ratios) of <0.8, ≥0.8 to <1.1, and ≥1.1 were interpreted as negative, indeterminate, and positive, respectively, per the instructions for use. All testing was performed on AGILITY™ automated ELISA analyzers (DYNEX Technologies Inc., Chantilly, VA).

Epitope Diagnostics Inc. Novel Coronavirus COVID-19 IgG ELISA (San Diego, CA). The Epitope Diagnostics Inc. (EDI) COVID-19 IgG ELISA employs a full length recombinant nucleocapsid protein from SARS-CoV-2. All samples were tested in singlet and the assay was performed on the AGILITY™ automated ELISA analyzers. Testing was performed at room temperature, with 100 µl of undiluted negative and positive control, and patient sera diluted 1:101 in sample diluent, added to antigen-coated microwells and allowed to incubate for 30 minutes. Following a wash step, 100 µl of horseradish peroxidase-conjugated anti-human IgG was added and incubated for 30 minutes. After washing, 100 µl of chromogen/substrate (TMB/ H₂O₂) was added and incubated for 20 minutes, followed by addition of 100 µl of 0.5M sulfuric acid stop solution, and OD measurement at 450nm. Patient index values were calculated by dividing patient sera OD values by the mean of the triplicate negative control OD values plus 0.18. The qualitative index value cutoff thresholds used for negative, indeterminate and positive results were <1.01, ≥1.01 to <1.21, and ≥1.21, respectively.
Abbott Laboratories SARS-CoV-2 IgG Chemiluminescent Microparticle Immunoassay (CMIA; Abbott Park, IL). The Abbott Laboratories SARS-CoV-2 IgG assay is a two-step qualitative CMIA which employs acridinium ester mediated chemiluminescence and is performed on the ARCHITECT i2000SR automated immunoassay analyzer. Testing was performed in accordance with manufacturer instructions. A combination of sample, SARS-CoV-2 nucleocapsid antigen coated paramagnetic microparticles, and assay diluent are added to a reaction vessel which allows specific antibodies present in patient sample bind to the antigen coated microparticles. Following an incubation step the mixture is washed and anti-human IgG acridinium-labeled conjugate is added to create a reaction mixture. After incubation and a wash step, H₂O₂ and NaOH are added and the resulting chemiluminescent reaction is measured by the ARCHITECT i2000SR system optics. The patient sample signal is divided by the calibrator signal, with calculated signal to cutoff (S/C) values of <1.4 and ≥1.4 reported as negative and positive, respectively.

Ortho-Clinical Diagnostics VITROS Anti-SARS-CoV-2 IgG Chemiluminescent Immunoassay (CLIA; Rochester, NY). The Ortho-Clinical Diagnostics anti-SARS-CoV-2 IgG assay is a qualitative CLIA utilizing luminol-horseradish peroxidase (HRP) mediated chemiluminescence that is performed on the VITROS 3600 automated immunoassay analyzer. Testing was performed in accordance with manufacturer instructions. In the first stage, specific antibodies present in patient sample react with recombinant SARS-CoV-2 spike antigen coated to reaction wells, and after an incubation period, unbound materials are removed by a washing step. For the second stage, horseradish peroxidase (HRP)-labeled anti-human IgG antibody conjugate is added, allowed to incubate, and followed with a wash step to remove unbound conjugate. Finally, luminogenic substrate reagent and electron transfer agents are added to the reaction wells. The HRP in the bound conjugate catalyzes oxidation of the luminol which produces a chemiluminescent reaction measured by a luminometer. The patient sample signal is divided by the calibrator signal, with the resulted signal to cutoff (S/C) values of <1.00 and ≥1.00 corresponding to non-reactive and reactive results, respectively.
A summary of the attributes of these four assays, including assay principle, EUA status, timing to first result and throughput, are summarized in Table 1.

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

Results

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

Sensitivity of the anti-SARS-CoV-2 IgG serologic assays. Among samples collected from hospitalized patients, the Abbott, Epitope, Euroimmun and Ortho-Clinical anti-SARS-CoV-2 IgG assays were positive 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 symptom onset (Table 3). Sensitivity increased for all four assays in samples collected eight to 14 days...
post symptom onset (range: 27.5% to 49.5%), and all except the Abbott assay achieved 100% sensitivity in samples collected at least 15 days following initial disease manifestation. Sensitivity of the Abbott assay was 91.8% (56/61) in samples collected 15 days or longer post symptom onset, however the five negative sera were all from a single patient who seroconverted at day 20 post onset. At the individual patient level, all in-patients who had a serum sample available after 14 days of symptoms (N=14), seroconverted by all four IgG assays. The temporal kinetics of the anti-SARS-CoV-2 IgG antibody response for each of the four evaluated assays is shown in Figure 1 for in-patients who seroconverted during their hospital stay and who had at least five serial draws available (N=11). The S/Co ratios increased over time for all patients and assays, and while seroconversion was observed as early as days six to nine by all methods, the median time to seroconversion was day 12 for the Abbott and Epitope assays, day 13 for the Ortho-Clinical assay and day 14 for the Euroimmun assay.

Among the 23 out-patients, the Abbott, Epitope, Euroimmun and Ortho-Clinical anti-SARS-CoV-2 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 seven days or less post initial positive SARS-CoV-2 RT-PCR assay (Table 3). Similar to the in-patient samples, sensitivity of the Abbott, Euroimmun, and Ortho-Clinical IgG assays increased significantly to 95.7% (22/23), 91.3% (21/23) and 95.7% (22/23), respectively, in samples collected 20 to 31 days following an initial positive molecular test (Tables 3). While sensitivity of the Epitope anti-SARS-CoV-2 IgG ELISA also increased in this convalescent sample set to 56.5% (13/23), it was notably lower than the other three assays. Combining both in-patient and out-patient sensitivity rates in convalescent serum samples collected at least 15 days post symptom onset or post initial positive RT-PCR result, the overall sensitivity of the Abbott, Epitope, Euroimmun and Ortho-Clinical IgG assays was 92.9% (78/84), 88.1% (74/84), 97.6% (82/84) and 98.8% (83/84), respectively, with overlapping 95% confidence intervals suggesting that these differences are insignificant (Table 4). At the individual patient level, among the 37 patients with at least one serum sample collected 15 days post symptom onset (N= 14) or following the
first positive SARS-CoV-2 molecular test (N=23), the sensitivity of the Abbott, Epitope, Euroimmun and
Ortho-Clinical anti-SARS-CoV-2 IgG assays was 97.3% (36/37), 73% (27/37), 94.6% (35/37) and 97.3%
(36/37), respectively (Table 4). Notably, the 95% confidence intervals for the Abbott and Ortho-Clinical
assays did not overlap with the Epitope ELISA. Although all four assays are qualitative in design, the
mean S/Co ratios for the Epitope, Euroimmun and Ortho-Clinical anti-SARS-CoV-2 IgG assays were nearly
double in convalescent sera from hospitalized patients versus convalescent sera from out-patients: S/Co
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-
Clinical, respectively (Figure 2). The difference in mean S/Co ratios in convalescent samples from in-
patients and out-patients (S/Co 5.5 versus 4.3) for the Abbott anti-SARS-CoV-2 IgG assay was not as
high.

Specificity of the anti-SARS-CoV-2 IgG Assays. Among 149 sera collected from healthy adults in 2018,
the Abbott, Epitope, Euroimmun and Ortho-Clinical anti-SARS-CoV-2 IgG assays were negative in 100%
(149/149), 100% (149/149), 99.3% (148/149) and 99.3% (148/149) of samples, respectively (Table 3).
Using a set of 105 sera collected in late January to early February 2020, which were positive for
antibodies to or nucleic acid from other respiratory viral or bacterial pathogens, the Abbott, Epitope,
Euroimmun and Ortho-Clinical IgG assays were negative in 99% (104/105), 99% (104/105; 3
indeterminate), 96.2% (101/105; 1 indeterminate) and 100% (105/105) of these samples, respectively.
When combined with the healthy donor results, overall specificity was 99.6% (95% CI: 97.6% - 100%),
99.6% (95% CI: 97.6% - 100%), 98.0% (95% CI: 95.3% - 99.3%) and 99.6% (95% CI: 97.6% - 100%),
respectively (Table 3). For this analysis, ‘indeterminate’ results yielded by the Epitope and Euroimmun
anti-SARS-CoV-2 IgG ELISAs were considered ‘negative,’ however if samples with ‘indeterminate’ results
were grouped as ‘positive’, the overall specificity of these two assays decreases to 98% (249/254; 95%
CI: 95.3% - 99.3%) and 97.2% (247/254; 95% CI: 94.3% - 98.8%), respectively. The Abbott CMIA was
positive (S/Co: 1.77) in a single serum sample positive for IgM and IgG antibodies against C. pneumoniae,
the Epitope ELISA was positive in one serum sample with antibodies to *C. pneumoniae* (S/Co 1.67), the Euroimmun ELISA was positive in one anti-*C. pneumoniae* IgG positive sera (S/Co 2.09), one serum sample (S/Co 1.91) from an RSV RT-PCR positive patient, and two sera (S/Co 2.89, 2.98) from patients positive for *Streptococcus pneumoniae* antigen in urine (Figure 2; data not shown). Notably, none of the samples positive by one assay were positive by any of the other three assays, suggesting that these are all false-positive results. Using a prevalence rate of 5%, our reported convalescent sensitivity in individual patients and the overall specificity values, the positive predictive values of the Abbott, Epitope, Euroimmun and Ortho-Clinical anti-SARS-CoV-2 IgG immunoassays is 92.8% (95% CI: 81.6% - 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% - 97.5%), respectively.

Discussion

This study presents a head-to-head comparison of four high-throughput, commercially available anti-SARS-CoV-2 IgG serologic tests from Abbott Laboratories, Epitope Diagnostics Inc, Euroimmun, and Ortho-Clinical Diagnostics, using serially collected acute and convalescent sera from both hospitalized patients and out-patients with RT-PCR confirmed COVID-19. We show that less than 20% (range 0 – 18.2%) of serum samples collected within seven days of symptom onset or first positive SARS-CoV-2 RT-PCR result as positive by any of the four evaluated methods. This is consistent with other studies evaluating the Abbott, Epitope and Euroimmun anti-SARS-CoV-2 IgG assays (6-9). Seroconversion to anti-SARS-CoV-2 IgG positive in most individuals occurs towards the end of week two post infection, and our data continue to underscore the importance of not solely relying on such testing to diagnose infection in acutely symptomatic patients (10, 11). As with other infections however, detecting seroconversion between acute and convalescent samples, collected 7 to 14 days apart, may be helpful
to diagnose recent COVID-19 infection in certain, challenging scenarios. Sensitivity increased significantly in sera collected 15 days or longer post symptom onset, and with the exception of the Epitope anti-SARS-CoV-2 IgG ELISA, all three remaining assays were positive in 92% to 97% of convalescent samples. At the individual patient level, the Abbott, Euroimmun and Ortho-Clinical anti-SARS-CoV-2 IgG immunoassays were positive in 94% to 97% of patients tested during the convalescent phase. Although neither of these tests achieved 100% sensitivity, with one to two COVID-19 confirmed out-patients remaining seronegative or indeterminate at day 22 to 27 post initial diagnosis, our findings indicate that the majority of infected individuals develop an immune response to SARS-CoV-2, irrespective of disease severity or the viral antigen used in the immunoassay.

The notable exception to this is the Epitope Diagnostics anti-SARS-CoV-2 IgG ELISA, which although positive in all serum samples collected from hospitalized patients with more than 15 days of symptoms, yielded negative results in 43.5% of out-patients tested during the convalescent phase. Additional serial serum samples to determine whether seroconversion by this assay occurred at a later time point were unavailable. The overall sensitivity of the Epitope IgG assay in convalescent sera was 88% in our study, similar to the 90% sensitivity reported by a recent pre-print manuscript in sera collected 20 days or longer post symptom onset (9). The Epitope IgG assay is based on a recombinant SARS-CoV-2 nucleocapsid protein, considered the most abundant coronavirus protein. Although prior studies suggest later seroconversion of anti-nucleocapsid based assays, we did not observe a notable delay with the Abbott IgG CMIA, which also targets antibodies to the nucleocapsid, and the median time to seroconversion between all assays was similar (12-14 days) (10, 12). Collectively, these data suggest lower sensitivity of the Epitope IgG ELISA, which is potentially limited to mildly ill patients, although a definitive reason for this remains unclear.
The specificity of the four evaluated assays was high, above 97%, for all assays using sera from both healthy donors collected prior to the pandemic and samples positive for antibodies to or nucleic acid from other viral or bacterial pathogens. While we identified positive results for three of these assays in a number of samples positive for antibodies to bacterial pathogens, it is unlikely that they are causing the anti-SARS-CoV-2 IgG positivity. Although possible that these samples, collected in January and February of 2020, were from patients previously infected with SARS-CoV-2, none of them were positive by more than one anti-SARS-CoV-2 IgG assay, suggesting that the observed reactivity was non-specific. Specificity of the Abbott anti-SARS-CoV-2 IgG assay was recently reported to be 99.4% and 99.9% by two separate studies, consistent with our findings (6, 8). Two prior published studies have evaluated the Euroimmun anti-SARS-CoV-2 IgG ELISA and shown specificity ranges of approximately 95% (145/153) to 96% (195/203), which is lower than what we report here (97% to 98%) and may be attributed to differences in the populations tested, inter-lab performance or lot-to-lot test kit variability (7, 8). Specificity of the Epitope anti-SARS-CoV-2 IgG ELISA was reported to range from 85% to 90% by the aforementioned pre-print study, which is significantly lower than our overall finding of 99.6% (9). Specificity differences may be due to multiple factors, including our use of an automated ELISA platform to perform the testing, which may provide higher processing consistency, and our use of a higher S/Co threshold for positive results (≥1.21) compared to those recommended by the manufacturer (≥1.01). Notably, application of the lower S/Co threshold for positive results also yielded high overall specificity of 98% using our sample set. Finally, to our knowledge, this is the first study to independently report on the specificity of the Ortho-Clinical anti-SARS-CoV-2 IgG immunoassay, which we found to be 99.6%.

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

There are a number of limitations associated with this study. First, the number of unique COVID-19 patients included in the in-patient and out-patient groups was low. However, aside from the Epitope anti-SARS-CoV-2 IgG ELISA, differences in sensitivity between these two groups were not notable for the other three assays. Second, specificity of these assays was not evaluated using samples which were known to be positive for antibodies to the commonly circulating human coronaviruses (i.e., 229E, NL63, OC43, HKU1). Prior seroprevalence studies show that 65% to 75% of children have antibodies to at least one of the commonly circulating coronaviruses, and over 90% of adults over the age of 50 years are seropositive for antibodies to all four common coronaviruses (13, 14). Collectively, based on these seroprevalence findings and the overall low false positivity rates identified in this study, we anticipate that although false positive reactions due to antibodies against other common coronaviruses can occur by these assays, the rate appears to be low. Additionally, samples in the cross-reactivity panel were collected during January and early February of 2020, thus preventing us from definitively ruling out prior
infection with SARS-CoV-2, and potentially negatively biasing our overall specificity rate. However, applying the FDA suggested approach of confirming positive samples with an assay based on an alternative SARS-CoV-2 antigen, all samples with positive results in this panel would be considered falsely positive. Third, due to lack of reporting, timing of serum collection for out-patients was compared to the first positive SARS-CoV-2 RT-PCR result, which may have led to an overestimation of anti-SARS-CoV-2 IgG assay sensitivity compared to true date of infection. Similarly, symptom onset in hospitalized patients was extrapolated based on date reported by the patient or assumed by the provider, which may be subject to recall inaccuracies. Finally, correlation of results from these assays relative to a neutralizing antibody (NAbs) test was not performed. Anti-SARS-CoV-2 NAbs primarily develop against the spike protein, specifically to the S1 receptor-binding domain, and initial studies indicate that NAb titers correlate better to spike-based assays as compared to nucleocapsid assays (10, 15). While one study has shown high correlation of NAbs with the Euroimmun IgG assay, as NAb assays become increasingly accessible, more detailed correlation studies across assays will be possible (7).

In conclusion, we show that the Abbott, Epitope, Euroimmun and Ortho-Clinical anti-SARS-CoV-2 IgG immunoassays perform similarly with respect to sensitivity in COVID-19 hospitalized patients, and with the exception of the Epitope assay, also in individuals with milder forms of the infection. The Abbott and Ortho-Clinical anti-SARS-CoV-2 IgG immunoassays provided the highest overall specificity at over 99%. Given that one of the primary uses of serologic assays (at this time) is for the purpose of monitoring local and regional seroprevalence, and that SARS-CoV-2 prevalence rates remain largely unknown, it will be important to use immunoassay(s) with the highest specificity in an effort to maximize the PPV of results. With the unprecedented influx of commercially available test kits for detection of antibodies against SARS-CoV-2, it remains imperative that laboratories thoroughly evaluate these assays for accuracy prior to implementation.
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### Table 1: Attributes for the Evaluated Anti-SARS-CoV-2 IgG Serologic Assays

| Assay principle | Abbott | Epitope | Euroimmun | Ortho-Clinical |
|-----------------|--------|---------|-----------|----------------|
| Assay principle | 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 |

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

*All assays are indirect in format*

*Laboratory determined cut-off threshold*

*Testing performed on the Dynex Agility automated ELISA processor (Chantilly, VA)*

### 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:**

| Period | In-Patient | Out-Patient |
|--------|------------|-------------|
| 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:**

| Period | In-Patient | Out-Patient |
|--------|------------|-------------|
| 0 to 7 days | NA | 11 |
| 20 to 31 days | NA | 23 |
### Table 3. Performance Characteristics of Four Commercially Available anti-SARS-CoV-2 IgG Assays

|                          | % Sensitivity (No. of Samples) | % Specificity (No. of Samples) | Healthy Donors | Cross-Reactivity Panel | Overall (95% CI) |
|--------------------------|--------------------------------|--------------------------------|----------------|-------------------------|-----------------|
|                          | Serum Samples from In-Patients, Days Post-Symptom Onset | Serum Samples from Out-Patients, Days Post First RT-PCR Positive Result |                |                         |                 |
|                          | ≤ 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%) |
| 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%) |
| 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%) |

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

*Overall specificity is 98% (95% CI: 95.3% - 99.3%) if indeterminate results are counted as ‘positive’.

One 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.

### 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) | Individuals Patients (N=37) |
|--------------------------|----------------------|-----------------------------|
|                          | % Positive | 95% CI | % Positive | 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) | 92.0% - 99.9% | 97.3% (36/37) | 85% - 100% |

*The 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).

*The 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.

One 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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Figure Legends

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

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

Figure 2. Signal to Cut-off (Index Value) Distribution Among SARS-CoV-2 RT-PCR Confirmed Patients and Controls. Signal to cut-off (S/Co) ratios are shown relative to days post symptom onset for in-patients (A) or days post first SARS-CoV-2 RT-PCR positive result in out-patients (B). Signal intensities are also shown for healthy control samples collected in 2018 and for cross-reactivity panel (C). Grey shaded bars represent the S/Co range for positive results by the four assays (range: 1.0 to 1.4 S/Co).

Abbreviations: AB, Abbott Laboratories; EDI, Epitope Diagnostics Inc.; EI, Euroimmun; OCD, Ortho-Clinical Diagnostics.
