FOCUSED REPORT

Specificity and Confirmation of SARS-CoV-2 Serological Test Methods in Emergency Department Populations across the United States

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Background: Serological testing for SARS-CoV-2 is integral for understanding prevalence of disease, tracking of infections, confirming humoral response to vaccines, and determining timing and efficacy of boosters. The study objective was to compare the specificity of serology assays in emergency department populations across the United States in 2019 (pre-pandemic) and early 2020, incorporating an automated confirmatory assay.

Methods: Patient specimens (n = 1954) were from 4 regions in the United States: New York, NY; Milwaukee, WI; Miami, FL; and Los Angeles, CA. Specimens were tested with SARS-CoV-2 anti-spike receptor-binding domain assays: SARS-CoV-2 IgG on the Abbott Alinity i (AdviseDx SARS-Cov-2 IgG II) and Beckman Coulter Access 2 (SARS-CoV-2 IgG II), and SARS-CoV-2 IgM on the Abbott Alinity i (AdviseDx SARS-CoV-2 IgM). Reactive samples were tested with a research use only angiotensin-converting enzyme 2 binding inhibition assay (Abbott ARCHITECT) for confirmation of SARS-CoV-2 neutralizing antibodies. Assay specificity was determined and comparisons performed with Fisher's exact test.

Results: Overall SARS-CoV-2 IgG specificity was 99.28% (95% confidence interval, 98.80%–99.61%), 99.39% (98.93%–99.68%), and 99.44% (98.99%–99.72%) for SARS-CoV-2 IgG by Abbott and Beckman, and SARS-CoV-2 IgM, respectively. Overall agreement for the two IgG assays was 99.28% (range for the 4 sites: 98.21% to 100%). There were no specificity differences between assays or sites.

Conclusions: The specificity of the serological assays evaluated in a large, diverse emergency department population was >99% and did not vary by geographical site. A confirmatory algorithm with an automated pseudo-neutralization assay allowed testing on the same specimen while reducing the false positivity rate and increasing the value of serology screening methods.

INTRODUCTION

The SARS-CoV-2 virus has significantly impacted populations across the globe, starting in Wuhan, China in late 2019, and spreading quickly to the US and globally in 2019 and early 2020 (1, 2). The pandemic, which was announced by the WHO in March of 2020, has claimed the lives of

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IMPACT STATEMENT

COVID-19 serological assays have clinically relevant high specificity that minimizes false-positive rates while providing insights into antibody responses during a global pandemic.

over 6 million people and has had catastrophic impact on economies worldwide (3). Several studies have demonstrated that the prevalence of infection is underestimated by counting only confirmed COVID-19 cases, especially among younger people (4), while the reports indicate the first cases may have been as early as December 2019 (5). Although the utility of serological assays has been a matter of debate since the beginning of the pandemic, serological testing has become an integral part of understanding the prevalence of the disease and the tracking of infections across the globe, as well as confirming humoral response to the vaccines as they have emerged. These assays are also essential in determining the timing and efficacy of boosters in vulnerable populations as antibody levels wane from the first rounds of vaccination. Therefore, accuracy of these diagnostic methods is an important tool for assessing different aspects of the pandemic.

Not only do antibodies reflect the number of individuals who have been infected by the virus (seroprevalence) in recent months/years, but they can be utilized in several other venues such as management of patients in the intensive care unit (severity of disease) in combination with antigen or PCR testing to enable early discharge, and in immune-suppressed/-compromised patient cohorts to manage risk. Antibodies that effectively inhibit the linkage of the coronavirus to the host cell receptor represent only a small portion of all clones stimulated after SARS-CoV-2 infection (6). A neutralizing antibody response develops very early after infection, often concomitantly with binding antibodies (7–9), and the great majority of these neutralizing antibodies target the receptor-binding domain (RBD) region (10). This is not too surprising since that portion of Spike 1 links to the human angiotensin-converting enzyme (ACE2) receptor. Unlike the classical response to other viruses, the IgM and IgG responses to SARS-CoV-2 happen with approximately 1 to 3 days separating the appearance of the IgM from the IgG response (11). Measuring the subset of IgG antibodies that are neutralizing can be used as a confirmatory method to verify that the antibodies detected are neutralizing to the SARS-CoV-2 specifically (10). The objective of this study was to compare the specificity of commonly used serology assays in emergency department populations from 4 geographic regions across the United States in 2019 (pre-pandemic) and into the early days of the pandemic in 2020 incorporating an automated confirmatory assay.

MATERIALS AND METHODS

Specimens in this study (n = 1954) were from patients presenting to emergency departments between April 2019 and January 2020 in 4 geographic regions in the United States: New York, NY (Site 1); Milwaukee, WI (Site 2); Miami, FL (Site 3); and Los Angeles, CA (Site 4) as described in Table 1. Prior to testing, specimens were stored frozen in a central location at −80° C and mixed thoroughly and centrifuged at 10 000*g* for 10 min following thaw. Specimens were tested with assays for the SARS-CoV-2 anti-spike RBD including SARS-CoV-2 IgG on the Abbott Alinity i (AdviseDx SARS-Cov-2 IgG II) and Beckman Coulter Access 2 (SARS-CoV-2 IgG II), and SARS-CoV-2 IgM on the

Table 1. Descriptive statistics and SARS-CoV-2 serology results for the geographic sites.

Site	n	Specimen collection dates	Male/Female (%)[a, b]	Age (Mean ± SD)[a, c]	SARS-Cov-2 IgG II % Specificity (95% confidence interval)			SARS-CoV-2 IgM (Abbott), % specificity (95% confidence interval)
					Abbott	Beckman Coulter	% Assay agreement (95% confidence interval)	
1	500	April 2019–May 2019	44/56	51.4±20.6	99.80 (98.89–99.99)	99.80 (98.89–99.99)	100.00 (99.26–100.00)	99.80 (98.89–99.99)
2	544	May 2019–Aug 2019	42/58	53.7±18.8	99.63 (98.68–99.96)	98.90 (97.61–99.59)	99.26 (98.13–99.80)	99.08 (97.86–99.70)
3	504	June 2019–Aug 2019	50/50	54.4±18.5	98.41 (96.90–99.31)	99.40 (98.27–99.88)	98.21 (96.64–99.18)	99.21 (97.98–99.78)
4	406	Dec 2019–Jan 2020	44/56	51.8±22.0	99.26 (97.86–99.85)	99.51 (98.23–99.94)	98.75 (98.64–99.99)	99.75 (98.64–99.99)
All	1954	April 2019–Jan 2020	45/55	52.9±20.5	99.28 (98.80–99.61)	99.39 (98.93–99.68)	99.28 (98.80–99.61)	99.44 (98.99–99.72)[d]

[a]Numbers differ due to missing demographic data in 28 cases.
[b]Site 1: n = 483.
[c]Site 1: n = 483; Site 2: n = 535; Site 3: n = 502.
[d]Of the 13 samples positive for SARS-CoV-2 IgM, 2 had insufficient volume for confirmatory testing.

Abbott Alinity i (AdviseDx SARS-CoV-2 IgM) on serum except for Site 2 (heparinized plasma). Positive/reactive cutoffs were ≥50 AU/mL, ≥10 AU/mL, and ≥1.00 index (signal-to-cutoff) for the 3 assays, respectively. Initially positive/reactive samples were repeated in duplicate with the same assay. Repeat reactive samples on any of the three assays were further tested with a research use only ACE2 binding inhibition assay on the Abbott ARCHITECT (11). The assay is a delayed 1-step immunoassay for the quantitative assessment of neutralizing antibodies against SARS-CoV-2 that block the interaction between the viral spike RBD and the ACE2 cell surface receptor. Sample and SARS-CoV-2 spike RBD antigen-coated paramagnetic microparticles are combined and incubated. IgG antibodies to SARS-CoV-2 present in the sample bind to the microparticles. ACE2 receptor antigen acridinium-labeled conjugate is added and incubated. Following a wash cycle, Pre-Trigger and Trigger Solutions are added. The resulting chemiluminescent reaction is measured as relative light unit (RLU). There is an inverse relationship between the amount of IgG antibodies to SARS-CoV-2 in the sample and RLU. A minimum of 12.5% inhibition of ACE2 binding is required to be considered positive. Assay specificity was determined for each site and comparison between assays and across sites assessed with Fisher's exact test. This study was determined to not constitute human subjects research by the Johns Hopkins Medicine Institutional Review Board (#IRB00258550).

RESULTS

SARS-CoV-2 IgG and IgM results for the 1954 specimens from patients presenting to emergency departments in 4 geographic areas are presented in Table 1. There were 14 positive SARS-CoV-2 IgG results with the Abbott assay and 12 with the Beckman Coulter SARS-CoV-2 IgG assay (range 1 to 8 and 1 to 6 per site for the 2 assays, respectively). Overall agreement for the 2 assays was 99.28% (range for the 4 sites: 98.21% to 100%). There were 13 positive SARS-CoV-2 IgM results (range 1 to 7 per site) with 1 specimen positive for both SARS-CoV-2 IgG and IgM. Positive antibody results were spread throughout the study (May 2019 to January 2020). Confirmatory testing for 30 of the 32 samples with a positive result by any of the 3 methods and sufficient sample volume available was performed using a lab-developed ACE2 receptor inhibition assay. No or minimal neutralization (8/30 samples) occurred in the ACE2 receptor inhibition assay. Thus, the overall specificity for the entire cohort was 99.28% (95% confidence interval, 98.80%–99.61%), 99.39% (95% confidence interval, 98.93%–99.68%), and 99.44% (95% confidence interval, 98.99%–99.72%) for SARS-CoV-2 IgG by Abbott and Beckman, and SARS-CoV-2 IgM, respectively. There was no significant difference in specificity between the 2 SARS-CoV-2 IgG assays nor across the 4 geographic sites for the IgG and IgM assays.

DISCUSSION

In this study, the specificity of 3 serological methods was compared using samples collected from 4 geographically distinct emergency departments in the USA. Samples, collected in 2019 and early 2020, represent a presumed pre-pandemic population. The extremely small number of antibody reactive samples did not confirm with the ACE2 receptor inhibition assay and a small number of those with minimal inhibition were most likely weak interactions from other viral exposures transiently binding to the COVID spike RBD. Specificity results for all 3 methods were comparable to one another, also supporting previously reported specificity data in the literature and product package inserts (12, 13).

The impact of vaccines and vaccine boosters along with global infection rates on the detection

of antibodies to SARS-CoV-2 is significantly higher than at the beginning of the pandemic. This exposure, through vaccination and or infection, has had a significant impact on the prevalence of circulating antibodies, and as a result, the specificity of antibody testing. As an example, the poor specificity of an assay in a low-prevalence population can create a burden on the laboratory from false-positive rates and subsequent confirmatory testing. As the prevalence increases, this issue is reduced since fewer false positives are generated. In clinical practice, the algorithm can aid in confirming vaccine efficacy in patients with vulnerabilities (transplant, oncology, immunosuppressed) and for confirmation of neutralization ability when variants emerge.

Our suggested testing algorithm provides a user-friendly approach to confirm screened positives by automating an ACE2 inhibition neutralization surrogate assay that quantitates the percent inhibition on the same instrument as the serological assay screening result. This confirmatory algorithm allows for precise results that can significantly shorten the wait time of a typical neutralization assay (biosafety level-3, culturing and growth of virus, etc.). Testing can be performed on the specimen as a reflex on the automated platform, further reducing the false positivity rate and thereby increasing the value of serological screening methods. In summary, utilizing this testing algorithm, we determined the specificity of the serological assays evaluated in a large diverse emergency department population was excellent at >99%, did not statistically differ from one another, and did not vary by geographical site.

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