Head-to-Head Comparison of Two SARS-CoV-2 Serology Assays

Running Title: SARS-CoV-2 Serology Assay Comparison

Anna E. Merrill[1*], J. Brooks Jackson[1], Alexandra Ehlers[1], Dena Voss[1], Matthew D. Krasowski[1]

[1] Department of Pathology, University of Iowa Hospitals and Clinics, Iowa City, IA, USA

* Corresponding Author:

Anna E. Merrill, PhD, DABCC

Address:

University of Iowa Hospitals and Clinics

Department of Pathology

200 Hawkins Drive

6234 RCP

Iowa City, IA 52242

Phone: 319-678-8400; **Fax:** 319-384-9613

Email: anna-merrill@uiowa.edu

Key words: SARS-CoV-2, COVID-19, serology, immunoassay

ABSTRACT

Background: While molecular techniques remain the gold standard for diagnosis of acute SARS-CoV-2 infection, serological tests have the unique potential to ascertain how much of the population has been exposed to the COVID-19 pathogen. There have been limited published studies to date documenting the performance of SARS-CoV-2 antibody assays.

Methods: We compared the DiaSorin Liaison SARS-CoV-2 S1/S2 IgG and Roche Diagnostics Elecsys Anti-SARS-CoV-2 assays using 228 samples spanning patients with positive PCR for SARS-CoV-2, patients with compatible symptoms but negative PCR, pre-COVID specimens, and potential cross-reactives.

Results: Both assays detected antibodies in 18/19 samples collected at least one week after a positive PCR result. Neither method consistently detected antibodies in specimens collected within one week of a positive PCR result (sensitivity < 50%), but antibodies were detected by only Roche in four samples in this time frame. Using 139 pre-COVID and 35 PCR-negative samples, the Roche and DiaSorin assays demonstrated specificities of 100.0% and 98.9%, respectively. Neither assay demonstrated cross-reactivity from other coronaviruses (229E, HKU1, NL63, OC43), respiratory pathogens (adenovirus, metapneumovirus, rhinovirus/enterovirus), or antibodies to other viruses (HIV, EBV, CMV, HBV, HCV, HAV).

Discussion: Overall, the qualitative interpretations afforded by the Roche and DiaSorin assays agreed for 99% of samples evaluated. Minor discrepancies in sensitivity and specificity were observed between methods, with the differences in specificity more

clinically significant for our low-prevalence population. For the DiaSorin assay, all disagreements with the Roche assay occurred in samples with quantitative signals near the cut-off determining positivity.

IMPACT STATEMENT

Automated serological assays detecting antibodies to SARS-CoV-2 have recently become available, though few published studies exist documenting their performance. In this report, we compare the DiaSorin Liaison SARS-CoV-2 S1/S2 IgG and Roche Diagnostics Elecsys Anti-SARS-CoV-2 assays using 228 samples. We observed slight differences in sensitivity and specificity, but the assays demonstrated very good agreement overall.

Introduction

In the ongoing COVID-19 pandemic, laboratory testing for the COVID-19 pathogen, SARS-CoV-2, has focused primarily on molecular detection of the viral genome by polymerase chain reaction (PCR) methods *(1, 2)*. Molecular techniques remain the gold standard for diagnosis of acute infection *(3, 4)*. More recently, serological assays for antibodies to SARS-CoV-2 have become available. As of May 28th, 2020, 12 SARS-CoV-2 serological assays have acquired Emergency Use Authorization (EUA) from the United States Food and Drug Administration (FDA) as part of the federal government's response to the COVID-19 public health emergency *(5)*. Although their interpretation can be challenging, antibody tests have the unique potential to ascertain how much of the population has been exposed to SARS-CoV-2. Furthermore, the availability of serological assays on high-throughput, automated clinical analyzers enables large-scale surveys that are logistically much easier than molecular testing, especially related to pre-analytical sample collection and analytical testing supplies.

There have been limited published studies to date of SARS-CoV-2 serological assays. A study in China demonstrated variable IgG and IgM responses in the first few weeks following SARS-CoV-2 infection, but documented IgG positivity for all cases more than 17 – 19 days from onset of symptoms *(6)*. Another study also documented seroconversion within two weeks of disease onset in most PCR-confirmed SARS-CoV-2 cases *(7)*. A group of researchers evaluated ten lateral flow assays and two ELISAs and found heterogeneous assay performance, with diagnostic sensitivity ranging from 81.8 – 100% and diagnostic specificity ranging from 84.3 – 100% *(8)*. A study

conducted by the University of Washington in Seattle showed very good analytical performance of the Abbott Architect SARS-CoV-2 IgG assay, particularly a high specificity of 99.9%, and utilized the assay to establish a 1.8% positivity rate in Boise, Idaho *(9)*. More recent studies comparing multiple high-throughput serological assays – Abbott, Epitope Diagnostics Inc., Euroimmun, Ortho-Clinical Diagnostics, and Roche Diagnostics – have generally revealed sensitivities surpassing 75% for samples collected more than 14 days post symptom onset or initial positive PCR result; these same studies have also documented specificities ranging from 94.8% to 99.6% *(10-12)*.

As more serological assays enter the market and are harnessed to test larger populations, differences in assay performance will emerge. In the present study at an academic medical center in a state with low prevalence for COVID-19, we evaluated two automated SARS-CoV-2 serological assays: DiaSorin Liaison SARS-CoV-2 S1/S2 IgG and Roche Diagnostics Elecsys Anti-SARS-CoV-2. As part of our method validation effort, we compared 228 samples, including from patients with positive PCR for SARS-CoV-2, patients with compatible symptoms but negative PCR, pre-COVID specimens, and potential cross-reactives. We hypothesized that differences between the two assays would be most likely in the early phase of infection (e.g., non-IgG antibody detected by Roche but not DiaSorin) and that false positives in one assay would not replicate in the other.

Materials and Methods

The IRB-approved study (protocol # 202005416) was conducted at the University of Iowa Hospitals and Clinics (UIHC), an 811-bed tertiary/quaternary care academic medical center located in Iowa City, Iowa, USA. Paired serological testing was performed using the Elecsys Anti-SARS-CoV-2 and Liaison SARS-CoV-2 S1/S2 IgG assays on Roche cobas e602 (Roche Diagnostics, Basel, Switzerland) and DiaSorin Liaison XL (DiaSorin, Saluggia, Italy) instrumentation, respectively. The Roche electrochemiluminescence immunoassay targets total antibodies (IgG, IgM, IgA) to the nucleocapsid (N) protein using a sandwich format, with a cut-off index (COI) of 1.0 or higher indicating a positive result; this qualitative assay was issued EUA on May 2nd, 2020. The DiaSorin chemiluminescent immunoassay targets IgG antibodies to the S1 and S2 domains of the spike (S) protein using an indirect format, with a signal of 15 AU/mL or higher indicating a positive result; this qualitative assay was issued EUA on April 24th, 2020. The plasma samples (lithium heparin and EDTA) utilized in this study included remnant clinical specimens from individuals with SARS-CoV-2 PCR performed at our institution and specimens collected prior to December 2019 for research and/or clinical assay validation studies. The electronic medical record (Epic Systems, version 2017, Verona, WI) was accessed for information regarding history and/or symptoms suggestive of SARS-CoV-2 infection, results of SARS-CoV-2 PCR testing, and results of other pertinent laboratory testing.

Results

Assay sensitivity was evaluated using 54 specimens from 32 unique patients with SARS-CoV-2 infection confirmed by PCR at our institution. Overall, the Roche and

DiaSorin serological assays demonstrated sensitivities of 65% and 57%, respectively, in our study (Table 1). Of note, 35 of these 54 samples were collected within one week after a positive PCR result. Both methods detected antibodies in all but one sample collected at least one week post-PCR positive (n = 19). Neither assay consistently detected antibodies in specimens collected within one week of a positive PCR result (sensitivity of both assays < 50%). Antibodies were detected by Roche, but not DiaSorin, in four samples collected within one week of a positive PCR result; of note, the signal from the DiaSorin assay for three of these four specimens was just below the cut-off determining positivity (Fig. 1, Supplemental Data).

Assay specificity was examined using two cohorts of samples: 35 specimens from patients with negative SARS-CoV-2 PCR testing and 139 specimens collected prior to December 2019 (i.e., pre-COVID). In addition, 12 of the 139 pre-COVID samples were HIV-positive. Overall, the Roche and DiaSorin serological assays demonstrated specificities of 100.0% and 98.9%, respectively, in our study (Table 1). Neither assay demonstrated cross-reactivity from other coronaviruses (229E, HKU1, NL63, OC43), respiratory pathogens (adenovirus, metapneumovirus, rhinovirus/enterovirus), or antibodies to other viruses (HIV, EBV, CMV, HBV, HCV, HAV). In two samples – one pre-COVID and one from a patient repeatedly negative by PCR with respiratory symptoms eventually attributed to cardiac causes – DiaSorin detected antibodies to SARS-CoV-2, while the Roche assay did not; of note, the signal from the DiaSorin assay for these two specimens was just above the cut-off determining positivity (Fig. 1, Supplemental Data).

Overall, the Roche and DiaSorin serological assays demonstrated excellent concordance given the sample cohort used in this study (Cohen's kappa 0.93, 95% CI 0.87 – 0.98). Of the 56 samples with antibodies detected by one or more serologic method, 50 (89%) were positive by both. Of the 178 samples where antibodies were not detected by one or more serologic method, 172 (97%) were negative by both.

Discussion

While the Roche and DiaSorin assays demonstrated identical sensitivity in antibody detection at least one week after positive PCR for SARS-CoV-2 (18/19 positive), Roche exhibited slightly higher sensitivity within one week of PCR diagnosis (49% vs. 37%; Table 1). This could be due to detection of non-IgG antibody classes more abundant earlier in the infection time course and/or more analytically sensitive detection of IgG by the Roche assay. Two of the four discrepant samples were from patients less than one week post-PCR diagnosis and less than two weeks post-symptom onset, while the other discrepant samples were from asymptomatic individuals. Interestingly, both assays failed to detect antibodies in one asymptomatic patient 15 days after PCR-confirmed SARS-CoV-2 infection. The diagnostic sensitivity of the Roche assay determined in our study (92%) corroborates a recent study (sensitivity 89%) for specimens collected at least two weeks after symptom onset, evidencing that the majority of symptomatic individuals will seroconvert within this timeframe *(12)*.

The Roche assay also displayed higher specificity in our study (100.0% vs. 98.9%, Table 1). Though this difference is not statistically significant, it was expected based on information provided by the assay manufacturers in the package inserts; in a Roche study of 5,272 pre-COVID specimens, antibodies were detected in only 10 cases (specificity 99.8%, 95% CI 99.7 – 99.9%), whereas a DiaSorin study detected antibodies in 8 of 1090 pre-COVID specimens (specificity 99.3%, 95% CI 98.6 – 99.6%). Possible reasons for variations in assay specificity include differences in target antigen (nucleocapsid vs. spike protein) and immunoassay format (sandwich vs. indirect) between the Roche and DiaSorin methods. Even seemingly minor differences in diagnostic specificity can lead to significant disparities in positive-predictive value (PPV) when testing low-prevalence communities *(13, 14)*. Considering the possible risks of reporting false-positive serological results, caution is warranted when testing patient populations where the prevalence of SARS-CoV-2 exposure is low or unknown *(15, 16)*. The Centers for Disease Control and Prevention (CDC) suggest three strategies for improving the PPV of SARS-CoV-2 serology, one being the use of two independent serological tests to confirm a positive result *(17)*.

Both the DiaSorin and Roche assays are intended for qualitative detection of antibodies to SARS-CoV-2. As such, assay performance around the cut-off is important, since even minor differences in quantitative signal can drastically change the qualitative interpretation reported. We considered DiaSorin specimens with raw signal between 7.5 – 30 AU/mL and Roche specimens with raw signal between COI 0.5 – 2.0 to be close to the cut-off (Supplemental Data). For DiaSorin, 13 of 228 samples (6%) met this

criterion, including five of six discrepancies relative to the Roche assay; only three samples fell into this range for the Roche assay. This indicates that results close to the cut-off are more likely to be observed on the DiaSorin assay relative to the Roche assay. Based on the results of our comparison, DiaSorin results in this range are at higher risk of being false-positives or false-negatives. Furthermore, specimens in this range are more likely to be impacted in a clinically significant way by changes in quantitative signal due to variability in calibration, reagent lot, or other assay parameters.

The present study compares the performance of two automated SARS-CoV-2 serological assays using 228 specimens. Overall, the qualitative interpretations afforded by the Roche and DiaSorin assays agreed for 99% of samples evaluated, which mimics the very good qualitative concordance between high-throughput serologic assays observed in other studies *(10-12)*. Minor discrepancies in sensitivity and specificity were observed between methods, with the differences in specificity more clinically significant for our low-prevalence population. One limitation of our study is the small sample size, which precludes a more robust statistical analysis. Another limitation is that we were unable to include multiple reagent lots in our direct comparison due to limited reagent availability. Finally, the performance of these serological tests in asymptomatic individuals is still largely unknown.

Acknowledgments

We acknowledge Renee Eigsti and Daniel Pelletier for their assistance identifying residual patient samples for this study. We also acknowledge Jeff Kulhavy and Rebecca Nelson for their efforts to procure necessary reagents and instrumentation.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

A.E. Merrill, statistical analysis, provision of study material or patients; J.B. Jackson, financial support; M.D. Krasowski, administrative support.

Authors' Disclosures or Potential Conflicts of Interest: *No authors declared any potential conflicts of interest.*

Role of Sponsor: No sponsor was declared.

References

1. Chu DKW, Pan Y, Cheng SMS, Hui KPY, Krishnan P, Liu Y, et al. Molecular diagnosis of a novel coronavirus (2019-ncov) causing an outbreak of pneumonia. Clin Chem 2020;66:549-55.
2. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al. Detection of 2019 novel coronavirus (2019-ncov) by real-time rt-pcr. Euro Surveill 2020;25.
3. Coronavirus disease (covid-19) technical guidance: Laboratory testing for 2019-ncov in humans. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance (Accessed May 29 2020).
4. Cdc 2019-novel coronavirus (2019-ncov) real-time rt-pcr diagnostic panel for emergency use only instructions for use. Vol. Atlanta, GA: Centers for Disease and Control and Prevention
2019.
5. Emergency use authorizations. https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations (Accessed May 29 2020).
6. Long QX, Liu BZ, Deng HJ, Wu GC, Deng K, Chen YK, et al. Antibody responses to sars-cov-2 in patients with covid-19. Nat Med 2020.
7. Okba NMA, Muller MA, Li W, Wang C, GeurtsvanKessel CH, Corman VM, et al. Severe acute respiratory syndrome coronavirus 2-specific antibody responses in coronavirus disease 2019 patients. Emerg Infect Dis 2020;26.
8. Whitman JD, Hiatt J, Mowery CT, Shy BR, Yu R, Yamamoto TN, et al. Test performance evaluation of sars-cov-2 serological assays. medRxiv 2020:2020.04.25.20074856.
9. Bryan A, Pepper G, Wener MH, Fink SL, Morishima C, Chaudhary A, et al. Performance characteristics of the abbott architect sars-cov-2 igg assay and seroprevalence in boise, idaho. J Clin Microbiol 2020.
10. Theel ES, Harring J, Hilgart H, Granger D. Performance characteristics of four high-throughput immunoassays for detection of igg antibodies against sars-cov-2. J Clin Microbiol 2020.
11. Tang MS, Hock KG, Logsdon NM, Hayes JE, Gronowski AM, Anderson NW, Farnsworth CW. Clinical performance of two sars-cov-2 serologic assays. Clin Chem 2020.
12. Tang MS, Hock KG, Logsdon NM, Hayes JE, Gronowski AM, Anderson NW, Farnsworth CW. Clinical performance of the roche sars-cov-2 serologic assay. Clin Chem 2020.
13. Farnsworth CW, Anderson NW. Sars-cov-2 serology: Much hype, little data. Clin Chem 2020.
14. Suhandynata RT, Hoffman MA, Kelner MJ, McLawhon RW, Reed SL, Fitzgerald RL. Longitudinal monitoring of sars-cov-2 igm and igg seropositivity to detect covid-19. J Appl Lab Med 2020.
15. Torres R, Rinder HM. Double-edged spike-are sars-cov-2 serologic tests safe right now? Lab Med 2020;51:236-8.
16. Mathur G, Mathur S. Antibody testing for covid-19. Am J Clin Pathol 2020.

17. Interim guidelines for covid-19 antibody testing. https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html (Accessed May 29 2020).

Table 1. Method comparison summary for Roche and DiaSorin assays.

Sample Category		Roche	DiaSorin
Positive		35/54 positive sensitivity 64.8% (50.6-77.3%)	31/54 positive sensitivity 57.4% (43.2-70.8%)
Relative to positive PCR	*<7 days*	17/35	13/35
	7-13 days	13/13	13/13
	>13 days	5/6	5/6
Relative to symptom onset	*<7 days*	1/5	1/5
	7-13 days	8/12	7/12
	>13 days	11/12	10/12
	Unknown	10/12	10/12
	Asymptomatic	5/13	3/13
Negative		174/174 negative specificity 100.0% (97.9-100.0%)	172/174 negative specificity 98.9% (95.9-99.9%)
	Negative PCR	30/30	29/30
	Pre-COVID	139/139	138/139

Figure 1. Distributions of normalized quantitative signals for Roche and DiaSorin assays. Roche results are shown in red circles. For Roche, a normalized signal of 1.0 equals COI 1.0, the cut-off for a positive result. DiaSorin results are shown in blue circles. For DiaSorin, a normalized signal of 1.0 equals 15 AU/mL (i.e., divided result in AU/mL by a factor of 15), the cut-off for a positive result. The normalized results are plotted on a logarithmic scale. Six samples (four PCR-positive and two pre-COVID/PCR-negative) with discrepant interpretations between assays are circled.