SCIENCE

Efficacy of POC Antibody Assays after COVID-19 Infection and Potential Utility for "Immunity Passports"

Akram Shalaby, MD,[1,] Hansini Laharwani, MD,[1] John T. Bates, PhD,[1] Patrick B. Kyle, PhD[1]*

[1]University of Mississippi Medical Center, Jackson, Mississippi, US; *To whom correspondence should be addressed. akramshalaby91@gmail.com

Keywords: COVID-19, SARS-CoV-2, antibody tests, ELISA, lateral flow assays

Abbreviations: ELISA, enzyme-linked immunosorbent assay; RT-PCR, real-time polymerase chain reaction; UMMC, University of Mississippi Medical Center; RBD, receptor binding domain; PBS, phosphate-buffered saline.

Laboratory Medicine 2021;XX:e0–e0; https://doi.org/10.1093/labmed/lmab093

ABSTRACT

Objective: Numerous manufacturers market lateral flow assays for the detection of SARS-CoV-2 antibodies, but there are many questions about the reliability and efficacy of these tests.

Materials and Methods: Serum specimens from 60 individuals were analyzed using 2 lateral flow antibody assays, an in-house enzyme-linked immunosorbent assay (ELISA), and the Abbott SARS-CoV-2 IgG chemiluminescent immunoassay.

Results: The BioMedomics and Premier Biotech lateral flow assays were positive for IgM in 73.3% and 70% and for IgG in 80% and 73.3% of specimens, respectively. The ELISA assay was positive for IgM and IgG in 73.3% and 86.7% of specimens from infected individuals, whereas the Abbott assay was positive in 80%. The specificities of the 4 assays ranged from 96.7% to 100% for IgM and from 93.3% to 100% for IgG.

Conclusion: Results of the 2 lateral flow assays were comparable to those of the ELISA and Abbott assays. Assay efficacy depended on length of time after SARS-CoV-2 infection.

COVID-19, caused by the SARS-CoV-2 virus, rapidly spread globally and was declared a worldwide pandemic in March 2020. The gold standard test methodology for the diagnosis of SARS-CoV-2 infection involves real-time polymerase chain reaction (RT-PCR) of viral RNA collected via a nasopharyngeal swab.[1] The detection of antibodies formed in response to SARS-CoV-2 could be a useful methodology to safely return adults to the workplace and children to school. Given estimates of asymptomatic COVID-19 infections ranging from 16% to 30%,[2] antibody tests may help us understand how the epidemic has progressed and provide crucial information about the true mortality of the disease. Early data suggested that convalescent plasma infusion and antibody tests were used to identify potential plasma donors as a treatment option for patients with COVID-19.[3-5]

Some reservations exist regarding the accuracy of available antibody tests, which became evident when the United Kingdom determined that 1 million test kits purchased from China lacked sufficient accuracy and could not be used for testing.[6] In addition, not all point-of-care tests have been properly vetted, and the results of these assays may vary.[7] A study comparing the performance of 7 lateral-flow IgM/IgG assays found sensitivities ranging from 50% to 97.4% in specimens collected 14 to 25 days after symptom onset.[8] In the current study, we sought to evaluate 2 point-of-care assays manufactured for the detection of human antibodies to the SARS-CoV-2 virus by comparing them to an in-house enzyme-linked immunosorbent assay (ELISA) and a commercially available assay.

Materials and Methods

Sixty previously tested patient specimens designated for disposal were obtained from the University of Mississippi Medical Center (UMMC) laboratory for this evaluation. Each specimen was collected from patients who presented to UMMC with symptoms suspicious for COVID-19 from late March to mid-April 2020. Of the 60 specimens, 30 originated from individuals positive for SARS-CoV-2 and 30 from negative individuals as determined by RT-PCR using the Abbott RealTime SARS-CoV-2 on an Abbott M2000 analyzer. The serum specimens were collected at a mean of 13.4 days after symptom onset (range, 7–30 days; lower quartile, 9.75; upper quartile, 15.5). The mean age of all patients included in the study was 54 years (range, 5–95 years). Thirty-one of the patients in the study were males and 29 were females. See **TABLE 1** and **TABLE 2** for patient demographics.

BioMedomics and Premier Biotech Rapid IgG-IgM Antibody Assays

Two commercially available point-of-care lateral flow assays manufactured in China and distributed in the United States by BioMedomics (Morrisville, NC) and Premier Biotech (Minneapolis, MN) were evaluated. Each of the assays are qualitative in nature and are designed to detect IgG and IgM antibodies specific to SARS-CoV-2 in serum, plasma, or whole blood. Package inserts do not state the antigen(s) used in either test or the expression systems used to generate the antigens. Each test cassette was received

sealed in a foil pouch with a desiccant and buffer. An alcohol pad and lancet were also provided with each Premier Biotech test cassette. Each test cassette has 3 regions that contain reaction antigen for IgG antibodies, IgM antibodies, and a positive control. The presence of antibodies is indicated by the appearance of a purple line in the IgM or IgG regions, which indicates a functioning test. Testing was performed as per each manufacturer's instructions. We added 10 μL of serum to the sample well in the respective device, followed by 2 drops of buffer solution in the buffer well. Results were determined visually after 15 minutes had elapsed.

IgM and IgG ELISA Assays

Per Stadlbauer et al,[9] ELISAs were developed for the measurement of human IgM and IgG antibodies specific for the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein. We coated 384-well MaxiSorp plates (Thermo Fisher Scientific) with purified recombinant RBD at a concentration of 3 μg/mL in phosphate-buffered saline (PBS). Recombinant RBD that was produced in Sf9 insect cells was purchased from Genescript. The coating volume and reaction volumes were 25 μL per well. Plates were incubated overnight at 4°C, washed 3 times with PBS containing 0.1% Tween20, and blocked with PBS containing 3% dry milk for 1 hour at room temperature. The blocking buffer was removed, and specimens (1-log dilutions in blocking buffer, 5×10^{1}-5×10^{4}) were added to the wells. Plates were incubated for 2 hours at room temperature before washing 3 times. Horseradish peroxidase conjugated to anti-human IgG FC (Southern Biotech) or anti-human IgM (Southern Biotech) was diluted 1:3000 in PBS containing 1% dry milk, added to the wells, and incubated at room temperature for 1 hour. The plates were washed 5 times and developed with tetramethyl-benzidine (Southern Biotech). After 30 minutes, development was stopped by adding 25 μL of 2N H_2SO_4 to each well. Absorbance was measured at 450 nm. The endpoint dilution titer was set to the serum dilution that resulted in an absorbance of 0.2 absorbance units over background. A specimen was counted as having a positive result if the reciprocal of the endpoint dilution was >5000 absorbance units.

Abbott SARS-CoV-2 IgG Assay

The SARS-CoV-2 IgG assay (Abbott Diagnostics, Abbott Park, IL) is a chemiluminescent microparticle immunoassay designed for the detection of IgG antibodies to the nucleocapsid protein of SARS-CoV-2. For this assay, 150 μL of serum or plasma is mixed with SARS-CoV-2 antigen-coated microparticles and allowed to react. After a wash step, anti-human IgG acridinium-labeled conjugate is added, is allowed to incubate, and is washed. Chemiluminescence is measured as relative light units. The presence of antibodies is associated with increasing luminescence. A cutoff of 1.4 S/C was used for positivity as per the manufacturer. All analyses were performed on an Architect i2000SR (Abbott Diagnostics) after proper calibration as recommended by the assay manufacturer.

TABLE 1. Demographics of Patients Who Tested Positive by RT-PCR (n = 30)

Feature	n (%)
Sex	
Male	14 (47)
Female	16 (53)
Age at time of presentation (y)	
Range	5–95
Mean	57
Race/ethnicity	
Black	23 (77)
White	5 (17)
Hispanic	1 (3)
Asian	0 (0)
Unknown	1 (3)

RT-PCR, real-time polymerase chain reaction.

TABLE 2. Demographics of Patients Who Tested Negative by RT-PCR (n = 30)

Feature	n (%)
Sex	
Male	17 (57)
Female	16 (43)
Age at time of presentation (y)	
Range	18–91
Mean	51
Race/ethnicity	
Black	18 (60)
White	10 (33)
Hispanic	1 (3)
Asian	1 (3)
Unknown	0 (0)

RT-PCR, real-time polymerase chain reaction.

Results

Of the 30 specimens from infected patients, the in-house ELISA assay was positive for IgM and IgG in 22 (73.3%) and 26 (86.7%) patients, respectively. The BioMedomics antibody assay found 73.3% and 80% positivity for IgM and IgG, respectively, whereas the Premier Biotech assay found 70% and 73.3% positivity for IgM and IgG, respectively. The Abbott chemiluminescence microparticle assay was positive in 24 patients who were RT-PCR–positive and negative in all patients who were RT-PCR–negative. The 4 serological assays showed a specificity ranging from 96.7% to 100% for IgM and from 93.3% to 100% for IgG. The control line formed in each of the BioMedomics and Premier Biotech tests, indicating that the assays were properly functioning (**FIGURE 1**). See **TABLE 3** for the sensitivity and specificity of all antibody assays included in our study.

Our results also showed an increased sensitivity and specificity of the BioMedomics and Premier Biotech assays over time. The sensitivity and specificity of both assays for IgM and/or IgG in specimens collected >14 days after RT-PCR testing was 100%. See **TABLE 4** for the sensitivity and specificity of the BioMedomics and Premier Biotech assays for IgM and/or IgG in specimens collected at different time intervals from the RT-PCR testing date.

Discussion

Point-of-care antibody tests for SARS-CoV-2 have several advantages: They are inexpensive, easy to perform, and offer rapid results. Antibody tests for SARS-CoV-2 antibodies may provide an insight into the prevalence of COVID-19 in specific geographic locations. This concept was shown in a pilot study in Chelsea, MA, involving 200 apparently healthy individuals, of whom 64 (approximately 30%) were positive for antibodies to SARS-CoV-2. These findings provided a valuable snapshot in a community known

to have a high prevalence of disease at the time.[10] The Florida Department of Health reported that 4.4% of 123,552 healthcare workers, firefighters, police officers, and first responders were positive for antibodies.[11,12]

In this study, 2 of the immunoassays, ELISA and BioMedomics, exhibited false-positive results that reduced their specificities to 93.3% and 96.7%, respectively. Assays with very high specificities are required for population screening because when prevalence is low, even a few false-positive results cause a significant overestimation of disease.[13] For example, if the prevalence of the disease in the population is 5%, the positive predictive value of a test that exhibits 95% sensitivity and 95% specificity will be 50.0%, essentially a coin toss. Meanwhile, tests that exhibit 95% sensitivity but 99% and 99.5% specificity would exhibit positive predictive values of 83.3% and 90.8%, respectively. Therefore, positive results from assays that exhibit low specificity should be followed with another test whenever the prevalence of disease is low.[14] Although these antibody tests correlated well with RT-PCR results, they did not meet the 99% specificity recommended for population surveillance. In this dataset, a follow-up test would have eliminated all false-positive results.

Interestingly, 3 patients with COVID-19 who tested positive by RT-PCR were negative on all 4 serological assays. This outcome could have resulted from antibody concentrations below the detection limit, which could occur with a weak immune response to the virus or during the early stages of disease before sufficient antibody concentrations have formed. A study of sailors infected during the USS Theodore Roosevelt outbreak found that only 90% of infected individuals who reported a positive RT-PCR test before the study made detectable levels of SARS-CoV-2 spike-specific antibody after infection,[15] which generally agrees with the 87% seroconversion we observed. Reasons for the low seroconversion rate among RT-PCR–confirmed infections remain unknown but are likely related to the length of time after infection.

Our results showed sensitivities of 50% and 100% for both the BioMedomics and Premier Biotech assays in specimens collected <7 days and >14 days after the RT-PCR testing, respectively. Others have attributed negative SARS-CoV-2 antibody results after confirmed infection to delayed specific antibody responses in patients with severe illness.[16,17] The 3 specimens that were negative by both lateral flow assays, ELISA, and the Abbott ARCHITECT but collected from patients who were RT-PCR–confirmed positive were collected at 7, 9, and 10 days after the onset of symptoms. These specimens would have been expected to contain sufficient concentrations of IgM, if not IgG.[18] Given the reported variability of the antibody response to SARS-CoV-2 infection, the agreement between these assays raises the possibility that these specimens represent true biological negatives rather than false negatives.

FIGURE 1. Premier Diagnostics (top row) and BioMedomics (bottom row) lateral flow assays. Cartridges 13–15 illustrate positive results for both IgG and IgM, whereas cartridge 16 illustrates negative results for both IgG and IgM.

TABLE 3. Results of Serologic Antibody Assays

	BioMedomics	Premier Biotech	ELISA	Abbott
Sensitivity				
IgM	73.3%	70%	73.3%	N/A
IgG	80%	73.3%	86.7%	80%
Specificity				
IgM	96.7%	100%	100%	N/A
IgG	96.7%	100%	93.3%	100%

ELISA, enzyme-linked immunosorbent assay.

TABLE 4. Sensitivity and Specificity of BioMedomics and Premier Biotech Assays for SARS-CoV-2 IgG and/or IgM Antibodies at Different Time Intervals From RT-PCR Testing Date

	BioMedomics	Premier Biotech
Sensitivity		
<7 days	50%	50%
7–14 days	93%	93%
>14 days	100%	100%
Specificity		
<7 days	86%	86%
7–14 days	100%	100%
>14 days	100%	100%

RT-PCR, polymerase chain reaction.

Antibodies are likely to offer protection against reinfection with SARS-CoV-2; therefore, antibody assays may have a role in identifying immune individuals. One study involving 3.2 million people who had undergone SARS-CoV-2 antibody testing concluded that seropositive individuals have a significantly decreased risk for future SARS-CoV-2 infection.[19] Animal studies investigating immune response in rhesus macaque monkeys[20,21] also showed humoral and cellular immune response to SARS-CoV-2, suggesting that some level of protective immunity may occur. The transfer of sera from immunized primates to hACE2 transgenic mice in another study protected against a challenge with SARS-CoV-2.[22] Antibodies are the correlate of protection for the great majority of viral infections and seem to correlate with protection against SARS CoV-2 infection.[23]

There is an ongoing debate about the use of "immunity passports" and their role in travel restrictions.[24] Several European countries began issuing certificates of travel to verify vaccination against SARS-CoV-2, receipt of a negative test result, or recovery from the virus.[25] Italy, Iceland, Spain, Greece, and other countries were opening their borders to travelers who have been vaccinated or recently tested negative for COVID-19.[26] Note that at this time, the U.S. Food & Drug Administration does not recommend antibody tests to assess immunity or protection from vaccination.[27] Because

the presence of antibodies to COVID-19 likely indicates immunity,[19] antibody tests could play a role as one modality to prove immunity in previously infected individuals. This testing could benefit travelers from underserved countries with limited vaccination rates or limited COVID-19 testing. Given the results observed in this study, antibody tests may provide variable results within the first 10 days after the development of symptoms of SARS-CoV-2 infection. Point-of-care tests are inexpensive, easy to perform, offer rapid results, and may have a utility in screening for immunity and resuming international travel even at the point of travel. A limitation of antibody tests could involve the potential misinterpretation of results in that (1) days or weeks are required for seroconversion after infection and (2) assay sensitivities and specificities may vary considerably.

Conclusion

One limitation of our study is the small sample size, in that we only examined 30 specimens from patients who were RT-PCR–positive and 30 specimens from patients who were negative. In addition, all our specimens were collected within 21 days of the RT-PCR testing. Larger studies evaluating specimens collected at longer time intervals are needed to assess the utility of serological assays in confirming past SARS-CoV-2 infection and immunity.

Conflict of Interest Statement

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest in the subject matter or materials discussed in this article.

REFERENCES

1. Corman V, Landt O, Kaiser M, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill*. 2020;25(3):2000045.
2. Byambasuren O, Cardona M, Bell K, et al. Estimating the extent of asymptomatic COVID-19 and its potential for community transmission: systematic review and meta-analysis. Preprint. Posted online September 13, 2020. *medRxiv*. doi: 10.1101/2020.05.10.20097543.
3. Duan K, Liu B, Li C, et al. Effectiveness of convalescent plasma therapy in severe COVID-19 patients. *Proc Natl Acad Sci U S A.* 2020;117(17):9490–9496.
4. Shen C, Wang Z, Zhao F, et al. Treatment of 5 critically ill patients with COVID-19 with convalescent plasma. *JAMA.* 2020;323(16):1582–1589.
5. Bloch EM, Shoham S, Casadevall A, et al. Deployment of convalescent plasma for the prevention and treatment of COVID-19. *J Clin Invest.* 2020;130(6):2757–2765.
6. Kirkpatrick DD, Bradley J. UK paid $20 million for new coronavirus tests. They didn't work. https://www.nytimes.com/2020/04/16/world/europe/coronavirus-antibody-test-uk.html. Updated December 17, 2020. Accessed September 29, 2021.
7. Li Z, Yi Y, Luo X, et al. Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis. *J Med Virol.* 2020;92(9):1518–1524.
8. Van Elslande J, Houben E, Depypere M, et al. Diagnostic performance of seven rapid IgG/IgM antibody tests and the Euroimmun IgA/IgG ELISA in COVID-19 patients. *Clin Microbiol Infect.* 2020;26(8):1082–1087.
9. Stadlbauer D, Amanat F, Chromikova V, et al. SARS-CoV-2 seroconversion in humans: a detailed protocol for a serological assay, antigen production, and test setup. *Curr Protoc Microbiol.* 2020;57(1):e100.
10. Saltzman J. Nearly a third of 200 blood samples taken in Chelsea show exposure to coronavirus. https://www.bostonglobe.com/2020/04/17/business/nearly-third-200-blood-samples-taken-chelsea-show-exposure-coronavirus/. Updated April 17, 2020. Accessed September 29, 2021.
11. Florida Department of Health now reporting antibody test results. https://www.capecoralbreeze.com/news/local-news/2020/05/29/florida-department-of-health-now-reporting-antibody-test-results/. Updated May 29, 2020. Accessed September 29, 2021.
12. Chang D. After weeks of antibody testing, Florida to report results for the first time Friday. https://www.miamiherald.com/news/health-care/article243028726.html. Updated May 28, 2020. Accessed September 29, 2021.
13. Horvath K, Semlitsch T, Jeitler K, Krause R, Siebenhofer A. Antibody tests for COVID-19: what the results tell us [in German]. *Z Evid Fortbild Qual Gesundhwes.* 2020;153:54–59.
14. Centers for Disease Control and Prevention. Interim guidelines for COVID-19 antibody testing. https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html. Updated September 21, 2021. Accessed September 29, 2021.
15. Payne DC, Smith-Jeffcoat SE, Nowak G, et al; CDC COVID-19 Surge Laboratory Group. SARS-CoV-2 infections and serologic responses from a sample of U.S. Navy service members—USS Theodore Roosevelt, April 2020. *MMWR Morb Mortal Wkly Rep.* 2020;69(23):714–721.
16. Xie J, Ding C, Li J, et al. Characteristics of patients with coronavirus disease (COVID-19) confirmed using an IgM-IgG antibody test. *J Med Virol.* 2020;92(10):2004–2010.
17. Shen L, Wang C, Zhao J, et al. Delayed specific IgM antibody responses observed among COVID-19 patients with severe progression. *Emerg Microbes Infect.* 2020;9(1):1096–1101.
18. Padoan A, Cosma C, Sciacovelli L, Faggian D, Plebani M. Analytical performances of a chemiluminescence immunoassay for SARS-CoV-2 IgM/IgG and antibody kinetics. *Clin Chem Lab Med.* 2020;58(7):1081–1088.
19. Harvey RA, Rassen JA, Kabelac CA, et al. Association of SARS-CoV-2 seropositive antibody test with risk of future infection. *JAMA Intern Med.* 2021;181(5):672–679.
20. Chandrashekar A, Liu J, Martinot AJ, et al. SARS-CoV-2 infection protects against rechallenge in rhesus macaques. *Science.* 2020;369(6505):812–817.
21. Bongiovanni M, Basile F. Re-infection by COVID-19: a real threat for the future management of pandemia? *Infect Dis (Lond).* 2020;52(8):581–582.
22. Yang J, Wang W, Chen Z, et al. A vaccine targeting the RBD of the S protein of SARS-CoV-2 induces protective immunity. *Nature.* 2020;586(7830):572–577.
23. Plotkin SA. Correlates of protection induced by vaccination. *Clin Vaccine Immunol.* 2010;17(7):1055–1065.
24. Brown RCH, Kelly D, Wilkinson D, Savulescu J. The scientific and ethical feasibility of immunity passports. *Lancet Infect Dis.* 2021;21(3):e58–e63.
25. Zakrzewski C. The Technology 202: European countries are starting to issue vaccine passports. The system is very different than the U.S. approach. https://www.washingtonpost.com/politics/2021/06/02/technology-202-european-countries-are-starting-issue-vaccine-passports-system-is-very-different-than-us-approach/. Updated June 2, 2021. Accessed September 29, 2021.
26. Josephs L. Covid vaccine passports: everything we know so far. https://www.cnbc.com/2021/05/22/covid-vaccine-passports-everything-we-know-so-far.html. Updated May 22, 2021. Accessed September 29, 2021.
27. U.S. Food & Drug Administration. FDA in brief: FDA advises against use of SARS-CoV-2 antibody test results to evaluate immunity or protection from COVID-19, including after vaccination. https://www.fda.gov/news-events/press-announcements/fda-brief-fda-advises-against-use-sars-cov-2-antibody-test-results-evaluate-immunity-or-protection. Updated May 19, 2021. Accessed September 29, 2021.