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COMMENTARY

Evaluation of High-Throughput Serological Tests for SARS-CoV-2

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ABSTRACT Serologic methods are an important part of a clinical laboratory's portfolio of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) tests and are essential to the broader response to coronavirus infectious disease 2019 (COVID-19), including epidemiological studies and vaccine development. There are currently a number of commercial SARS-CoV-2 antibody tests with emergency use authorization (EUA) from the U.S. Food and Drug Administration. In this issue of the *Journal of Clinical Microbiology*, H. E. Prince, T. S. Givens, M. Lapé-Nixon, N. J. Clarke, et al. (J Clin Microbiol 58:e01742-20, https://doi.org/10.1128/JCM.01742-20, 2020) report the results of their evaluation of the agreement of 4 high-throughput EUA tests for SARS-CoV-2 IgG: Abbott Architect, DiaSorin Liaison, Euroimmun, and Ortho Vitros. They showed excellent agreement between the tests and rare false-positive reactivity for all tests.

The global pandemic of coronavirus infectious disease 2019 (COVID-19) has placed unprecedented demands on the capacity of laboratory medicine and public health systems. An adequate public health response to COVID-19 requires broad testing with accurate and reliable laboratory methods, particularly given the high proportion of asymptomatic infections (1, 2). Acute COVID-19 cases are diagnosed using nucleic acid amplification and antigen detection tests. Serologic tests generally provide accurate diagnosis when performed on specimens collected at least 10 to 14 days after symptom onset (3), but performance varies widely among tests and methods (4). There are now a number of commercially available serologic tests that have received emergency use authorization from the U.S. Food and Drug Administration (https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas#individual-serological; accessed 18 August 2020). Commercial serologic methods target host antibodies specific to several severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) epitopes, including the nucleocapsid protein, the spike protein, and the receptor binding domain of the spike protein. Given the novelty of SARS-CoV-2 and the laboratory methods used to detect SARS-CoV-2 infections, it is important to thoroughly vet their performance using rigorously designed studies in real-world settings. Our knowledge of SARS-CoV-2, COVID-19, and the performance of laboratory diagnostic tests for SARS-CoV-2 is expanding rapidly. There are a large number of published studies describing the performance of serologic tests for SARS-CoV-2, but many suffer from design flaws, including small sample size and patient selection bias (4). High-quality studies evaluating the performance of serologic tests for SARS-CoV-2 are needed.

Prince et al. evaluated the performance and concordance of 4 high-throughput serologic tests for detection of SARS-CoV-2 IgG targeting either nucleocapsid or spike protein (5). These immunoassays included a chemiluminescent microparticle immunoassay that targets the nucleocapsid protein (Architect SARS-CoV-2 IgG test [Abbott Laboratories, Abbott Park, IL]), 2 chemiluminescent immunoassays that target the spike

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protein (Liaison SARS-CoV-2 S1/S2 IgG [DiaSorin, Stillwater, MN] and Vitros anti-SARS-CoV-2 IgG [Ortho Clinical Diagnostics, Raritan, NJ]), and an enzyme-linked immunosorbent assay that targets the spike protein (anti-SARS-CoV-2 ELISA [IgG] [Euroimmun, Mountain Lakes, NJ]). The investigators selected consecutive serum specimens with either positive ($n = 600$) or negative ($n = 600$) results by the Architect test for subsequent analysis by the 3 spike protein-based tests. Of note, the study design incorporated inhibition assays using soluble SARS-CoV-2 proteins to robustly assess the specificity of the Architect, Liaison, and Euroimmun tests. The investigators used a consensus reference standard—agreement of at least 3 of the 4 tests evaluated—against which individual tests were compared. They found a high level of agreement, within the consensus interpretation, across all tests for 1,194 (99.5%) specimens. Of these, 1,109 specimens (92.9%) showed complete agreement. Agreement of the individual tests with the negative consensus interpretation ranged from 96.7% for the Euroimmun test to 100% for Vitros. Agreement with the positive consensus interpretation was lowest for Liaison at 94.3%. Of 36 specimens with false-negative results, 33 were with Liaison. Agreement with the positive consensus interpretation for the remaining tests ranged from 99.7% for Architect to 100% for Vitros. There were 49 specimens that were positive in only one assay. Retesting of these specimens in the respective instrument platform by inhibition assays showed that only 2 of these specimens were true positives. Nonetheless, false-positive reactivity was rare ($\leq$1.7% of all specimens for a given test). A limitation of the study was a lack of clinical information for patients from whom the serum specimens were collected. The ability to disaggregate data based on timing of specimen collection relative to symptom onset or exposure can provide valuable context for evaluating test sensitivity. The presence and severity of symptoms are also helpful information when serologic tests are assessed, as persons with asymptomatic or mild infections are known to exhibit a less robust antibody response (6).

Other investigators have reported similar performance among these 4 commercial tests in head-to-head comparisons (7, 8). Jääskeläinen et al. reported more variability in sensitivity and specificity among the Architect, Euroimmun, and Liaison tests compared to a microneutralization test, but this study included a relatively small number of specimens, and most were collected less than 15 days after onset of symptoms (9). The time from symptom onset to specimen collection will affect the sensitivity of SARS-CoV-2 serologic tests. Several investigators have reported higher sensitivity of the Architect (10–12) and Euroimmun (13) tests when they were performed on specimens collected later in the course of infection.

Prince et al. compared a nucleocapsid-based test (Architect) and 3 spike-based tests (Euroimmun, Liaison, and Vitros) and showed a high level of agreement (5). Similarly, others have reported similar performance between nucleocapsid and spike-based IgG immunoassays (6, 14). However, Burbelo et al. reported that nucleocapsid antibodies emerge before spike antibodies, resulting in higher sensitivity of a nucleocapsid-based test in some specimens (15).

Some evaluations of commercial SARS-CoV-2 serologic tests have used neutralization tests such as microneutralization (9) and plaque reduction (6) as the reference method and basis for reporting sensitivity and specificity of the tests being evaluated. Prince et al. used a different approach, by selecting specimens based on results from the Architect test and then assessing agreement between the immunoassay methods using a consensus reference standard.

Together, these studies demonstrate that these 4 high-throughput commercial SARS-CoV-2 IgG EUA tests targeting either nucleocapsid or spike protein and representing different assay formats have excellent agreement.

In summary, serologic tests are an important part of a laboratory's portfolio of SARS-CoV-2 tests. A number of commercial EUA SARS-CoV-2 IgG tests are available for implementation in clinical laboratories. Prince et al. provide important real-world data to help laboratory scientists and administrators make decisions on which test systems to select and implement.

REFERENCES

1. Kim GU, Kim MJ, Ra SH, Lee J, Bae S, Jung J, Kim SH. 2020. Clinical characteristics of asymptomatic and symptomatic patients with mild COVID-19. Clin Microbiol Infect 26:948.E1–948.E3. https://doi.org/10.1016/j.cmi.2020.04.040.
2. Tang Y-W, Schmitz JE, Persing DH, Stratton CW. 2020. Laboratory diagnosis of COVID-19: current issues and challenges. J Clin Microbiol 58:e00512-20. https://doi.org/10.1128/JCM.00512-20.
3. Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, Wang X, Yuan J, Li T, Li J, Qian S, Hong C, Wang F, Liu Y, Wang Z, He Q, Li Z, He B, Zhang T, Fu Y, Ge S, Liu L, Zhang J, Xia N, Zhang Z. 2020. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. Clin Infect Dis Epub ahead of print. https://doi.org/10.1093/cid/ciaa344.
4. Lisboa Bastos M, Tavaziva G, Abidi SK, Campbell JR, Haraoui L-P, Johnston JC, Lan Z, Law S, MacLean E, Trajman A, Menzies D, Benedetti A, Ahmad Khan F. 2020. Diagnostic accuracy of serological tests for covid-19: systematic review and meta-analysis. BMJ 370:m2516. https://doi.org/10.1136/bmj.m2516.
5. Prince HE, Givens TS, Lapé-Nixon M, Clarke NJ, Schwab DA, Batterman HJ, Jones RS, Meyer WA, III, Kapoor H, Rowland CM, Haji-Sheikhi F, Marlowe EM. 2020. Detection of SARS-CoV-2 IgG targeting nucleocapsid or spike protein by four high-throughput immunoassays authorized for emergency use. J Clin Microbiol 58:e01742-20. https://doi.org/10.1128/JCM.01742-20.
6. Okba NMA, Müller MA, Li W, Wang C, GeurtsvanKessel CH, Corman VM, Lamers MM, Sikkema RS, de Bruin E, Chandler FD, Yazdanpanah Y, Le Hingrat Q, Descamps D, Houhou-Fidouh N, Reusken CBEM, Bosch B-J, Drosten C, Koopmans MPG, Haagmans BL. 2020. Severe acute respiratory syndrome coronavirus 2-specific antibody responses in coronavirus disease patients. Emerg Infect Dis 26:1478–1488. https://doi.org/10.3201/eid2607.200841.
7. Kohmer N, Westhaus S, Rühl C, Ciesek S, Rabenau HF. 2020. Brief clinical evaluation of six high-throughput SARS-CoV-2 IgG antibody assays. J Clin Virol 129:104480. https://doi.org/10.1016/j.jcv.2020.104480.
8. Theel ES, Harring J, Hilgart H, Granger D. 2020. Performance characteristics of four high-throughput immunoassays for detection of IgG antibodies against SARS-CoV-2. J Clin Microbiol 58:e01243-20. https://doi.org/10.1128/JCM.01243-20.
9. Jääskeläinen AJ, Kuivanen S, Kekäläinen E, Ahava MJ, Loginov R, Kallio-Kokko H, Vapalahti O, Jarva H, Kurkela S, Lappalainen M. 2020. Performance of six SARS-CoV-2 immunoassays in comparison with microneutralisation. J Clin Virol 129:104512. https://doi.org/10.1016/j.jcv.2020.104512.
10. Bryan A, Pepper G, Wener MH, Fink SL, Morishima C, Chaudhary A, Jerome KR, Mathias PC, Greninger AL. 2020. Performance characteristics of the Abbott Architect SARS-CoV-2 IgG assay and seroprevalence in Boise, Idaho. J Clin Microbiol 58:e00941-20. https://doi.org/10.1128/JCM.00941-20.
11. Chew KL, Tan SS, Saw S, Pajarillaga A, Zaine S, Khoo C, Wang W, Tambyah P, Jureen R, Sethi SK. 2020. Clinical evaluation of serological IgG antibody response on the Abbott Architect for established SARS-CoV-2 infection. Clin Microbiol Infect 26:1256.e9–1256.e11. https://doi.org/10.1016/j.cmi.2020.05.036.
12. Hamilton F, Muir P, Attwood M, Vipond ANB, Hopes R, Moran E, Maskell N, Warwick D, Albur M, Turner J, MacGowan A, Arnold D. 30 July 2020. Kinetics and performance of the Abbott architect SARS-CoV-2 IgG antibody assay. J Infect. https://doi.org/10.1016/j.jinf.2020.07.031.
13. Beavis KG, Matushek SM, Abeleda APF, Bethel C, Hunt C, Gillen S, Moran A, Tesic V. 2020. Evaluation of the EUROIMMUN anti-SARS-CoV-2 ELISA assay for detection of IgA and IgG antibodies. J Clin Virol 129:104468. https://doi.org/10.1016/j.jcv.2020.104468.
14. Liu W, Liu L, Kou G, Zheng Y, Ding Y, Ni W, Wang Q, Tan L, Wu W, Tang S, Xiong Z, Zheng S. 2020. Evaluation of nucleocapsid and spike protein-based enzyme-linked immunosorbent assays for detecting antibodies against SARS-CoV-2. J Clin Microbiol 58:e00461-20. https://doi.org/10.1128/JCM.00461-20.
15. Burbelo PD, Riedo FX, Morishima C, Rawlings S, Smith D, Das S, Strich JR, Chertow DS, Davey RT, Cohen JI. 2020. Sensitivity in detection of antibodies to nucleocapsid and spike proteins of severe acute respiratory syndrome coronavirus 2 in patients with coronavirus disease 2019. J Infect Dis 222:206–213. https://doi.org/10.1093/infdis/jiaa273.