Unexpected False-Positive Rates in Pediatric SARS-CoV-2 Serology Using the EUROIMMUN Anti-SARS-CoV-2 ELISA IgG Assay

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Key Words: SARS-CoV-2; Orthogonal testing; Pediatric population; Seroprevalence

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ABSTRACT

Objectives: Serologic assay performance studies for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in pediatric populations are lacking, and few seroprevalence studies have routinely incorporated orthogonal testing to improve accuracy.

Methods: Remnant serum samples for routine bloodwork from 2,338 pediatric patients at UPMC Children's Hospital of Pittsburgh were assessed using the EUROIMMUN Anti-SARS-CoV-2 ELISA IgG (EuroIGG) assay. Reactive cases with sufficient volume were also tested using 3 additional commercial assays.

Results: Eighty-five specimens were reactive according to the EuroIGG, yielding 3.64% (95% confidence interval [CI], 2.91%-4.48%) seropositivity, of which 73 specimens had sufficient remaining volume for confirmation by orthogonal testing. Overall, 19.18% (95% CI, 10.18%-28.18%) of samples were positive on a second and/or third orthogonal assay. This 80.82% false positivity rate is disproportionate to the expected false positivity rate of 50% given our pediatric population prevalence and assay performance.

Conclusions: In pediatric populations, false-positive SARS-CoV-2 serology may be more common than assay and prevalence parameters would predict, and further studies are needed to establish the performance of SARS-CoV-2 serology in children.

Key Points

- SARS-CoV-2 serologic assay performance studies in the pediatric population are lacking, and neither adult nor pediatric seroprevalence studies incorporate orthogonal testing to improve accuracy.
- In pediatric populations, false positives may be more common than assay and prevalence parameters would predict.
- Orthogonal testing may be important in pediatric seroprevalence analyses for accurate results.

Serologic assays for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)–specific antibodies provide several important applications in monitoring and responding to the coronavirus disease 2019 (COVID-19) pandemic. SARS-CoV-2–specific antibody testing assists in surveillance, complex diagnoses, convalescent plasma donation, and confirmation of appropriate vaccine response. Characterization of the humoral response to SARS-CoV-2 and specific, robust detection methods are essential for meeting these needs. Prior validation studies across multiple testing platforms have demonstrated sufficient specificity (>99%) in the detection of anti–SARS-CoV-2 immunoglobulin G (IgG) antibodies, which included patients with prior common endemic coronavirus infections detected by nucleic acid testing. Large-scale seroprevalence surveys are underway, however, and minor cross-reactivities can lead to significant inaccuracies in prevalence estimates because of low pretest probabilities. To minimize false-positive tests, the current Centers for Disease Control and Prevention (CDC) interim clinical testing guidelines for SARS-CoV-2...
antibody testing recommend orthogonal testing, which employs 2 sequential independent tests when the initial test yields positive results. Seroprevalence studies in the pediatric population are especially lacking, and neither adult nor pediatric studies have routinely incorporated orthogonal testing. Between February 12, 2020, and April 20, 2020, only 1.7% of reported cases in the United States were in children younger than 18 years of age. Children generally develop milder disease, raising the possibility that seroprevalence studies are underestimating true seroprevalence. SARS-CoV-2 serologic assay validation in the pediatric population is lacking. We present a subset of cases from our pediatric SARS-CoV-2 seroprevalence study using an orthogonal testing strategy to assess false-positive SARS-CoV-2 antibody detection rates in our low-prevalence population and maximize analytic specificity.

Materials and Methods

The full cohort has been described elsewhere. Briefly, we used remnant serum samples from 2,338 consecutive patients younger than 19 years of age at the UPMC Children’s Hospital of Pittsburgh clinical laboratory received for routine testing for the study under the auspices of UPMC Quality Assurance for Clinical Laboratories and University of Pittsburgh institutional review board study number 20040072. Samples were collected beginning 2 weeks after the March 2020 peak of COVID-19 cases in Allegheny County during 2 phases: from April 27, 2020, to May 19, 2020, and from June 22, 2020, to July 4, 2020. These phases corresponded to Allegheny County’s initial period of containment (Red Phase) and subsequent less restrictive reopening phase (Yellow Phase). The sample cohorts were analyzed using enzyme-linked immunosorbent assay (ELISA)–based tests for anti–SARS-CoV-2 spike protein on the EUROIMMUN Anti-SARS-CoV-2 ELISA IgG (EuroIGG) assay, the Beckman Coulter Access SARS-CoV-2 IgG test on the AU5800 analyzer (Beckman), the Siemens ADVIA Centaur XP SARS-CoV-2 Total Antibody assay (Siemens-C), and the Siemens Dimension Vista 1500 SARS-CoV-2 Total Antibody assay (Siemens-V) according to the manufacturers’ instructions. These ELISA tests are for antibody against the S1 subunit/domain of the spike protein of SARS-CoV-2.

Results

Eighty-five of 2,338 specimens were reactive by the EuroIGG, yielding a 3.64% (95% confidence interval [CI], 2.91%-4.48%) seropositivity rate. Seventy-three of 85 EuroIGG reactive specimens had sufficient remaining sample volume for confirmation by orthogonal repeat testing. Samples with sufficient volume were tested using a third assay. The concordance between reactive EuroIGG samples and additional assays listed were 19.18% (Beckman, n = 73), 0% (Siemens-V, n = 9), and 10.0% (Siemens-C, n = 20) Table 1. Interestingly, concordance rates between the subset of reactive EuroIGG and Beckman samples with Siemens-V and Siemens-C assays were 100% (n = 9) and 95% (n = 20), respectively. Overall, only 19.18% (95% CI, 10.18%-28.18%) of samples were reactive on a second and/or third orthogonal assay, equating to a false positivity rate of 81.82%. This low concordance is disproportionate to the expected false-positive rate given our pediatric population prevalence (approximately 1%) and our validation of the assay sensitivity (98.7% at >14 days) and specificity (98.9%).

Discussion

Determining seroprevalence is a critical component of the COVID-19 response, and understanding the strengths and limitations of serologic testing is important for the application of these tests. We anticipated a false positivity rate of approximately 50% but found this pediatric population to have a false positivity rate of 81.82%. The expected false positivity rate of 50% we anticipated is consistent with that reported by other limited studies. A recent study found no significant difference in the false positivity rate in children across multiple assays compared with adults in a select cohort with a high prevalence. The positive predictive value of an assay is correlated with disease prevalence and specificity. Even tests with high specificity, when tested in low-prevalence populations, have a high probability of false positives. Currently, studies analyzing COVID-19 immunologic responses and validation of serologic assays have been carried out almost entirely in the adult population, indicating that assay parameters may be different in
children than in adults. Differences in immune response and cross-reactivity between pediatric and adult populations may help explain disproportionately increased false-positive rates.3,12 Discordant results among assays could be explained by immune responses directed toward a specific viral protein or assays detecting different Ig classes.1 Additionally, the increased false-positive rate could be attributed to the specific assay comparison (eg, EuroIGG vs Beckman), although prior work shows concordance between both the EuroIGG and Beckman assays to the assay consensus of 6 serologic assays to be 95% and 96%, respectively, in polymerase chain reaction–positive specimens.7 In a cohort of specimens with other known infectious disease processes, the EuroIGG had a specificity of 98.9%.7 A limitation of the study is that all assays used were against the S1 subunit/domain of the spike protein, though specific antigenic sites may vary. Orthogonal testing using multiple independent, antigenically distinct serologic assays has been shown to further promote increased specificity.13,14 Our results cause concern in low-prevalence pediatric populations because false positives may be more common than assay parameters and prevalence would dictate. These results support the CDC recommendation for orthogonal testing to improve study accuracy, especially in children and adolescents. Further studies are needed to establish the performance of SARS-CoV-2 serology in children.

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References

1. Theel ES, Slev P, Wheeler S, et al. The role of antibody testing for SARS-CoV-2: is there one? J Clin Microbiol. 2020;58:e00797-e00820.
2. Wheeler SE, Shurin GV, Keetch C, et al. Evaluation of SARS-CoV-2 prototype serologic test in hospitalized patients. Clin Biochem. 2020;86:8-14.
3. 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. Accessed March 20, 2021.
4. Centers for Disease Control and Prevention. COVID-19 case surveillance public use data profile. https://data.cdc.gov/Covid-19-Case-Surveillance/Public-Use-Data-Profile/xigx-wn5e. Accessed March 20, 2021.
5. Freeman MC, Rapsinski GJ, Zilla ML, et al. Immunocompromised seroprevalence and course of illness of SARS-CoV-2 in one pediatric quaternary care center [published online ahead of print October 13, 2020]. J Pediatric Infect Dis Soc. doi:10.1093/jpids/piaa123.
6. Allegheny County. COVID-19. https://www.alleghenycounty.us/Health-Department/Resources/COVID-19/COVID-19.aspx. Accessed March 20, 2021.
7. Zilla M, Wheeler BJ, Keetch C, et al. Variable performance in 6 commercial SARS-CoV-2 antibody assays may affect convalescent plasma and seroprevalence screening. Am J Clin Pathol. 2021;155:343-353.
8. Watson J, Richter A, Deeks J. Testing for SARS-CoV-2 antibodies [published online ahead of print September 8, 2020]. BMJ. doi:10.1136/bmj.m3325.
9. Turbett SE, Anahtar M, Dighe AS, et al. Evaluation of three commercial SARS-CoV-2 serologic assays and their performance in two-test algorithms. J Clin Microbiol. 2020;59:e01892-e01920.
10. Farnsworth CW, Anderson NW. SARS-CoV-2 serology: much hype, little data. Clin Chem. 2020;66:875-877.
11. Oved K, Olmer L, Shemer-Avni Y, et al. Multi-center nationwide comparison of seven serology assays reveals a SARS-CoV-2 non-responding seronegative subpopulation. EClinicalMedicine. 2020;29:j10651.
12. Carlotti APCP, de Carvalho WB, Johnston C, et al. Update on the diagnosis and management of COVID-19 in pediatric patients. Clinics (Sao Paulo). 2020;75:e2353.
13. Ripperger TJ, Uhrlaub JL, Watanabe M, et al. Orthogonal SARS-CoV-2 serological assays enable surveillance of low-prevalence communities and reveal durable humoral immunity. Immunity. 2020;53:925-933.e4.
14. Xu G, Emanuel AJ, Nadig S, et al. Evaluation of orthogonal testing algorithm for detection of SARS-CoV-2 IgG antibodies. Clin Chem. 2020;66:1531-1537.