Correctly Interpreting SARS-CoV-2 Serologic Assays

To the Editor:

The authors of Tang et al. *(1)* have incorrectly concluded that the Abbott SARS-CoV-2 assay demonstrated higher sensitivity and specificity than the EUROIMMUN Anti-SARS-CoV-2 IgG assay, despite also stating that overlapping of confidence intervals showed differences were statistically insignificant. Therefore, the authors contradict their own conclusions as they found no significant difference between the EUROIMMUN and the Abbott assays for detection of IgG antibodies to SARS-CoV-2.

Of concern, for the specificity controls, the authors used 153 samples. Of these, 50 were from uncharacterized donors sampled in 2015 and the other 103 samples used were without description of the sampling date. We can only assume that the 80 samples from symptomatic patients with negative RT-PCR for SARS-CoV-2 noted in the study were sampled during the pandemic. The sampling date for the remaining 23 specimens is unclear: 5 samples from patients with positive RT-PCR for other human coronaviruses without sampling date, 4 samples from patients with Influenza A or B without characterization of the type or conformation for the disease and 14 random samples with known outcomes of unrelated antibodies against CMV or EBV and rheumatoid factor.

We regard the use of PCR negative samples taken during the pandemic for negative controls in the calculation as problematic and believe that most in the field would agree with that conclusion. RT-PCR cannot be used as a "gold standard" in this setting, given that the "false" negative rate of RT-PCR is approximately 20%, depending on the study *(2)*. Negative results in individuals that have already been infected may arise because of analytical failure, but may also be attributable to timing of sampling, low nasal carriage of virus, or low viral load. It therefore remains plausible that positive results in this group are true positives. Overall, 5 patients compared with one patient were positive out of the 80 symptomatic RT-PCR negative individuals using EUROIMMUN and Abbott assays, respectively. Additional serial sampling of these patients would be required to accurately determine their immune response against SARS-CoV-2. In a study of 1756 pre- and post-pandemic normal individuals, specificity of the EUROIMMUN Anti-SARS-CoV-2 IgG assay was 99.0% *(3)*.

We further believe that the condensed report of the sensitivity based on all 103 samples from only 48 patients taken at different time points postonset of symptoms (PSO) and/or positive results in RT-PCR also portrays a biased picture. In general, IgG antibodies can be detected at a median of 14 days' PSO and IgG peaks at 21–25 days PSO. Long et al. 2020 showed an increase in virus-specific IgG was observed during the first 3 weeks PSO, and the positivity rate reached approximately 100% at 17–19 days PSO *(4)*. Segregation of time points for determining sensitivity of IgG assays is imprecise as published in this study, and mostly before IgG response maturation. CDC recommendations call for medical use of IgG serology to determine past infection, not an acute infection, and use of acute rather than convalescent sera to determine "sensitivity" of an IgG serology is incorrect. Beavis et al. demonstrated a EUROIMMUN Anti-SARS-CoV-2 IgG sensitivity of 100% in convalescent sera of previously documented RT-PCR positive individuals *(5)*.

The study cites and was performed according to an earlier CE-marked assay package insert. While the kit and assay instructions remained unchanged, the currently available kit was FDA EUA approved based on additional data *(3)*. We recognize that the literature on serology is rapidly evolving, but FDA websites are constantly updated *(3)*. In total, 597 COVID-19 patient samples were included in our EUA submission. Additional studies have been published or are ongoing.

The authors state it is unclear whether neutralizing antibodies target the spike protein or the nucleocapsid protein. On the contrary, experience with SARS-CoV-1 and recent studies on SARS-CoV-2 suggest that neutralizing antibodies target the external S spike protein, particularly the S1 subunit (the antigen in the EUROIMMUN assay) and the receptor binding domain contained within the S1 domain, preventing viral entry into the host cell, rather than the internal N nucleocapsid protein (the antigen in the Abbott assay). We and others continue to study neutralization.

In conclusion, we believe strongly the authors' study does not accurately represent true sensitivity and specificity of the EUROIMMUN IgG ELISA.

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.*

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:*

Employment or Leadership: S.J. Naides, EUROIMMUN US and, formerly, Quest Diagnostics, Inc.
Consultant or Advisory Role: None declared.
Stock Ownership: S.J. Naides, Quest Diagnostics, Inc.
Honoraria: None declared.
Research Funding: None declared.
Expert Testimony: None declared.
Patents: None declared.

References

1. 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;66:1055–62.
2. Liu L, Liu W, Zheng Y, Jiang X, Kou G, Ding J, et al. A preliminary study on serological assay for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in 238 admitted hospital patients. [Epub ahead of print] Microbes Infect May 18, 2020 as doi:10.1016/j.micinf.2020.05.008.
3. US Food and Drug Administration. Anti-SARS-CoV-2 ELISA (IgG). Instruction for use. https://www.fda.gov/media/137609/download (Accessed June 2020).
4. 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;26:845–8.
5. Beavis KG, Matushek SM, Abeleda APF, Bethel C, Hunt C, Gillen S, et al. Evaluation of the EUROIMMUN anti-SARS-CoV-2 ELISA assay for detection of IgA and IgG antibodies. [Epub ahead of print] J Clin Virol May 23, 2020 as doi:10.1016/j.jcv.2020.104468.

Stanley J. Naides*[a]
[a]Scientific Affairs, EUROIMMUN US, Mountain Lakes, NJ

*Address correspondence to this author at: Euroimmun US, Scientific Affairs, 1 Bloomfield Ave., Mountain Lakes, NJ 07046. E-mail stanley.naides@euroimmun.us.

DOI: 10.1093/clinchem/hvaa165