Serology testing demonstrates that antibodies to SARS-CoV-2 S1-RBD correlate with neutralization of virus infection of Vero E6 cells

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To the Editor:

Coronavirus disease 2019 (COVID-19) patients develop antibodies against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) proteins, including the highly immunogenic Spike (S) and Nucleocapsid (N) proteins used in most serology assays. The S-protein S1-domain harbors the receptor-binding domain (S1-RBD) that targets its cellular receptor, ACE2. Receptor binding is followed by viral fusion, cell entry, and viral replication. Studies have shown that anti-SARS-CoV-2 antibodies are elicited in COVID-19 patients, and that antibody neutralizing activity predominantly targets S1-RBD and total S-protein. Several groups have reported correlations of neutralizing antibody titers with SARS-CoV-2 and various anti-RBD or anti-S IgG (or total IgG) assay titers [1-4]; and, immune and neutralizing titers have correlated strongly with disease severity—the highest titers found in patients with the most severe disease [3-5]. Current mRNA, DNA, and protein vaccines target S-protein as antigen, known to elicit neutralizing titers in vaccinated individuals. Such neutralizing antibodies can potentially prevent viral infection by blocking receptor binding—although the level required for immune protection is not yet known.

On July 31, 2020, the Atellica® IM SARS-CoV-2 IgG (COV2G) Assay (Siemens Healthineers, Tarrytown, New York, United States [U.S.]) was granted Emergency Use Authorization by the U.S. Food and Drug

Administration. The assay is a fully automated chemiluminescent immunoassay that detects IgG to S1-RBD in serum and plasma. Results are reported in Index Values: Samples with ≥1.0 Index Values are considered reactive and positive for antibodies. Semi-quantitative measurements can be reported for reactive results with numeric Index Values. Here, we evaluated the extent to which the COV2G test results reflected the presence in serum of antibodies with ability to neutralize SARS-CoV-2.

Serum samples for this retrospective study were from 26 symptomatic subjects with RT-PCR and serology (Siemens assays, COV2G and SARS-CoV-2 Total [COV2T])-confirmed SARS-CoV-2 infection. Samples were provided by Siemens Healthineers, from BocaBiolistics (www.bocabio.com) and Xera Med Research (www.xeramed.com), with Institutional Review Board approvals and patient consent. Time from blood draw to symptom onset and RT-PCR positive date were recorded. Microneutralization endpoint-dilution assays were performed at ZeptoMetrix (Buffalo, New York, U.S.) [8]. SARS-CoV-2 virus, Isolate USA-WA1/2020, NR-52281 (Centers for Disease Control and Prevention and, BEI Resources, NIAID, NIH). The virus was grown in VeroE6 cells with MEM/2% FBS/sodium pyruvate/MEM-NEAA/Penicillin-Streptomycin. Cultures were harvested, frozen, then titered in VeroE6 cells using the endpoint-dilution $TCID_{50}$ assay. The day before assay set-up, Vero E6 cells (1×10^4) were plated in 96-well plates. Low (1-20), medium (20-60),

and high (>60) index samples (previously heat inactivated) were diluted 1:25, 1:100, and 1:1000 in MEM, respectively, before serially diluting 1:2 across fresh plates. SARS-CoV-2 (100 $TCID_{50}$ units) was added to each well and plates were incubated, one hour at 37°C. Virus-only control was infectious virus incubated with MEM for one hour. Serum-virus mixture or virus control (150 µL) was then added to Vero E6 cells (in 50 µL of MEM) and plates were incubated for two days. Column 12 of all plates contained cell-only control Vero E6 cells in MEM. After two days, wells were photographed. Cytopathic effects (CPE) and neutralization of CPE were quantified vs. controls. Supernatant was replaced with Neutral Red staining solution (Sigma, St. Louis, MO) (0.02% in D-PBS) and plates were incubated, one hour at room temperature. Wells were washed twice with D-PBS, lysis buffer added for 15 minutes, and absorbance at 540 nM recorded (BioTek Epoch Plate Reader, Gen5 software). The highest serum dilution showing an absorbance reading greater than the cut-off value was considered the neutralization titer. To calculate the cut-off, absorbance values of cell-only control wells were averaged and divided by two.

Results demonstrated that SARS-CoV-2 transfection caused CPE which were reduced in the presence of various patient sera. Furthermore, a strong relationship was found between the COV2G assay Index Values and microneutralization titer (Spearman’s correlation coefficient=0.81) (Figure 1). These results corroborate those of other studies that reported

strong correlations between levels of various anti-RBD or anti-S IgG serology assays and neutralizing titers with SARS-CoV-2 [1-4] . Correlations between immune and neutralizing titers and disease severity, as reported by others [3-5] , could not be assessed due to small sample size (severity level only available for four patients). However, this is a goal for future studies—relevant to populations that may benefit from this kind of serology testing as a proxy for neutralization titers. No correlation was found between Index Value to days from symptom onset or days from RT-PCR positive result.

To our knowledge, these data are the first to demonstrate that COV2G test results correlate with in vitro neutralization using CPE. Future studies should determine whether serology assays can be a suitable substitute for cumbersome ‘gold-standard’ plaque-reduction neutralization tests (PRNT) and the protective level of antibody required for immunity by vaccines.

Disclaimer: This test has not been FDA cleared or approved. This test has been authorized by FDA under an EUA for use by authorized laboratories. This test has been authorized only for detecting the presence of antibodies against SARS-CoV-2, not for any other viruses or pathogens. This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under

Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner. Product availability may vary from country to country and is subject to varying regulatory requirements.

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Figure Caption

Figure 1. The Atellica IM COV2G Assay Index Values correlate with neutralization titer using a viral neutralization test (VNT) [10]; R=0.81. Samples tested were from 26 subjects with a clinical diagnosis of COVID-19 based on positive SARS-CoV-2 RT-PCR method results. CPE: Cytopathic effects.