SARS-CoV-2 Anti-Spike IgG Antibody and ACE2 Receptor Binding Inhibition Levels among Breakthrough Stage Veteran Patients

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ABSTRACT

SARS-CoV-2 mRNA vaccines have been critical to curbing pandemic COVID-19; however, a major shortcoming has been the inability to assess levels of protection after vaccination. This study assessed serologic status of breakthrough infections in vaccinated patients at a Veterans Administration medical center from June through December 2021 during a SARS-CoV-2 delta variant wave. Breakthrough occurred mostly beyond 150 days after two-dose vaccination with a mean of 239 days. Anti-SARS-CoV-2 spike (S) IgG levels were low at 0 to 2 days postsymptoms but increased in subjects presenting thereafter. Population measurements of anti-S IgG and angiotensin converting enzyme-2 receptor (ACE2-R) binding inhibition among uninfected, vaccinated patients suggested immune decay occurred after 150 days with 62% having anti-S IgG levels at or below 1,000 AU comparable with breakthrough patients at 0 to 2 days postsymptom onset. In contrast, vaccination after resolved infection conferred robust enduring anti-S IgG levels (5,000 to >50,000 AU) with >90% ACE2-R binding inhibition. However, monoclonal antibody (MAb)-treated patients did not benefit from their prior infection suggesting impaired establishment of B cell memory. Analysis of boosted patients confirmed the benefit of a third vaccine dose with most having anti-S IgG levels above 5,000 AU with >90% ACE2-R binding inhibition, but a subset had levels <5,000 AU. Anti-S IgG levels >5,000 AU were associated with >90% ACE2-R binding inhibition and no documented breakthrough infections, whereas levels falling below 5,000 AU and approaching 1,000 AU were associated with breakthrough infections. Thus, quantitative antibody measurements may provide a means to guide vaccination intervals for the individual.

IMPORTANCE

Currently, clinicians have no guidance for the serologic assessment of SARS-CoV-2 postvaccination status regarding protection and risk of infection. Vaccination and boosters are administered blindly without evaluation of need or outcome at the individual level. The recent development of automated quantitative assays for anti-SARS-CoV-2 spike protein IgG antibodies permits accurate measurement of humoral immunity in standardized units. Clinical studies, such as reported here, will help establish protective antibody levels allowing identification and targeted management of poor vaccine responders and vaccinated subjects undergoing immune decay.

KEYWORDS

ACE2 receptor, COVID-19, SARS-CoV-2, antibody assay, breakthrough infection, immunization, serology

Understanding the efficacy and duration of SARS-CoV-2 mRNA vaccine elicited antibodies remains an important subject of investigation. RNA-based vaccines were not in wide use prior to the COVID-19 pandemic. The recent extensive implementation of these agents
has provided unique opportunities to study this novel vaccine approach. Several studies have described anti-SARS-CoV-2 antibody induction kinetics following mRNA vaccination (1–6). However, questions remain concerning the relationship between antibody levels and clinical protection, and to date, target levels of protective vaccine-induced antibodies have not been defined. Likewise, the potential influence of prior SARS-CoV-2-targeted monoclonal antibody (MAb) therapy on vaccine efficacy is unknown.

Soon after the emergency use authorization (EUA) of SARS-CoV-2 mRNA vaccines, the Veterans Health Administration (VA) initiated a national vaccination program for veteran patients and employees. The VA Ann Arbor Healthcare System (VAAAHS), Ann Arbor, Michigan implemented local vaccinations in December 2020 with vaccination histories stored in electronic medical records. Over 25,000 veterans in its catchment area had completed a two-dose vaccination protocol by June 1, 2021, by which time cases of COVID-19 had declined to a nadir in the state. Subsequently, the SARS-CoV-2 delta (B.1.617.2) variant emerged and became the dominant variant for the remainder of 2021 (7). This latter period was associated with a notable number of breakthrough infections among vaccinated patients. The present study was undertaken to assess the durability of the vaccine-elicited antibody response and potential effects of prior infection and treatments. Using chart review and testing of blood specimens, the present study examined the anti-SARS-CoV-2 serologic status of vaccinated patients during this active breakthrough period. Serologic testing included assessment of anti-SARS-CoV-2 receptor binding domain (RBD) IgG antibodies, anti-nucleocapsid (N) IgG antibodies and angiotensin converting enzyme-2 receptor (ACE2-R) binding inhibition. The findings reveal important insights into immune decay, the effects of prior infection, monoclonal antibody therapy, and potential use of serological testing to assess risk of breakthrough infection.

**RESULTS**

Breakthrough infections. Between June and December 2021, 246 breakthrough infections documented by RT-PCR or viral antigen testing were identified among patients who had undergone either Pfizer or Moderna two-dose vaccination protocol. None had yet to receive a third booster injection. As shown in Fig. 1, the number of breakthrough infections increased rapidly 6 months after completion of the initial vaccination series, with the average interval from second injection to breakthrough infection of 239 ± 43 days. Breakthrough rates declined after 8 months as the delta variant infection wave waned and booster vaccinations were instituted.

A subset of both vaccinated and unvaccinated infected patients underwent testing for anti-SARS-CoV-2 antibodies at the time of presentation. Antibody levels were plotted against days after symptom onset for each patient. Fig. 2 shows the semiquantitative anti-spike (S) and anti-nucleocapsid (N) IgG levels in these populations. Both vaccinated (n = 22) and unvaccinated (n = 11) patients who presented 0 to 2 days after symptom onset had
low levels of anti-S IgG antibodies, with an average of 5.9 ± 7.8 U and 1.4 ± 3.2 U, respectively. The higher level among vaccinated subjects was consistent with a residual level of vaccine-elicted immunity. Thereafter, compared with unvaccinated patients who remained at near baseline over 10 days, vaccinated patients displayed what appeared to be a rapid anti-S IgG memory response with some patients exceeding the reportable range of the assay by day 4 (Fig. 2A). RT-PCR cycle threshold (Ct) values were comparable among vaccinated and unvaccinated patients suggesting this difference was not due to differences in viral load (Fig. S1). In addition, vaccinated breakthrough patients at each postsymptom day showed no differences in postvaccination interval (Fig. S2), indicating the increasing anti-S IgG was not due to differences in vaccination intervals.

As shown in Fig. 2B, anti-N IgG antibodies appeared with similar kinetics in both groups with most individuals positive after day 10, as we previously reported for a primary response to SARS-CoV-2 nucleocapsid antigen (8). Because there was no evidence of a secondary anamnestic anti-N response, it indicated that no previous occult infections had occurred. The potential for a rapid endogenous anti-S IgG memory response illustrates the difficulty of determining risk levels of anti-SARS-CoV-2 antibodies in breakthrough patients if measurements are not related to time of symptom onset.

**Serologic status of patients in postvaccine breakthrough period.** Having established a substantial onset of breakthrough infections beyond 6 months after a two-dose vaccination, we assessed the serologic status of uninfected, vaccinated veteran patients sampled at the population level. Anti-S (RBD) IgG and ACE2 receptor (ACE2-R) binding inhibition (a surrogate of
viral neutralizing activity) were measured by quantitative Abbott assays in patient serum and plasma samples provided by the VAAAHS clinical laboratory.

Fig. 3 shows quantitative levels of anti-S (RBD) IgG and ACE2-R binding inhibition in 107 vaccinated individuals plotted against days since completing their second vaccine dose. These included naive patients with no prior SARS-CoV-2 infection as well as subjects with a known history of infection before undergoing Pfizer or Moderna vaccination among which some had anti-SARS-CoV-2 monoclonal antibody (MAb) therapy, either bamlanivimab alone or combination preparations of casirivimab plus imdevimab or bamlanivimab plus etesevimab. Fig. 3A shows that anti-S (RBD) IgG levels were in the 5,000 to >50,000 AU range for subjects in the under 150 days postvaccination period. This was associated with potent 95% to 100% ACE2-R binding inhibition (Fig. 3B). Among naive vaccinated patients 150 to 450 days after completing atwo-dose Pfizer or Moderna vaccination, 50 of 81 (62%) subjects had anti-S (RBD) IgG of less than 1,000 AU and 73 of 81 (90%) less than 5,000. This corresponded to a clearly demarcated reduction in ACE2-R binding inhibition levels in the 0% to 50% range. Pfizer and Moderna vaccine responses were comparable among naive vaccinated subjects. Many patients underwent booster vaccination after sample collection. However, seven subjects had documented breakthrough infections shortly after sample collection (Fig. 3 diamond enclosed circles), all of which had anti-S (RBD) IgG levels below 5,000 AU (969 ± 1,232 AU) with ACE2-R binding inhibition of 21% ± 29%. No subsequent breakthrough infections were charted during the study period among subjects with >5,000 AU anti-S (RBD) IgG levels.

![Fig 3](image-url)

**FIG 3** Quantitative SARS-CoV-2 anti-S (RBD) IgG and ACE2 receptor binding inhibition among vaccinated subjects after two-dose vaccination. (A) anti-S (RBD) IgG. (B) ACE2-R binding inhibition. Solid circles are Pfizer and Moderna vaccinated subjects (N = 85) with no documented prior or current viral infection. Diamond enclosed circles are seven individuals who developed breakthrough infections after sampling. Open squares and triangles are respectively monoclonal antibody (MAb) untreated (N = 10) and treated (N = 12) subjects vaccinated after documented resolved infection.
To compare Abbott with Siemens semiquantitative Anti-S (RBD) IgG assay results, we performed a correlation study and showed excellent correlation with a correlation coefficient of 0.935 (Fig. S3). Based on this, the average of Siemens values for anti-S (RBD) IgG at 0 to 2 days (Fig. 2A) after breakthrough converted to an equivalent 815 ± 642 quantitative Abbott units. This compared favorably with levels observed among the 62% of uninfected naive vaccinated subjects after 150 days and suggested that anti-S (RBD) IgG levels dropping below 5,000 AU and approaching 1,000 AU may increase risk for breakthrough infection.

Serologic status of vaccinated patients with prior infection and monoclonal antibody treatment. Our serologic survey also identified patients who had been vaccinated after a resolved SARS-CoV-2 infection. All were vaccinated more than 100 days after infection. As shown in Fig. 3A and B, previously infected patients with no history of MAb treatment had enduring robust anti-S (RBD) IgG levels of 5,000 to >50,000 AU with ACE2-R binding inhibition of 90% to 100%. This is fully consistent with recent reports that vaccination after natural infection elicits a strong anti-SARS-CoV-2 antibody response (1, 9, 10). In contrast, previously infected individuals who had undergone anti-SARS-CoV-2 MAb treatment did not show the same enduring response in the >150-day period, but rather had a pattern more like naive subjects with significant reductions of ACE2-R binding inhibition. As a group, anti-S (RBD) IgG levels among eight MAb treated subjects sampled in the >150-day breakthrough period had significantly lower anti-S (RBD) IgG and ACE2-R binding inhibition levels compared to infected subjects who were not treated with MAb (Table 1). Interestingly, when sorted by specific MAb therapy, patients who received combination preparations of casirivimab plus imdevimab or bamlanivimab plus etesevimab appeared to be more severely impacted by the treatment than those receiving bamlanivimab alone (Table 1; Fig. S4). While levels in vaccinated patients with prior MAb were higher than unvaccinated subjects with no prior infection, they were not statistically different from previously infected, unvaccinated patients treated with MAb.

Serologic status of veterans after booster vaccination. Our samples also included specimens from patients who received booster vaccinations. Fig. 4A and B show plots of 90 vaccinated individuals who received a third booster up to 300 days before sample collection. Among the COVID-naïve vaccinated group, only three Moderna vaccinations were identified precluding comparisons with Pfizer; however, 98% of boosted subjects had anti-S (RBD) levels of 1,000 AU or above with 74% greater than 5,000 AU. All 12 previously infected patients not treated with MAb had robust serologic status with 99% to 100% ACE2-R binding inhibition. Only five MAb treated patients were identified but all appeared to respond comparably to COVID-naïve vaccinated subjects. The findings supported the benefit of a booster as it appeared to confer a potent and enduring antibody response in most subjects. However, after day 90, 15 patients had ACE2-R binding inhibition <80% suggesting either immune decay or suboptimal vaccine response among some individuals. A review of the clinical histories of these individuals revealed a variety of chronic illnesses with no major differences from the overall group that would readily explain the reduced responses. In addition to immune decay, suboptimal vaccine administration, genetic factors, or undiagnosed medical comorbidities.

| Group                        | N   | Days since vaccination #2 (range) | Days since MAb treatment (range) | Anti-S (RBD) IgG (AU) Mean ± SD | ACE2-R binding inhibition (%) Mean ± SD |
|------------------------------|-----|-----------------------------------|----------------------------------|---------------------------------|----------------------------------------|
| No MAb vaccinated            | 7   | 216 to 399                        | n/a                              | 31,889 ± 33,437                | 98.2 ± 2.9                             |
| MAb treated vaccinated       | 8   | 165 to 257                        | 277 to 321                       | 6,007 ± 9,532                 | 44.2 ± 29.7                          |
| BAM                          | 3   | 185 to 257                        |                                   | 14,000 ± 12,825               | 75.4 ± 24.4                          |
| BAM + ETE                    | 2   | 191 to 217                        |                                   | 951 ± 420                     | 27.9 ± 15.8                          |
| CAS + IMD                    | 3   | 165 to 188                        |                                   | 1,387 ± 146                   | 23.7 ± 2.5                           |
| MAb treated unvaccinated     | 6   | n/a                               | 264 to 402                       | 657 ± 459                    | 21.2 ± 17.7                           |
| No infection unvaccinated    | 8   | n/a                               | n/a                              | 22 ± 42                       | 1.0 ± 0.7                             |

*BAM, bamlanivimab; ETE, etesevimab; CAS, casirivimab; IMD, imdevimab; n/a, not applicable.

Significantly different from previously infected, MAb untreated vaccinated (top row) and uninfected, unvaccinated (bottom row) (P < 0.05), but not from MAb treated unvaccinated (P > 0.05).

Significantly increased compared with no MAb unvaccinated, uninfected control group (bottom row) (P < 0.05).

Kruskal-Wallis rank test performed for group comparisons.
would be among etiologic considerations. Follow-up studies to assess breakthrough among these patients were not performed. Nevertheless, the results indicate that serologic studies may be useful for identifying suboptimal antibody levels that may require additional booster interventions.

**Relationship of anti-RBD IgG levels and ACE-2 binding inhibition.** The quantitative ACE2-R binding inhibition assay highly correlates with the in vitro viral plaque reduction neutralization test (11). Fig. 5 shows the ACE2-R binding inhibition plotted as function of the quantitative anti-S (RBD) units in each of 142 patient specimens. A distinct S-shaped curve is generated with a steep slope between 800 and 5,000 AU corresponding to ACE2-R binding inhibition of 20% to 90% with 50% inhibition at approximately 2,000 anti-S (RBD) IgG AU. Our analyses suggest that anti-S (RBD) IgG levels declining to the 1,000 AU (20% ACE2-R binding inhibition) range are associated with breakthrough infections, providing some guidance for setting desirable target levels. While midrange ACE2-R binding inhibition (50%) may offer protection, we suggest setting clinical target anti-S (RBD) IgG level of >5,000 AU that should achieve ACE2-R binding inhibition of >90%. This appears to be easily achievable with a third booster vaccination for most subjects, but in our sample approximately 20% of patients did not reach or fell below 5,000 AU beyond 90 days after booster.

**DISCUSSION**

Numerous studies have established the protective effect of SARS-CoV-2 mRNA vaccine-elicited antibodies (6, 12–14). Despite this, serologic measurement to assess protective levels of anti-S (RBD) antibodies is not currently recommended by the Food and Drug Administration (15). This is due to confusion in interpreting serologic results without defined protective levels.

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**FIG 4** Quantitative SARS-CoV-2 anti-S (RBD) IgG and ACE2 receptor binding inhibition among vaccinated subjects after third booster vaccination. (A) anti-S (RBD) IgG. (B) ACE2-R binding inhibition. Solid circles are Pfizer and Moderna vaccinated subjects (N = 74) with no documented prior or current viral infection. Open squares and triangles are respectively monoclonal antibody (MAb) untreated (N = 12) and treated (N = 4) subjects vaccinated after documented resolved infection.
Moreover, it is difficult to implement prospective studies to ascertain protective antibody levels that would require repeated blood sampling of subjects with random viral exposure monitored over long periods. Yet, the clinical need of having defined levels of protection is clear. Currently, there is no standard means to identify poor vaccine responders or monitor decay of protective antibodies. A recent study employed a retrospective population-based analysis to establish target threshold protective anti-spike(S) IgG levels for different vaccines using a pseudoviral neutralization assay (16). We employed a similar retrospective study of veterans at a tertiary care VA Healthcare System to assess the relationship of antibody levels to breakthrough infections.

SARS-CoV-2 RNA vaccine antibody induction kinetics and efficacy are well-characterized with peak efficacy occurring about 1 month after the second booster dose (6). After that time, efficacy appears to decay at varying rates with a recent meta-analysis reporting an approximate 21% drop in efficacy for all ages and genders by 6 months after two-dose vaccinations (13). In accord with published reports of immune decay kinetics (17), our analysis revealed diminished levels of anti-S (RBD) IgG and ACE2-R binding inhibition among most vaccinated veterans in the period beyond 6 months after vaccination which was associated with a notable onset of breakthrough infections.

The recent development of high-throughput automated assays to quantify anti-SARS-CoV-2 antibodies provides the ability to reproducibly measure anti-spike (S) glycoprotein antibody levels. While several manufacturers offer assays, these often have different output units. The World Health Organization has established guidance for conversion to standard binding antibody units (BAU), but this is not yet widely utilized by clinical laboratories (18, 19). The Abbott anti-S (RBD) IgG assay employed in this study has a wide reportable range of 6.8 to 50,000 AU/mL corresponding to 0.97 to 7,100 BAU allowing for accurate measurement of both high and low levels of antibodies. Our study suggested that anti-S IgG levels dwindling near and below 1,000 AU (142 BAU) were associated with corresponding low ACE2-R binding inhibition and breakthrough infections. At present, the ACE2-R binding inhibition assay is not approved for clinical application necessitating the use of anti-S (RBD) IgG levels as the measure of immunity. Thus, quantitative measurement of antibody levels could potentially identify subjects at risk for breakthrough infection. Additionally, based on the relationship of antibody levels to ACE2-R binding inhibition, levels of 5,000 AU (710 BAU) or higher would seem to be reasonable target for protection as they were associated with ACE2-R binding inhibition of >90%.

Using anti-SARS-CoV-2 serologic status to assess protection is controversial as it represents only one aspect of the immune response. The currently accepted paradigm

![Figure 5](image-url)
is that immunity to viral infection involves innate as well as adaptive humoral and cytotoxic T cell immune mechanisms. Vaccines induce both humoral and cytotoxic T cell immunity. The latter is thought to eliminate virally infected host cells and has been demonstrated to clear virus without antibodies in a mouse model; however, in the presence of antibodies, cytotoxic T cells appear redundant (20). Protective antibodies are highly effective in curtailing virally induced severe disease likely by reducing downstream cell-mediated cytokine production (21). From a clinical perspective, having an easily measurable parameter of protection would be of value despite the contribution of other immune mechanisms. Currently, clinicians have no objective measure to identify individuals with adequate or suboptimal vaccine responses. For example, repeated “blind” boosting is inefficient, leading to both under- and overdosing a notable portion of the population. With utilization of an evidence-based quantitative metric, vaccination intervals could be tailored to an individual’s needs, based on objective laboratory studies. As scalable tests for cytotoxic T cell immunity become available (22), combined measurement of humoral- and cell-mediated immunity would be optimal for monitoring postvaccination immune status.

A critical point to address is the role of viral variants. The SARS-CoV-2 omicron variant and its subvariants became dominant in 2022 causing widespread breakthrough infections. Mutations in the spike glycoprotein of this variant augmented infectiousness (23). The assays used in this study were optimized to detect responses to earlier variants. Presumably, anti-S (RBD) antibody levels effective against the delta variant would be insufficient to fully block omicron variants. Studies indicate that a third first-generation booster vaccination provides some protection against the omicron variant and is as effective as omicron targeted vaccines (24–26). Our quantitative serologic analysis of patients having received a third booster showed the majority (80%) having anti-S (RBD) IgG levels above 5,000 AU (710 BAU) and >90% ACE2-R binding inhibition. This would support the notion of setting target IgG levels that achieve >90% ACE2-R binding inhibition. However, the duration of protection against the omicron variant by first-generation vaccine boosting has yet to be established. To fully address protection and monitoring, updated, broad spectrum antiviral antibody assays targeting multiple variants are needed.

Our survey identified subjects that had SARS-CoV-2 infections prior to vaccination. The findings fully agreed with reports of others showing that previous infection notably augments the vaccine response (9, 27, 28). However, a novel finding was our observation that previously infected subjects that had undergone SARS-CoV-2 anti-S monoclonal antibody (MAb) therapy did not display this augmentation. We previously reported that MAb treatment suppressed the endogenous anti-S antibody response in what appeared to be an epitope-specific manner because the anti-N antibody response was largely unaffected (8). Our current findings agree with this and suggest that, regarding the anti-S antibody response, MAb therapy keeps subjects in a naive state during infection and recovery, presumably by preventing expansion of memory B cells by either epitope blockade or reduction of antigen load or possibly both. Consequently, these subjects responded to vaccination like COVID-naive subjects. It was intriguing that combination MAb preparations seemed to have greater effect than single MAb preparations, which would be consistent with combination preparations causing greater inhibition of primary B cell expansion. However, studies with larger group sizes would be required to confirm these findings.

A limitation of our study is that the patient population represents veterans consisting predominantly of males with an average age of >60 years having a broad spectrum of medical conditions. As such, findings and recommendations may be largely applicable to this population. Despite this, observed vaccine responses were comparable with those reported for other populations. In summary, this study is among the first to attempt to relate quantitative anti-S antibody levels to breakthrough and protection. We show the potential value of quantitative antibody measurement for clinical application, but
were also recorded. Patients with immunode
tive/reactive cutoff of $6.8$ to $50,000$ AU/mL and posi-
terior range of $6.8$ to $50,000$ AU/mL and posi-
99.6%. anti-N IgG, 100% and 99% (33). The quantitative anti-S (RBD) IgG assay measures relative levels of
microparticle assays with respective speci
formed on a high-throughput Abbott Alinity i automated analyzer (31, 32). Both are chemiluminescent
Abbott Laboratories, Chicago, IL), and anti-SARS-CoV-2 nucleocapsid (N) IgG (Alinity SARS-CoV-2 IgG) per-
(FDA) EUA assays for anti-spike SARS-CoV-2 receptor binding domain (RBD) IgG (AdviseDx SARS-CoV-2 IgG II,
have aberrant vaccine responses and abnormal immune decay (29, 30).
Table 2 summarizes group sizes, age, and gender distribution. As expected for a VA medical facility,
the patient population was predominantly male with average age greater than 60 years compared with
females averaging about 60 years. There were no significant age or gender distribution differences
among groups. Most individuals presented as outpatients at the time of evaluation. Uninfected subjects
had no current or recent SARS-CoV-2 infection, and any hospitalizations were unrelated to COVID-19.
Among infected subjects, concordant with the known benefit of vaccination, COVID-19-related hospital-
izations were significantly greater among unvaccinated compared with vaccinated infected subjects of
whom the latter generally presented with mild symptoms.

**Source of specimens and data collection.** Serum and plasma specimens were provided by the
VAAAHS clinical laboratory. These had undergone testing for other purposes and the residual was
retrieved for serologic studies. For each sample, the corresponding patient EMR was reviewed to deter-
mine vaccination and clinical status. Only patients with clearly documented dates of vaccination or
recovered SARS-CoV-2 infection occurring more than 2 months after a full
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mine vaccination and clinical status. Only patients with clearly documented dates of vaccination or
SARS-CoV-2 infection were included for analysis. For each subject, basic demographic, diagnostic, and
serologic analysis of retrieved blood specimens. The study was approved by the local Institutional Review
Board (IRB). The cohort consisted of patients presenting to the VA Ann Arbor Healthcare System (VAAAHS)
from June to December 2021. The population included SARS-CoV-2-infected and -uninfected patients who
were vaccinated or unvaccinated. Vaccinated patients received two injection courses of either Pfizer-BioNTech
COVID-19 (BNT162b2) or Moderna COVID-19 (mRNA-1273) mRNA vaccine with or without a third booster.
Recipients of the Janssen (Johnson & Johnson) adenoviral vector vaccine were not included. Breakthrough was
defined as new onset laboratory confirmed SARS-CoV-2 infection occurring more than 2 months after a full
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**MATERIALS AND METHODS**

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RBD and the ACE2-R (11). Sample and paramagnetic microparticles coated with SARS-CoV-2 spike RBD antigens are combined and incubated. IgG antibodies to SARS-CoV-2 RBD antigens present in the sample bind to the microparticles. ACE2-R antigen acridinium-labeled conjugate is then added and incubated. Following a wash cycle, Pretrigger and trigger solutions are added. The resulting chemiluminescent reaction is expressed in relative light units (RLU). There is an inverse relationship between the amount of IgG antibodies to SARS-CoV-2 in the sample and RLU. A minimum of 12.5% inhibition of ACE2-R binding is required to be considered positive.

Other SARS-CoV-2 testing assays. When available, test results were also collected from the EMR of those patients who underwent SARS-CoV-2 testing as part of their clinical care. Among these were RT-PCR, viral antigen, and serologic tests. Three FDA EUA RT-PCR assay platforms were employed at the VAAAHS, the Roche cobas 8800 SARS-CoV-2 test (Roche Diagnostics, Indianapolis, IN), the Xpert Xpress SARS-CoV-2 (Cepheid, Sunnyvale, CA), and the BioFire Respiratory 2.1 Panel (bioMérieux, Marcy l’Étoile, France) (34–36). These assays have reported lower limits of detection of 25, 250, and 500 viral gene copies/mL, respectively. The Roche cobas and Xpert Xpress platforms provide cycle threshold (Ct) values which represent a rough surrogate of viral load. Viral antigen was detected in nasal turbinate swabs using the FDA EUA BD Veritor System, a chromatographic digital immunoassay for the direct qualitative detection of SARS-CoV-2 nucleocapsid (N) antigens during the acute phase of infection (37). Serologic assays included chemiluminescent semiquantitative anti-SARS-CoV-2 spike (RBD) IgG (Siemens Laboratory Diagnostics, München, Germany) performed on high-throughput automated analyzers (38). Claimed assay specificity and sensitivity were reported as 100% and 99.9%, respectively (33). Positive cutoff index values for the assays are ≥1.0 U with a reportable range up to 100 U.

Statistics. Statistical analysis was performed using STATA/SE 17.0 software (StataCorp LLC, College Station, TX). One way analysis of variance (ANOVA) with Bonferroni correction and nonparametric Kruskal-Wallis rank test were used for comparing groups. Fisher’s exact test was used to analyze categorical parameters such as hospitalizations and gender. Pearson’s correlation analysis with linear regression was applied to compare anti-SARS-CoV-2 IgG test results from different testing platforms. Values of P < 0.05 were considered significant.

SUPPLEMENTAL MATERIAL
Supplemental material is available online only.
SUPPLEMENTAL FILE 1, PDF file, 0.7 MB.

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