Clinical Evaluation of the Abbott Alinity SARS-CoV-2 Spike-Specific Quantitative IgG and IgM Assays among Infected, Recovered, and Vaccinated Groups

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ABSTRACT

The coronavirus disease 19 (COVID-19) pandemic continues to impose a significant burden on global health infrastructure. While identification and containment of new cases remain important, laboratories must now pivot and consider an assessment of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) immunity in the setting of the recent availability of multiple COVID-19 vaccines. Here, we have utilized the latest Abbott Alinity semiquantitative IgM and quantitative IgG spike protein (SP) serology assays (IgMSP and IgGSP) in combination with Abbott Alinity IgG nucleocapsid (NC) antibody test (IgGNC) to assess antibody responses in a cohort of 1,236 unique participants comprised of naive, SARS-CoV-2-infected, and vaccinated (including both naive and recovered) individuals. The IgMSP and IgGSP assays were highly specific (100%) with no cross-reactivity to archived samples collected prior to the emergence of SARS-CoV-2, including those from individuals with seasonal coronavirus infections. Clinical sensitivity was 96% after 15 days for both IgMSP and IgGSP assays individually. When considered together, the sensitivity was 100%. A combination of NC- and SP-specific serologic assays clearly differentiated naive, SARS-CoV-2-infected, and vaccine-related immune responses. Vaccination resulted in a significant increase in IgGSP and IgMSP values, with a major rise in IgGSP following the booster (second) dose in the naive group. In contrast, SARS-CoV-2-recovered individuals had several-fold higher IgGSP responses than naive following the primary dose, with a comparatively dampened response following the booster. This work illustrates the strong clinical performance of these new serological assays and their utility in evaluating and distinguishing serological responses to infection and vaccination.

KEYWORDS COVID-19, SARS-CoV-2, IgG, IgM, spike, nucleocapsid, vaccine, antibody, immunoassays

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel human pathogen that causes coronavirus disease 19 (COVID-19). The global spread of this virus began in early 2020 and continues to dramatically impact global public health infrastructure. Until recently, identification of positive cases, treatment of a patient with severe or life-threatening symptomology, and containment to prevent disease transmission have been the most widely used epidemiological tools. However, the recent availability of vaccines engineered to provide protective humoral immunity represents a major step forward in international responses to SARS-CoV-2 and a pivoting point for the clinical laboratory.

Laboratory diagnostic focus must now expand to include a more thorough assessment of SARS-CoV-2 immunity due to vaccine availability. The molecular detection of SARS-CoV-2 RNA remains the gold standard for COVID-19 diagnosis. These methods are
highly sensitive in the immediate period post-symptom onset, but diagnostic sensitivity diminishes by about 50% after 1 week of symptoms (1–3). Conversely, the sensitivity of serological assays improves following 2 weeks after symptom onset (2, 4), making these overlapping methods helpful for establishing disease prevalence in a population.

Akin to natural infection, the process of vaccination stimulates the immune system to form memory B cells that produce neutralizing antibodies. The two successful mRNA-based COVID-19 vaccines approved and administered in the United States contain mRNA that encodes the spike (SP) protein of SARS-CoV-2 (Pfizer-BioNTech and Moderna). Serological methods are important for their roles in assessing immune status in vaccinated and unvaccinated (UV) individuals. In this setting, the use of tests that exhibit exceptional sensitivity and specificity with minimal cross-reactivity is essential.

In this work, we evaluated the clinical performance of two new serological assays that are designed to quantitatively evaluate the presence of SP-specific IgM (IgMSP) and IgG (Abbott SARS-CoV-2 IgG II [IgGSP]) with respect to precision, linearity, sensitivity, specificity, and cross-reactivity assessments. For clinical validation, patient samples collected prior to COVID-19 emergence, as well as samples from patients with confirmed respiratory viral infections, multiple myeloma, and COVID-19, were utilized in addition to the vendor-provided quality control materials. Additionally, to assess the serological dynamics following SARS-CoV-2 infection and vaccination, we evaluated the results of these assays in combination with an assay which detects IgG specific to the nucleocapsid (NC) protein (IgGNC) assay (Abbott) in SARS-CoV-2-infected, naive, and vaccinated groups. The NC-related antibody response is specific to present and past SARS-CoV-2 infection or exposure. These assays exhibited excellent analytical characteristics and could be used as reliable tools for the assessment of serological responses to COVID-19 infection versus vaccination.

**MATERIALS AND METHODS**

**Patient samples.** A total of 1,236 individuals (1,428 specimens) were included in this study. This included 263 inpatients with suspected SARS-CoV-2 infection (413 samples); 404 unique patients for specificity and cross-reactivity studies, including 338 patients with samples obtained prior to the emergence of SARS-CoV-2; and 569 individuals (611 samples) tested in outpatient settings with available COVID-19 vaccination information (Fig. 1). For the assessment of clinical sensitivity, only inpatients with suspected SARS-CoV-2 infection with PCR and documented dates of symptom onset were included. In order to accurately evaluate the serological response following vaccination, only outpatients with information concerning their vaccine status were included. The manuscript deals with two aspects, (i) assessing clinical performance of a newly approved quantitation assay, IgGSP, and (ii) application of this assay, along with IgMSP and IgGNC, to evaluate and compare the immune dynamics of SARS-CoV-2 infection.
and vaccination. This study was approved by the institutional review board of the University of Texas Southwestern Medical Center.

Validation of the new serological assays. Precision, linearity, sensitivity, specificity, and cross-reactivity assessments were performed to validate the two new serological assays that are designed to semiquantitatively evaluate the presence of IgMSP and IgGSP, recognizing SARS-CoV-2 SP protein (methods detailed below). The patient samples included those that were collected prior to the COVID-19 pandemic, patient samples with confirmed related respiratory viral infections, multiple myeloma, and COVID-19 samples alongside the vendor-provided quality controls materials. Reverse transcriptase PCR (RT-PCR) testing was used as the predicate/gold standard for assay validation.

Analytical precision. Precision testing was performed in accordance with Clinical Laboratory Improvement Amendment (CLIA) guidelines and with standard laboratory practices at the University of Texas Southwestern Medical Center. A typical directed 5-day precision study following CLSI EP06-A3 (5) guidelines was conducted. Two levels of vendor-provided quality controls were tested with 5 replicates in the morning and 5 replicates in the afternoon for 5 days for each test verified.

Measurement range and functional sensitivity. Linearity (analytical measurement range) testing was performed as a single-day study following CLSI EP06-A (6). Patient samples with elevated IgGSP and IgMSP values (as determined by the IgGSP and IgMSP assays described below) were chosen to prepare linearity panels per CLSI EP6-A and according to the manufacturer’s guidelines. Linearity panels consisted of 7 to 8 levels covering the linear range of the test, with one level within the 10th percentile of the lower reportable range, one level within the 90th percentile of the upper reportable range, and one level around the cutoff range of the assays. A minimum of three replicates were tested for each level.

The clinical reportable range was calculated by diluting samples with elevated values that were just below the upper analytical measurement range of the assays at 1:2 and 1:4 dilutions and comparing the results to their neat (undiluted) sample results (±10% acceptability criteria). Functional sensitivity of the quantitative IgGSP test (7 arbitrary units [AU]/ml) was verified by running a neat patient sample (8.3 AU/ml) and its 1:1 dilution (expected value, 4.15 AU/ml) 10 times each and calculating their percent coefficient of variation (CV) (acceptable criteria ≤ 20% imprecision) as recommended by the manufacturer.

Carryover and sample stability studies. Carryover studies were undertaken with a positive (high sample) and negative control (low sample). The high sample was run three times followed by three runs of low sample, and this was repeated three times. Ten patient samples, both SARS-CoV-2 positive and negative, were tested repeatedly over a period of 12 days for specimen stability analysis. An acceptance criterion of ≤10% CV deviation from the baseline/initial results or index values of ≤0.1 for IgMSP and ≤5 AU/ml for IgGSP were utilized.

IgMSP assay. The AdviseDx SARS-CoV-2 IgMSP assay has recently been granted emergency use authorization by the U.S. Food and Drug Administration. IgMSP testing was performed on the Abbott Alinity i platform per the manufacturer’s instructions. The test is a chemiluminescent microparticle (CMIA) assay for semiquantitative assessment of IgM antibodies to the spike protein of SARS-CoV-2 in human serum and plasma sample. A vendor-recommended cutoff of 1.0 (index value) for reactivity/positivity of infection (FDA approval nos. for CoV-2 IgM, 6SR87 and H14977R01) was applied.

IgGSP assay. AdviseDx SARS-CoV-2 IgG II (FDA approval nos. for CoV-2 IgG II, 6560 and H18575R01)/SARS-CoV-2 IgG II Quant assay (CE marked) were performed on the Abbott Alinity i platform in accordance with the manufacturer’s package insert. In this antibody CMIA test, the SARS-CoV-2 antigen-coated paramagnetic microparticles bind to the IgG antibodies that attach to the virus’s spike protein in human serum and plasma sample. The resulting chemiluminescence in relative light units (RLU) following the addition of anti-human IgG (mouse, monoclonal) acridinium labeled conjugate in comparison with the IgG II calibrator/standard indicates the strength of response, which reflects the quantity of IgGSP present. Fifty arbitrary units per milliliter and above in this test are considered positive. This quantitative measurement of IgGSP can be helpful to evaluate an individual’s humoral response to vaccines.

IgGNC assay. The Abbott Alinity i SARS-CoV-2 anti-nucleocapsid protein IgG assay is a semiquantitative CMIA assay that has been previously verified for routine patient testing in our institution’s clinical laboratory, and the index values of 1.4 and above are considered positive (7) per the manufacturer’s instructions.

RT-PCR testing. The Abbott M2000 or Abbott Alinity m RT-PCR-based molecular testing/confirmation for SARS-CoV-2 were performed using nasopharyngeal specimens collected in viral transport media as previously described (7).

Clinical specificity. Specificity was evaluated in the pre-COVID-19 era remnant-banked plasma samples from 217 unique patients collected from blood donors from September to November 2019 and early COVID-19 period samples from March to April 2020.

Cross-reactivity studies. The serum/plasma specimens for cross-reactivity studies included human leukocyte antigen (HLA) lab-confirmed positive samples (collected from 1 January 2015 to 30 September 2019) for cytomegalovirus (CMV) IgG, influenza A/B, respiratory syncytial virus, and endemic human coronaviruses (n = 92). Also, the samples that typically contain significant levels of antibodies, such as lupus patients (n = 29; collected between 2004 and 2007) positive for anti-nuclear antibodies (ANA) and anti-double-stranded DNA and hematological malignancies (HM) (n = 66; collected between March and October 2020), were included.

Statistical analysis. Data analysis was carried out using GraphPad Prism software (version 9.0.1; San Diego, CA, USA). Data are presented as median with range. When experiments involved more than two groups, one-way analysis of variance (ANOVA) followed by Tukey multiple-comparison post hoc analysis were used to analyze the statistical differences. For experiments involving only two groups, as
appropriate, paired or unpaired Student’s t tests were performed. A P value of <0.05 was considered statistically significant.

RESULTS

This study used a two-pronged approach to assess the potential utility of serological assays to distinguish prior infection- and vaccine-associated response by first validating the clinical performance of a newly FDA-approved quantitative IgGSP assay and, second, evaluating the role of this assay, along with IgMSP and IgGNC assays, to compare the immune dynamics of SARS-CoV-2 infection and vaccination.

**Analytical performance.** For both the IgGSP and IgMSP assays, the imprecision was less than 5%. The analytical measurement range of the IgGSP assay was from 4.2 to 50,000 AU/ml (clinical reportable range, up to 200,000 AU/ml), and for the IgMSP assay, we observed linearity up to an index value of 16. Manufacturer-recommended functional sensitivity of 7 AU/ml of the quantitative IgGSP assay was confirmed. There were no carryover issues with both of the assays. The samples kept refrigerated were stable over a 12-day test period.

**Clinical specificity and cross-reactivity.** Clinical specificity of the assay was evaluated with serum/plasma from blood donors (SARS-CoV-2 uninfected) obtained before the emergence of SARS-CoV-2. None of these samples were positive (0/217; 100% specificity) for SARS-CoV-2 spike-specific IgG (Fig. 2A) and spike-specific IgM (Fig. 2B) that matched with the manufacturer’s claimed specificity. The median IgGSP value and IgMSP index value were found to be 1.92 and 0.05, respectively, well below the corresponding positive cutoff limit of 50 AU/ml for IgGSP and index value of 1.0 for IgMSP.

Cross-reactivity of the Abbott SARS-CoV-2 IgGSP and IgMSP assays was also evaluated in the setting of respiratory illness and disease conditions where antibody production is significantly elevated (i.e., lupus, hematological malignancies [HM]), primarily multiple myeloma (Fig. 2C and D). One hundred twenty-one samples obtained prior to SARS-CoV-2 emergence did not cross-react with IgGSP. Interestingly, among 66 HM patient samples collected in 2020, 2 patients (1.1%) were found to produce a positive value for IgGSP, indicating some level of cross-reactivity (Fig. 2C). One of these cases (a multiple myeloma patient) was
confirmed by PCR as having recovered from COVID-19 upon chart review. The second positive patient was repeatedly negative for SARS-CoV-2 by PCR and strongly positive for hepatitis viral panel serology testing. As no history of SARS-CoV-2 exposure could be established for this patient, further investigation of this IgGSP-specific apparent cross-reactivity is warranted. No cross-reactivity was observed in the IgMSP assay (0/187 patients) (Fig. 2D).

Clinical sensitivity. We evaluated the clinical sensitivity of IgGSP and IgMSP assays in our RT-PCR-positive COVID-19 inpatient cohort where reliable information concerning the date of symptom onset and duration was available. Not surprisingly, we noted that the assay sensitivity increased proportionally over time following symptom onset, consistent with the developmental kinetics of a specific antibody response. The sensitivity of IgGSP was 39% and 58% at 5 and 10 days following symptom onset (Table 1 and Fig. S1A in the supplemental material). Extending the analysis of these patients to 16 to 20 days post-symptom onset and beyond revealed that clinical sensitivity of IgGSP assay was 96% and 98%, respectively. As expected, clinical sensitivity of IgGSP assay was much higher (74% within 10 days and 100% within 20 days) based on the RT-PCR-confirmed date of diagnosis (Table S1; Fig. S1B).

Clinical sensitivity of IgMSP test was comparable to that of the IgGSP test both within 10 days and 20 days post-symptom onset. At 20 days post-symptom onset and beyond, the sensitivity of the IgMSP test was 95% (Table 1) and 82% based on RT-PCR-confirmed date of diagnosis (Table S1). Importantly, when IgGSP and IgMSP results were combined and analyzed for assay positivity in either format, the sensitivity reached 100% after 15 days from symptom onset and RT-PCR-confirmed date (Table 1 and Table S1).

Longitudinal assessment in COVID-19 inpatients. We next used IgGSP values over 360 specimens to assess the change in IgGSP response over time as a function of days post-COVID-19 RT-PCR testing (DPCR) with the data plotted as a frequency distribution mapping (Fig. S2A). Within the first 15 DPCR, the median IgGSP values gradually increased and reached an average of 4,466 AU/ml. A dramatic increase in the antibody index value began to occur after 15 days (with a median average of 59,396 AU/ml) (Fig. S2A). Beyond the 27th DPCR, we noticed a decreasing trend in IgGSP values; however, these results remained above the positivity threshold (≥50 AU/ml). While a lower assay sensitivity was noted in the early period post-symptom onset, it is notable that in some samples, the IgGSP assay was able to detect positivity within the same time frame as detectable SARS-CoV-2 RNA by RT-PCR. Additionally, the IgGSP assay was sensitive enough to detect the positivity at day 0, which is the date of infection confirmation by PCR in three samples.

In the same cohort, akin to IgGSP, we observed the overall tendency of IgMSP to rise over the positivity threshold of index value of 1.0. Particularly within the first week of symptoms, the distribution was highly inconsistent, and the maximum median index was only 4.345 (Fig. S2B). In contrast, we noted the IgMSP distribution to progress regularly in tandem over the 2nd week with the median IgMSP consistently doubling. This subsequently reached as high as ~38 by the mid-2nd week (17th day). In four samples, IgMSP was sensitive enough to detect the positivity on day 0 of infection confirmation by PCR (Fig. S2B). While we were able to detect IgMSP until the 80th day, the IgMSP

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**TABLE 1** SARS-CoV-2 IgG<sub>SP</sub> and IgM<sub>SP</sub> positive agreement by days post-symptom onset

<table>
<thead>
<tr>
<th>DSO&lt;sup&gt;a&lt;/sup&gt;</th>
<th>IgG&lt;sub&gt;SP&lt;/sub&gt; positivity rate (no. of positive patients/total no. of patients [%])</th>
<th>IgM&lt;sub&gt;SP&lt;/sub&gt; positivity rate (no. of positive patients/total no. of patients [%])</th>
<th>IgG&lt;sub&gt;SP&lt;/sub&gt; or IgM&lt;sub&gt;SP&lt;/sub&gt; positivity rate (no. of positive patients/total no. of patients [%])</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;5</td>
<td>11/28 (39.3)</td>
<td>11/28 (39.3)</td>
<td>14/28 (50.0)</td>
</tr>
<tr>
<td>6–10</td>
<td>46/83 (57.8)</td>
<td>51/83 (55.4)</td>
<td>55/83 (66.3)</td>
</tr>
<tr>
<td>11–15</td>
<td>79/96 (82.3)</td>
<td>76/96 (79.2)</td>
<td>82/96 (85.4)</td>
</tr>
<tr>
<td>16–20</td>
<td>42/44 (95.5)</td>
<td>42/44 (95.5)</td>
<td>44/44 (100)</td>
</tr>
<tr>
<td>&gt;20</td>
<td>41/42 (97.6)</td>
<td>40/42 (95.2)</td>
<td>42/42 (100)</td>
</tr>
<tr>
<td>Total</td>
<td>221/293 (75.4)</td>
<td>220/293 (75.1)</td>
<td>237/293 (80.1)</td>
</tr>
</tbody>
</table>

<sup>a</sup>For RT-PCR-confirmed SARS-CoV-2 cases. DSO, days from symptom onset.
values were found to greatly oscillate beyond 23 days with a decreasing trend; however, they were still well above the cutoff index value of 1.0 (Fig. S2B).

**IgGNC assay cutoff for positivity much lower in prior COVID-19 (recovered) patients.** This study included 16 blood samples from known COVID-19-recovered patients (recovered more than 3 to 11 months prior to sampling). Production of NC-specific antibodies is a main differentiator of immune responses to natural SARS-CoV-2 infection versus those to spike-based vaccines. Analysis of this group of patient samples with the IgGNC assay revealed index values ranging from 0.2 to 1.4 (Table 2; Fig. 3). This is significantly lower than the manufacturer-recommended cutoff index level of ≥1.4 used to detect active (early) infection. However, during waning humoral response (recovery period and postrecovery), our data suggest an index value less than 1.4 and up to 0.2 may predict prior COVID-19 in recovered patients (Table 2; Fig. 3). We therefore applied an index value cutoff below 0.2 for IgGNC to establish the patients that were truly immunologically naive to SARS-CoV-2 infection (true negatives). This is important in the current setting of increasing numbers of COVID-19-recovered patients in the general population. More reliably, where a combination of tests is possible, the highest diagnostic accuracy or detecting truly negative individuals can be attained when combining an index value of <0.2 for IgGNC with <50 AU/ml for IgGsp and index value of <1.0 for IgMsp. Ninety-nine percent of the samples analyzed using this combination of results were confirmed as negative by RT-PCR or were from patients who were in an early period of infection. In contrast, COVID-19 patients tested between 10 and 20 days post-symptom onset and filtered with the manufacturer-recommended positive cutoff combination of index value of ≥1.4 for IgGNC with ≥50 AU/ml for IgGsp and an index value of ≥1.0 for IgMsp showed 99% positivity by PCR (Table 2).

**TABLE 2** Different antibody assay thresholds used (filtered in) to predict various conditions in our dataset

<table>
<thead>
<tr>
<th>Condition</th>
<th>Threshold of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgGsp assay (AU/ml)</td>
</tr>
<tr>
<td>Infected (recent)</td>
<td>≥50</td>
</tr>
<tr>
<td>Naive</td>
<td>&lt;50</td>
</tr>
<tr>
<td>Naive vaccinated</td>
<td></td>
</tr>
<tr>
<td>Prior COVID-19</td>
<td></td>
</tr>
<tr>
<td>Prior COVID-19 and vaccinated</td>
<td>≥50</td>
</tr>
</tbody>
</table>

**FIG 3** Derivation of new IgGNC threshold based on the prior COVID-19 data set. A total of 16 specimens from 11 unique COVID-19-recovered individuals were subjected to IgGNC analysis against the vendor-recommended cutoff (index value of ≥1.4).
Subsequently, we applied these filters wherever we analyzed naïve vaccinated and prior COVID-19-vaccinated groups.

**SARS-CoV-2 vaccination results in robust SARS-CoV-2 SP-specific antibody responses.** A novel facet of this work is the inclusion of vaccinated patient samples in our analysis. Among those vaccinated, 91% received the Pfizer-BioNTech formulation (*n* = 132), and 8% obtained the Moderna formulation (*n* = 13). Antibody responses in the vaccinated group (*n* = 145) were compared to that of the UV group (*n* = 424), which included patients that were both RT-PCR positive and negative for SARS-CoV-2 infection. IgG<sub>SP</sub> antibody responses ranged from 0 to 49,517 AU/ml, with a median of 6,396 AU/ml (95% confidence interval [CI], 3,814 to 9,729) (Fig. 4A) in the vaccinated group. The median antibody level for vaccinated patients was significantly higher (*P* < 0.0001) than for UV patients (Fig. 4A). Intriguingly, 18% (76/424) of UV patients had IgG<sub>SP</sub> values greater than the positive detection threshold despite RT-PCR negativity. IgM<sub>SP</sub> production was significantly higher (*P* < 0.0001) in the vaccinated than the UV group, with a more modest median fold increase (23%; 1.34 versus 0.06) (Fig. 4B). Nearly 13% (53/424) of UV patients were above the IgM<sub>SP</sub> positive threshold despite RT-PCR negativity.

The identification of patients with positive IgG<sub>SP</sub>/IgM<sub>SP</sub> results despite a lack of vaccination and historically negative RT-PCRs prompted us to investigate IgG<sub>Nc</sub> levels in these patients as a marker of natural SARS-CoV-2 infection. Surprisingly, about 12% (51/424) were positive (≥1.4), leaving 26 (7%) patients with positive IgG<sub>SP</sub> but without IgG<sub>Nc</sub> positivity. These patients could have silent infection (present or past) because they were also positive for either IgG<sub>SP</sub> or IgM<sub>SP</sub>. In a majority of UV patients with no known SARS-CoV-2 exposure (82%; 347/424), the IgG<sub>SP</sub> and IgM<sub>SP</sub> values were determined to be lower than the vendor-recommended cutoff.

Figure S3 illustrates the pattern of IgG<sub>SP</sub> antibody value relationships over the days following COVID-19 vaccination (DFD). This group (187 specimens from 145 unique individuals) included both RT-PCR-verified cases and subjects with no exposure to SARS-CoV-2. Within 10 days, the first dose yielded a minimal increase in vaccine-specific antibody response, irrespective of formulation in 2% (4/187) of specimens. More than 54% (102/187) of the vaccinated group had a 5-fold increase, 44% (81/187) had a 10-fold increase, and 17% had a 25-fold increase in the median IgG<sub>SP</sub> values compared to the UV group (Fig. S3A). Samples up to 21 days are inclusive of the initial vaccine dose and >21 days are inclusive of the booster. While an increase in value following the first dose of vaccination was clearly observed, not surprisingly, its distribution was variable (Fig. S3A, dark red rectangle). While the number of observations available at or less than 10 days following the first dose was modest, four of the individuals that were tested had values under the positive threshold, and two had values over 45,000 AU/ml. Clearly, after the second dose of vaccination, the IgG<sub>SP</sub> values were consistently high and comparatively exhibited a more uniform trend (Fig. S3A, green oval). Interestingly,
two patients exhibited a null response following 21 days postvaccination. One of these patients was an HM (multiple myeloma) patient, while the medical history of the other is unknown.

In parallel, SARS-CoV-2-specific IgMsp levels were also increased following vaccination in naive subjects (Fig. 4B and Fig. S3B). In particular, the distribution analysis demonstrated that following the primary dose of vaccination at week 1 and week 2, ~71% of the subjects had values above the positive threshold (Fig. S3B). However, at 21 days and beyond post-booster dose, IgMsp levels again rose, and a noticeable over-the-threshold cluster was seen compared to the primary vaccination (Fig. S3B). Although the number of Moderna-vaccinated recipients in this evaluation is modest, both the IgGsp and IgMsp responses appeared to be comparable to those of individuals who received the Pfizer-BioNTech formulation.

**Vaccination response in naive population.** Next, we compared the IgGsp response after the first and booster dose in the naive vaccinated group that was derived by filtering out for IgGnc of ≥0.2 index cutoff based on Table 2 and Fig. 3. Following the first dose, we observed IgGsp positivity with a median of 2,217 AU/ml (95% CI, 0 to 44,182), which was drastically increased by 8.2-fold following the booster to a median of 18,272 AU/ml (98% CI, 11,724 to 21,750) \((P < 0.001)\) (Fig. 5A). The cumulative IgMsp response after the primary dose of vaccination in the naive subjects was found to be with a median index value of 1.1, which is above the positive cutoff (95% CI, 0.87 to 1.4) (Fig. 5B). This was further significantly increased \((P = 0.0236)\) by 1.7-fold to a median of 1.95 (98% CI, 1.16 to 3.3) following the booster dose (Fig. 5B). The IgGnc remained unchanged between dose 1 and dose 2 in the naive vaccinated group (Fig. 5C). This result suggests that there is a robust response after the booster dose in the naive group.

**Vaccination response in prior COVID-19 (recovered) patients.** Currently, the CDC recommends vaccination in those cohorts that are recovered from COVID-19 3 months or prior. Therefore, understanding the immune response in this population is important. The prior COVID-19 group filter was derived from the known recovered patients (Table 2 and Fig. 3). The median IgGsp values following the first and second doses in the prior COVID-19 group were found to be 17,519 AU/ml (lane 2) and 20,760 AU/ml (lane 3), respectively (Fig. 6A). However, unlike the naive vaccinated first versus second doses (Fig. 5A, lane 1 versus 2), the booster dose in the prior COVID-19 group displayed only a dampened response (Fig. 6A, lane 2 versus lane 3). Similar to the tendency of

**FIG 5 Comparison of antibody response post-first and booster doses in naive (non-COVID-19) subjects.** (A) Evaluation of spike-specific antibody response in naive (non-COVID-19) subjects following primary and booster dose of COVID-19 vaccine. (B) IgMsp levels in naive (non-COVID-19) subjects administered first and second dose of COVID-19 vaccine. (C) IgGnc antibody levels following primary and booster vaccine dose in the naive vaccinated subjects. For panels A to C, the samples used for testing were collected on a convenient basis (as and when available). The VD1 samples for testing were collected starting at the 4th day until 21 days for Pfizer and until 28 days for Moderna, and VD2 samples were collected between 1 and 27 days after vaccination. V, vaccinated; N, naive (applying <0.2 for IgGnc filter; Table 2), VD1/VD2, vaccine dose 1/2. Extended dark blue line indicates the median.
IgGSP response between naive vaccinated and prior COVID-19 vaccinated, we noted a blunted and nonsignificant IgMSP response following booster dose in the prior COVID-19 vaccinated group (Fig. 6B, lane 3 versus lane 2) relative to that observed in the naive vaccinated group (Fig. 5B, lane 2 versus lane 1).

Next, we compared the vaccination response in prior COVID-19 against the immune response in those with active COVID-19 infection (UVC). The COVID-19 infection status was confirmed by the IgGNC levels that had a median of 3.3 (96% CI, 2.78 to 4.02), which is 2.4-fold above the positive threshold cutoff (UVC; Fig. 6C, lane 1). Correspondingly, the median IgGSP and IgMSP levels were found to be 1,046 (96% CI, 575.3 to 1,518) and 4.65 (96% CI, 2.78 to 4.02), respectively (Fig. 6A and B, lane 1). Both vaccine doses robustly increased the IgGSP response in relation to the infected group, while it was significant only after dose 1 (P < 0.0001; Fig. 6A, lane 2 versus lane 1). In contrast, IgMSP levels were significantly lower in the vaccinated group than the infected group (Fig. 6B, lanes 2 and 3 versus lane 1).

Interestingly, IgGNC median values remained much lower (by 0.73) than the positive cutoff of 1.4, suggesting IgGNC response is infection specific and not vaccination specific. The vaccinated prior COVID-19 groups (primary/first dose [D1] and booster/second dose [D2]) displayed significantly lower IgGNC response than the unvaccinated COVID-19 group and much below the positive threshold (Fig. 6C, lanes 2 and 3 versus lane 1).

Further examination of antibody levels confirmed the restrained response of booster dose among prior COVID-19-recovered patients (Fig. 7A and B, lane 3 versus lane 4) compared to the robust response exhibited by naive vaccinated individuals (Fig. 7A and B, lane 1 versus lane 2). Within-patient as well as within-group comparison revealed an unchanged response for IgGNC (Fig. 7C). As expected, patients with prior COVID-19 exposure...
Sure exhibited higher IgNC levels than those determined to be immunologically naive (Fig. 7C, lanes 3 and 4 versus lanes 1 and 2).

**DISCUSSION**

SARS-CoV-2 serology has played a secondary role in the clinical diagnosis of COVID-19 compared to molecular detection of viral RNA. However, the recent availability of sensitive and specific serologic assays to quantitatively evaluate vaccination responses elevates the role of these important diagnostic tools at this juncture in the COVID-19 pandemic. In this work, we systematically demonstrate that the Abbott Alinity quantitative IgSP and IgMsp assays are sensitive and specific and perform well in a routine clinical setting. Additionally, these assays, when used in combination with the currently marketed IgGNC assay, can measure and distinguish COVID-19 antibody responses from SARS-CoV-2 natural infection versus vaccination over a broad dynamic range.

The analytical performance of both the IgMsp and IgGSP assays met the acceptance criteria for implementation of the assay in the clinical laboratory. The specificity of these assays was 100% in the samples from the pre-COVID-19 period, with no serological cross-reactivity in patients infected with other respiratory viruses (including seasonal coronaviruses). For the IgMsp assay, our results closely mirrored data provided by the manufacturer. The high positive predictive value gained from minimal serological cross-reactivity in patients infected with other respiratory pathogens or autoimmune-associated antibodies is notable. Evaluation of the IgSP and IgMsp assays utilizing RT-PCR positivity and days post-symptom onset was to confirm the infection and antibody response following the infection, respectively. The low level of sensitivity in samples collected between 5 and 15 days after symptom onset or PCR positivity is not surprising given the kinetics of antibody generation at these time points. However, the significant increase in sensitivity to greater than 95% beyond 15 days highlights the accuracy of these assays in the setting of an activated and productive humoral immune response. Therefore, these serologic assays, when used in conjunction with a molecular method which exhibits a lower degree of sensitivity at periods where serology is strongest, allows for a broad and thorough interrogation of a patient’s COVID-19 status.

Interestingly, when the IgGSP with IgMsp assays were analyzed in tandem, the sensitivity of the combination was improved over either the IgMsp or IgGSP assay individually, with 100% sensitivity achieved after 15 days. Still, false negatives in this paired approach were identified within 15 days, which could be due to host-specific factors, variations in immune responses, low antibody values, or early SARS-CoV-2 infection.

While IgM is largely useful in determining the likelihood of a recent infection, the dynamics of how long this isotype remains detectable are ill-defined. In this study, we have detected IgMsp for a protracted period (beyond 20 days and up to 80 days after symptom onset) with an increased sensitivity. This observation is more consistent with other studies that have detected IgM after ≥90 days following infection of SARS-CoV-2 (8, 9). Interestingly, from our data set, simultaneous detection of IgM and IgG was also noted, particularly with the detection of IgG during the early period as well. This illustrates that seroconversion may occur simultaneously or shortly thereafter IgMsp induction in addition to the archetypal sequential class-switching responses (10).

The analysis of serum specimens from vaccinated patients is an exciting aspect of this work. These assays were able to detect and monitor vaccine-elicited antibody responses compared to a UV patient group. The elevated levels of specific IgMsp and IgGsp observed in this work are in agreement with clinical trial data (11, 12) for both currently available formulations of COVID-19 vaccines. Because it is not possible to parse if spike protein-specific antibodies arise because of vaccination or natural infection, we chose to utilize a currently marketed nucleocapsid-specific IgG test with the IgMsp/IgGsp assays, which would only be positive in the setting of a natural infection.

Our results suggest the manufacturer-established IgGNC assay cutoff can be adjusted to “rule in” or “rule out” the prior COVID-19 and recovered patients. Specifically, our preliminary
studies demonstrate a “gray zone” of IgGNC index values (0.2 to 1.4) in RT-PCR-confirmed COVID-19-recovered specimens (n = 16 samples from 11 patients, recovery period within 3 to 11 months). While IgGNC and IgGSP assays demonstrated high clinical specificity and sensitivity independently, using them in combination may facilitate differentiation of patients with prior COVID-19 exposure from vaccinated individuals. Hence, we believe it is possible to expand the utility of the marketed IgGNC assay to identify COVID-19-recovered individuals who are likely to present with waning antibody values (13, 14). A similar revision of the IgGNC threshold has been suggested to improve assay sensitivity for early stage (<6 days) of COVID-19 infection (15). Importantly, a model-based study recently proposed the use serostatus for prioritizing vaccination (16), and along these lines, the new cutoff range could improve the applicability with respect to identifying the early, weak, and previously infected and recovered (waning) cases. A recent study supports this idea that intermediate serology assay results below the diagnostic thresholds for positivity (manufacturer recommended) are still associated with the presentation of COVID-19-associated symptoms (17). By identifying the relative abundance and characteristics of a population that is susceptible, infected, and recovered would be critical to better inform and devise the immunization roadmap. However, the cutoffs’ modification must be carefully considered in the context of clinical need and deployment. It should be noted that by lowering assay thresholds, it would not be possible to detect all previous early, weak, and/or mild infections while maintaining adequate specificity. Studies to test the use of this cutoff algorithm on a larger scale are ongoing.

The combination of the new IgGSP assay with IgMSP and IgGNC can provide a rigorous evaluation of the vaccination and the antibody responses postadministration. Given the emergent circumstances and obvious clinical and economic need, COVID-19 vaccine deployment has been well timed and efficient. Limited knowledge concerning longitudinal outcomes, public uncertainty, and misinformation has created hesitancy among a few to get immunized (18). These assays could aid in (i) establishing the efficacy and kinetics of spike-specific antibody responses in infected or vaccinated populations, (ii) identifying infected individuals not previously identified by molecular testing, and (iii) evaluation of the effectiveness of the adjunct convalescent plasma therapy (CPT). All of these applications add to the breadth of knowledge concerning the COVID-19 vaccine and its effects, helping to fill in knowledge gaps and address lingering concerns. While the U.S. Centers for Disease Control and Prevention does not recommend serological testing to assess the COVID-19-driven immunity following mRNA vaccination, the use of a test that evaluates IgM/IgG to the NC antigen for ascertaining prior infection in case of COVID-19 vaccination has been suggested (https://www.cdc.gov/vaccines/covid-19/info-by-product/clinical-considerations.html). Our work highlights an improved utility of a combination of antibody assays (SP and NC) to accomplish this task.

Using these assays, while we observed an increased response to the vaccine following the primary/first dose (D1), the booster/second dose (D2) had a dramatic effect in the naïve subjects. Among vaccinated prior COVID-19 persons who had recovered from the infection, no significant difference is seen in IgGSP and IgGMSP values following D2. However, D1 in prior COVID-19-recovered individuals elicited strong IgGSP and IgGSP responses of equivalent robustness to that of the D2 of the naïve group. D1 and D2 specimens from the same individuals also confirmed this finding. The dramatic D1 response among prior COVID-19-recovered individuals could be ascribed to the natural SARS-Cov-2 infection functioning in a similar manner as acting as a priming dose. This observed response is supported by recent preprint reports that have demonstrated a single dose of mRNA vaccine in seropositive subjects is sufficient to elicit a robust immune response comparable to SARS-CoV-2 naïve subjects receiving two doses (19, 20). These data raise important questions concerning whether a single dose is sufficient among previously SARS-CoV-2-infected and/or COVID-19-recovered individuals; however, larger longitudinal studies are needed. Results of such work would have
profound implications on vaccination strategies, particularly in the current setting of high COVID-19 vaccine demand with limited supply.

In summary, our data demonstrate excellent performance of the Abbott’s latest IgGSP quantitative assay and the clinical utility of the latest IgGSP quantitative assay and IgMSP test in evaluating the COVID-19 vaccine response. In addition, the combination of IgGNC, IgGSP, and IgMsp points out the role/utility of serologic testing for the purpose of distinguishing prior infection status and vaccine-derived SP humoral responses. Furthermore, a newly defined IgGNC threshold reported here, when used in combination with the quantitative IgGSP, and IgMsp assays, can accurately identify COVID-19-recovered individuals, allowing improved diagnostic performance and enhancing public health surveillance and epidemiological initiatives. While antibody testing could be useful in select circumstances as mentioned above, until the body generates detectable antibody “levels” that are associated with humoral response, the role of antibody testing should be considered carefully.

SUPPLEMENTAL MATERIAL
Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 1.3 MB.

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REFERENCES


