

Comparison of two automated immunoassays for the detection of SARS-CoV-2 nucleocapsid antibodies

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Impact Statement

This study describes the relative performance characteristics of two automated immunoassays for the detection of anti-SARS-CoV-2 antibodies. Our findings indicate that both assays offer excellent specificity but exhibit important differences in sensitivity, particularly in immunocompromised patients. Information presented here will help guide laboratorians, clinicians and patients in the selection of assays for clinical use and will provide context to aid in the interpretation of anti-SARS-CoV-2 test results.

Abstract

Background. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel member of the coronavirus family that caused the global coronavirus 2019 (COVID-19) pandemic. The prevalence remains largely unknown because of early testing supply shortages. Although it cannot currently be used to determine level of immunity, antibody testing can contribute to epidemiological studies, identify convalescent plasma donors, or satisfy curiosity about previous exposure to the virus.

Methods. 407 samples collected from hospitalized inpatients with and without a confirmed SARS-CoV-2 infection, 170 remnant clinical specimens collected and frozen prior to the COVID-19 outbreak, and paired serum and plasma samples from 23 convalescent plasma donors were used to determine performance characteristics of the Abbott SARS-CoV-2 IgG and Roche Elecsys Anti-SARS-CoV-2 assays. The sensitivity, specificity, imprecision, interferences, and sample stability were determined. These assays were then used to characterize the antibody response in serial samples from 20 SARS-CoV-2 positive inpatients.

Results. Both assays exhibited 100% specificity (95% CI; 99.05 – 100.00), giving no positive results in 170 specimens collected before July 2019 and 215 specimens from patients without a confirmed SARS-CoV-2 infection. Differences between platforms were most notable in SARS-CoV-2 positive samples. Roche offered higher sensitivity in convalescent plasma donors at 95.7% (95% CI; 78.1 – 99.9) versus 91.3% (95% CI; 72.0 – 98.9) but Abbott detected antibodies in two immunocompromised patients whereas Roche did not. The Roche and Abbott platforms also exhibited different trends in antibody signal for a subset of patients.

Conclusions. Both the Abbott and Roche platforms offer excellent specificity but different trends in antibody signal may reflect qualitative differences in the types of antibodies recognized by the two assays. Negative serologic results do not exclude previous SARS-CoV-2 infection.

Introduction

A respiratory pneumonia caused by a novel human coronavirus was discovered in Wuhan, China in December 2019. Shortly thereafter, the genetic sequence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was released and confirmed to be a highly pathogenic virus causing the coronavirus disease 2019 (COVID-19) outbreak (1). The early outbreak exhibited exponential growth as it spread worldwide with a basic reproductive number (R_0) estimated to be between 1.4 – 6.5 (2–7). The first case reported in the United States was on January 19, 2020 (8) and the virus spread to all 50 states by mid-March. Given the degree of global spread, the World Health Organization declared a pandemic on March 11, 2020 (9).

The identifying clinical features of SARS-CoV-2 infection include fever and respiratory symptoms, accompanied by radiographic pulmonary features appearing three to seven days after exposure. Symptoms range from mild to critical and some present with only non-respiratory symptoms, such as mild fatigue, headache, or diarrhea, while others present with life threatening symptoms such as septic shock, multiple organ failure, or fatal pneumonia (10,11). Preliminary data suggest that after approximately two weeks of illness, there may be a positive correlation between clinical severity and antibody signal (12,13).

Active infection with SARS-CoV2 is diagnosed with nucleic acid amplification testing of the virus collected with either throat or nasopharyngeal swabs (14). Rapid development of molecular testing was critical in identifying affected individuals so they could self-quarantine and mitigate the spread of the disease. Due to shortages of collection and reagent supplies, this testing was not widely available at the start of the outbreak. As a result of these shortages, the total number of infected individuals remains unknown. However, it is possible to estimate prevalence of this virus with serology testing.

SARS-CoV-2 is an enveloped positive sense single-stranded RNA virus that, under microscopy, resembles the shape of a crown due to its spike structural proteins (10). The coronavirus

particle also contains a nucleoprotein (N) that encapsulates the RNA, a small envelope protein that surrounds the helical nucleocapsid, and a membrane glycoprotein that is embedded in the envelope (15). The S protein is an appealing target for antibody assay design because it is immunogenic and is the target of neutralizing antibodies. Despite this, many assays, including the Abbott IgG and Roche total antibody assays, target the N protein. This is likely due to the increased sensitivity (16) and specificity offered by the lack of sequence conservation throughout the N protein among coronaviruses (17).

To date, there are numerous SARS-CoV-2 serology assays available on the market. However, only a subset have received FDA Emergency Use Authorization (EUA), of which only a few are available on high throughput, automated platforms that can meet the demands of a large clinical laboratory. Similar to other recent publications (18–20), we conducted a head-to-head comparison of the Abbott SARS-CoV-2 IgG and Roche Elecsys Anti-SARS-CoV-2 assays. We compared the performance characteristics and used the validated testing platforms to characterize the antibody response of convalescent plasma donors and the seroconversion of inpatients with known SARS-CoV-2 infection.

Methods

Specimen Collection

Hospitalized Inpatients

Four hundred seven remnant serum and lithium heparin plasma specimens collected in gel separator tubes for clinical purposes from hospitalized patients with and without confirmed SARS-CoV-2 infection were removed from refrigerated storage within seven days of collection. All patients included in this study were tested for SARS-CoV-2 infection with one of three molecular diagnostic methods: the CDC format laboratory developed test, the Abbott RealTime SARS-CoV-2 m2000 assay, or the Diasorin Simplexa COVID-19 assay. Two hundred fifteen of

these specimens were collected from 155 unique patients with a documented negative SARS-CoV-2 molecular diagnostic result generated on the same day or one day prior to serum or plasma specimen collection. Both serum and plasma were available for 60 patients while only plasma was available for the remaining 94 patients. The remaining 193 remnant specimens were collected from 20 unique, symptomatic patients with a previously documented positive SARS-CoV-2 molecular diagnostic result. Serum and plasma samples were aliquoted into sealed plastic tubes and frozen at -80°C until further use (no longer than 3 weeks in frozen storage).

Pre-SARS-CoV-2 Outbreak

170 remnant serum and lithium heparin plasma specimens collected in gel separator tubes for clinical purposes between September 2017 and June 2019 had been sequestered in -20°C storage for future validation projects. Consistent with internal laboratory practice, the specimens had been removed from refrigerated storage within seven days of collection, transferred to sealed plastic tubes, and stored at -20°C until further use.

Convalescent Plasma Donors

Paired serum and lithium heparin plasma specimens collected in gel separator tubes were collected at least 14 days after resolution of symptoms from 23 unique individuals with a documented positive SARS-CoV-2 molecular diagnostic result. Serum and plasma samples were aliquoted into sealed plastic tubes and frozen at -80°C until further use (no longer than 3 weeks in frozen storage).

Stability Study

One serum and one lithium heparin specimen from each of three convalescent plasma donors were kept in the original gel separator tubes and stored at 4°C. The specimens were subjected to SARS-CoV-2 serologic testing on days 0-4, 6-7 and 14 after collection.

Institutional Review

This study was approved by the Dartmouth-Hitchcock Institutional Review Board.

Specimen Handling and Data Collection

With the exception of refrigerated specimens used for the stability study, specimens described above were thawed at ambient temperature and briefly centrifuged to remove precipitated material. Specimens were tested using the Abbott SARS-CoV-2 IgG assay (06R8620, Abbott Laboratories Diagnostics Division, Abbott Park, IL) on an Architect i1000 instrument and the Roche Elecsys Anti-SARS-CoV-2 assay (09203079190, Roche Diagnostics, Indianapolis, IN) on a cobas e801 instrument. Individual specimens were tested on both analytical platforms on the same day, and held at refrigerated temperature if they were not tested on the day they were removed from the freezer. All samples were tested within five days after removal from frozen storage.

Roche Elecsys Anti-SARS-CoV-2 Method

The Roche anti-SARS-CoV-2 method uses a bridge format in which patient antibody links biotinylated SARS-CoV-2-specific antigen and ruthenium-labeled SARS-CoV-2-specific antigen to form an antigen – antibody – antigen sandwich complex. (21) Following the initial incubation of patient sample and SARS-CoV-2-specific antigen, streptavidin-coated paramagnetic microparticles are added, allowing the antibody-SARS-CoV-2-specific antigen complex to bind to the solid phase via biotin-streptavidin interaction. After magnetic capture of the microparticles, unbound substances are removed by a wash step and application of a voltage stimulates the emission of chemiluminescent signal, which is directly proportional to the amount of anti-SARS-CoV-2 antibody present. Sample types approved for use with this assay include serum, Li-heparin and EDTA (dipotassium and tripotassium) anticoagulated plasma. Results are reported in a qualitative fashion with a cutoff index (COI) of <1.0 interpreted as non-reactive and ≥ 1.0

interpreted as reactive. In a study performed by the manufacturer, the assay demonstrated post-PCR confirmation sensitivities of 65.5% (76/116) at 0-6 days, 88.1% (52/59) at 7-13 days and 100% (29/29) at ≥ 14 days. The overall specificity was 99.7% (5262/5272) in samples collected before December 2019.

Abbott SARS-CoV-2 IgG Method

The Abbott SARS-CoV-2 IgG method is a two-step immunoassay in which SARS-CoV-2 antigen-coated paramagnetic microparticles are exposed to patient sample and bound by anti-SARS-CoV-2 antibodies. (22) Following a wash step, acridinium-labeled anti-human IgG is added and allowed to bind to IgG that remains bound to the washed microparticles. Following a second wash step and addition of pre-trigger and trigger solutions, chemiluminescent signal is generated, which is directly proportional to the amount of anti-SARS-CoV-2 IgG present. Results are reported in a qualitative fashion with a signal/calibrator (S/C index) of < 1.4 interpreted as negative and ≥ 1.4 interpreted as positive. Sample types approved for use with this assay include serum, as well as Li-heparin, Na-heparin, EDTA (dipotassium or tripotassium), and sodium citrate anticoagulated plasma. In a study performed by the manufacturer, the assay demonstrated post-symptom onset sensitivities of 0% (0/4) at < 3 days, 25.0% (2/8) at 3-7 days, 86.4% (19/22) at 8-13 days and 100% (88/88) at ≥ 14 days. The specificity was 99.6% (993/997) in samples collected "Pre-COVID-19 Outbreak" and 100.0% (73/73) in samples collected from patients with other respiratory illnesses.

Imprecision

Imprecision was assessed by performing five replicates of each level of quality control (QC) and patient pool, per day, for five days.

Quality Control (QC) Material

SARS-CoV-2 IgG control material from Abbott Diagnostics (06R8610) was used to establish imprecision characteristics and monitor daily performance of both assay platforms. The negative QC consists of human plasma whereas the positive control contains inactivated, cell-free, human blood-derived material that is reactive for anti-SARS-CoV-2 IgG. Expected results on the Abbott SARS-CoV-2 IgG assay as defined by the manufacturer were S/C values of ≤ 0.78 (Negative) and 1.65-8.40 (Positive).

Negative and Positive Patient Pools

Negative and positive pools were made by combining Li-heparin plasma samples from individuals with documented “Not Detected” or “Detected” SARS-CoV-2 molecular diagnostic test results. SARS-CoV-2 antibody testing was performed on the Abbott Architect to determine the absence or presence of antibody. Pools were aliquoted into sealed plastic vials and stored at -20°C until use.

Comparison of Serum vs. Li-Heparin plasma and Interfering Substances

To verify that serum and Li-Heparin plasma collected in gel-separator tubes would produce comparable results we performed a direct comparison of approximately 100 pairs of matched serum and plasma samples collected from convalescent plasma donors and inpatients as described above.

The effect of hemolysis (H-index) was determined by preparing a hemolysate from a discarded unit of type O negative packed red blood cells. After three washes with 0.9% saline, pelleted cells were resuspended in type 1 laboratory water and frozen overnight in a -20 °C freezer. The hemolysate was thawed at room temperature and centrifuged for 30 minutes. The supernatant

was added to separate aliquots of pooled SARS-CoV-2 antibody-negative or positive plasma samples to give H-indices (Roche cobas c701 module) of 34, 63, 114, 207, 393, 423 and 660. The effect of icterus (I-index), was determined by reconstituting bilirubin (Fisher Scientific) in 0.1 N NaOH to a final concentration of 10 g/L, which was added to separate aliquots of pooled SARS-CoV-2 antibody-negative or positive plasma samples to give I-indices (Roche cobas c701 module) of 5, 8, 16, 22, 32, 41 and 48.

The effect of lipemia (L-index) was assayed by diluting a solution of 20% intralipid (20 g/L) into separate aliquots of pooled SARS-CoV-2 antibody-negative or positive plasma samples to give L-indices (Roche cobas c701 module) of 187, 413, 884, 1184 and 1804.

Results

Inpatient seroconversion

The antibody signals from serial plasma samples collected from 20 inpatients with confirmed SARS-CoV-2 infection were measured on both the Abbott and Roche platforms (Figure 1). Overall, the Abbott and Roche assays demonstrated similar performance characteristics. The serial samples collected from 16/20 patients demonstrated consistent detected or not detected results across both platforms. The first two samples collected for Patient 8 were negative on the Abbott platform, but had detectable antibodies on Roche. Conversely, the first two samples collected for Patient 1 were detected by the Abbott platform but were negative on the Roche. Patient 11 and Patient 12 had consistently undetectable antibodies by the Roche assay but were consistently detected by Abbott. Interestingly, both of these patients were immunocompromised.

Patient 1 and Patient 2 both received convalescent plasma during their stay, producing a rapid increase in antibody signal in both cases. Patient 1 increased from a COI of 13.1 to 30.8 on Roche and from 6.44 to 6.95 S/C on Abbott after CP treatment. The Roche platform more than

doubled in COI whereas the Abbott platform only slightly increased. This could potentially be due to a limited dynamic range of the Abbott assay compared to Roche, however assessment of linearity material on both platforms is required to prove this hypothesis. Patient 2 exhibited a noticeable rise and immediate fall in antibody signal after CP treatment. Both patients continued to develop antibodies and exhibited a consistent rise in antibody signal after treatment.

The time until the first detectable antibody results for the 20 SARS-CoV-2 positive inpatients was tracked to determine the correlation between the Abbott and Roche platforms (Table 1). Symptom onset ranged from zero to twelve days prior to hospital admission. Most patients tested positive for SARS-CoV-2 infection by PCR the day of their admission, but three inpatients tested positive up to nine days prior to admission and three patients were not tested until 1-2 days after admission. Patients had detectable antibodies by both platforms at a median of 12.5 days after symptom onset and seven days after testing positive for a SARS-CoV-2 infection by PCR. It is important to note that the first sample collected from these inpatients may have been several weeks after symptom onset (i.e., Patients 6, 9, 12, 17, 18, and 20) or several days into their hospital stay (Figure 1). Therefore, antibodies may have developed prior to the date of study specimen collection. With the exception of Patients 1, 8, 11, and 12, all other patients developed detectable antibodies at the same time on both platforms.

Abbott and Roche performance characteristics

The performance characteristics of both the Abbott and Roche assays were evaluated (Table 2). We investigated the effects of sample hemolysis, icterus, and lipemia on both assays. Our studies did not demonstrate any interference (less than 10% bias) up to an H-index of 660, I-index of 48, or L-index of 1800, the highest concentrations tested for each interferent. To evaluate specimen stability, we tested three pairs of plasma and serum samples (n = 6 total) at concentrations spanning the dynamic range. When stored at 4°C, these samples were stable for at least 14 days. Both the Abbott and Roche assay exhibited 100% specificity (95% CI; 99.05 –

100.00) in our evaluation as none of the 170 remnant serum and plasma samples collected prior to the SARS-CoV-2 outbreak, or 155 plasma and 60 serum samples collected from 155 SARS-CoV-2-negative inpatients, generated positive results.

The sensitivity of the two assays for the detection of antibodies in the samples from 23 convalescent plasma donors was 91.3% (95% CI; 71.96 – 98.93) for Abbott and 95.7% (95% CI; 78.05 – 99.89) for Roche. One donor had negative antibody results on both platforms in a plasma specimen collected 41 days after the onset of symptoms. This patient had a low positive SARS-CoV-2 PCR diagnostic test result four days after the onset of symptoms, which consisted of diarrhea, body ache and prolonged cough but did not require hospitalization. A second donor with body ache, headache, diarrhea and shortness of breath and a positive SARS-CoV-2 PCR diagnostic test result had negative antibody results on the Abbott platform but was just above the positivity threshold on the Roche platform in a plasma specimen collected 38 days after the onset of symptoms.

The sensitivity for 10 inpatients ≥ 14 days post positive PCR was 100% (95% CI; 69.15 – 100.00%) for Abbott and 80% (95% CI; 72.16 – 87.84%) for Roche. The discrepancy is due to the two immunocompromised patients who had undetectable antibodies throughout the length of their hospital stay on the Roche platform. If those two patients are excluded, then the sensitivity is 100% for both platforms at ≥ 14 days post positive PCR within the inpatient population.

Imprecision was assessed using four different samples: Abbott SARS-CoV-2 IgG negative control, Abbott SARS-CoV-2 IgG positive control, a negative patient pool, and a positive patient pool. Imprecision was established by running five replicates of each material over five days. The mean and percent CV for each QC is listed in Table 3. The Abbott assay exhibited an imprecision ranging from 1.5-2.3% CV with the exception of the Abbott negative QC (7.9% CV). This is likely due to the low S/C of the negative Abbott QC, which ranged from just 0.05 – 0.07 with an SD of 0.004. The Roche assay exhibited a consistent imprecision ranging from 3.3-3.7% CV for all

material tested. Imprecision was determined over two separate lots and at least two calibrations on the Roche platform but only one lot and one calibration on the Abbott platform.

Matched serum and plasma samples were collected and compared to validate both as acceptable sample types. Consistent results were observed between matrices indicating that both were appropriate for clinical use. Of the approximately 100 matched serum and plasma samples tested, only a single pair of samples generated discrepant qualitative results on the Abbott platform, falling slightly above the threshold for positivity in plasma (S/C 2.11) but just below (S/C 1.29) in serum. Otherwise, the use of serum or plasma did not change the reported result on either platform. No statistically significant difference (two-tailed paired t-test) existed between serum and plasma samples.

Discussion

As it is yet unknown whether anti-SARS-CoV-2 antibodies confer immunity, nor is a vaccine available, serologic testing is currently limited to one of three applications: evaluating prospective convalescent plasma donors, conducting large-scale epidemiologic studies and satisfying the curiosity of individuals who experienced upper respiratory symptoms but were unable to obtain molecular diagnostic testing while symptomatic. According to the manufacturers' package inserts, both the Roche and Abbott assays are suitable for use in these applications as they demonstrated excellent and nearly identical diagnostic performance characteristics despite very different assay designs. In this study, using a single cohort of patient samples tested on both analytical platforms, we confirmed the manufacturers' findings of >99% specificity but observed somewhat lower sensitivity. In our study consisting of 385 SARS-CoV-2 PCR negative samples, we did not find any false positive test results for either assay. While this does confirm the manufacturers' stated specificity of >99%, we cannot claim a true specificity of 100% as it is understood that this likely represents a sampling artifact, and if a larger cohort were tested we would likely encounter some false positive results. Furthermore, we observed

important differences in assay performance that may help guide assay selection at a future date when serologic testing is used to monitor immune response in infected patients.

Two of 20 SARS-CoV-2-positive inpatients were immunocompromised (patient 11: chemotherapy, and patient 12: anti-CD20 monoclonal therapy) and both generated consistently positive results on the Abbott platform and negative results on the Roche platform throughout their admissions. While the exact reason for this discrepancy is unclear, it may have important implications for the selection of test methods to evaluate the serologic response in other immunocompromised patients. We did not attempt to assess the affinity of the antibodies produced in these patients and we acknowledge that one limitation of our study is the small number of immunocompromised patients. Further evaluation of these assays in this patient population will be required to definitively establish the cause of the discrepant results.

Different trends in antibody response were also observed between the two assays in the SARS-CoV-2-positive inpatient group. All patients exhibited either a continually increasing or unchanging antibody signal on the Abbott platform over the course of their admission. While 16 patients exhibited one of these two patterns on the Roche platform, four patients (Patients 7-10) showed an initial rise in antibody signal, followed by a gradual decrease to a steady state plateau. This may reflect disappearance of the IgM contribution to the Roche total antibody signal or may indicate a transition in the quality or number of IgG antibodies being produced, resulting in decreasing signal on the Roche platform and steady or increasing signal on the Abbott platform. It is also possible that the Abbott assay becomes saturated at high antibody concentrations (maximum positive signal ~ 7.5 S/C), resulting in falsely low “peak” S/C values and preventing the decreasing trend from becoming apparent. Importantly, these trends were not predictive of disease course or admission length as some patients with increasing antibody signals were discharged to home following resolution of symptoms (Patients 1, 3, 5-6, 19) while others with this same pattern remained admitted or were discharged to regional hospitals for continued care (Patients 2 and 4). Similarly, some patients with unchanging or decreasing signal

were discharged to home (Patients 7, 9, 1-11, 15-16, 18) while others remained admitted or were discharged to regional hospitals (Patients 8, 12,-14, 17).

A correlation between disease severity and antibody response in patients with PCR-confirmed SARS-CoV-2 infection has been suggested (13,23) and future work will be required to definitively establish this relationship. Anti-SARS-CoV-2 antibody was not detected on at least one assay platform in two convalescent plasma donors with PCR-confirmed infection. Both of these donors exhibited symptoms consistent with SARS-CoV-2 infection. One patient experienced headache, nausea, vomiting, diarrhea, and myalgias but did not experience upper respiratory symptoms or require hospitalization. 38 days after resolution of symptoms, antibody was not detected on the Abbott assay (0.8, cutoff ≥ 1.4) and was detected but close to the threshold for positivity on the Roche assay (1.9, cutoff ≥ 1.0). The second patient experienced diarrhea, body aches and prolonged cough but also did not require hospitalization. Interestingly, this patient's molecular diagnostic test was very weakly positive (cycle threshold value of 29.5, cutoff ≤ 31 cycles). 37 days after the positive PCR result (41 days after onset of symptoms), antibody was not detected on either the Abbott (0.02, cutoff ≥ 1.4) or the Roche (0.09, cutoff ≥ 1.0) platforms. These findings indicate that negative serologic results do not definitively exclude a previous SARS-CoV-2 infection in patients with consistent signs and symptoms.

One may expect that assays using a total antibody format capable of detecting IgM, IgG and IgA anti-SARS-CoV-2 antibodies would generate positive results sooner than assays that detect IgG only. This was not supported by our study as the Roche (total antibody) assay generated positive results sooner than the Abbott (IgG-specific) assay in one of 18 SARS-CoV-2-positive inpatients with at least one positive sample on both analytic platforms. Similarly, Abbott generated positive results sooner in one of these 18 SARS-CoV-2-positive inpatients. The qualitative results were concordant between the two platforms for the remaining 16 inpatients.

Lastly, our findings confirm previous observations by the assay manufacturers and other independent groups that antibody testing to assess convalescent plasma donors, perform epidemiologic studies or evaluate the cause of a previous uncharacterized respiratory illness should be performed several weeks after resolution of symptoms (24,25). While all immunocompetent hospitalized patients developed detectable anti-SARS-CoV-2 antibodies on both assay platforms at some point in their hospital stay, testing of individuals less than 10 days after the onset of symptoms (patients 1, 7-8, 11, 15, 19) generated antibody results near the detection threshold, resulting in a mixture of negative and weakly positive results.

Several questions remain to be answered during future evaluation of these two assays. As both assays detect antibodies to the viral nucleocapsid rather than spike protein, is either suitable for use in evaluating immune status following infection or immunization? Is one assay preferred for the evaluation of antibody persistence or waning immunity? Should the Roche assay be contraindicated or is it preferred in immunocompromised patients? Do negative serologic test results in patients with PCR-confirmed infection but mild or atypical symptoms suggest the presence of genetic variants that prevent or delay viral entry? Future work should determine whether these assays have a role in clinical laboratory testing beyond the three applications described above.

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Table 1: Time to first detected antibody result for 20 inpatients with confirmed SARS-CoV-2 infection

Patient Number	Symptom onset	Positive PCR	Abbott 1st positive from symptom onset	Roche 1st positive from symptom onset	Abbott 1st positive from positive PCR	Roche 1st positive from positive PCR
1	-6	0	8	10	2	4
2	-4*	0	12	12	8	8
3	-9	0	14	14	5	5
4	-8	0	12	12	4	4
5	-12	0	12	12	0	0
6	-8	0	24	24	16	16
7	-9*	-9	9	9	9	9
8	-7	0	9	7	2	0
9	-7	0	21	21	14	14
10	-9	-7	12	12	10	10
11	-5	0	5	N/A	0	N/A
12	-5	0	19	N/A	14	N/A
13	-6	0	13	13	7	7
14	-1*	1	15	15	13	13
15	0*	1	8	8	7	7
16	-7	0	13	13	6	6
17	-10	2	17	17	5	5
18	-8	-6	19	19	17	17
19	0	-1	3	3	4	4
20	-3	0	38	38	35	35

All values are days relative to date of admission (time point 0).

*An approximation of symptom onset was used in the absence of a definitive date on onset.

Table 2: Validation Summary

Validation characteristic	Abbott	Roche
H-index	660	660
I-Index	48	48
L-index	1804	1804
Stability	14 days	14 days
Specificity Pre-COVID	100%	100%
Specificity PCR-negative inpatients	100%	100%
Sensitivity Convalescent plasma	91.3%	95.7%
Sensitivity ≥14 d post pos PCR inpatient	100%	80%

Table 3: Imprecision

Quality Control	Abbott Mean S/C	Abbott %CV	Roche Mean COI	Roche %CV
Abbott negative	0.05	7.9	0.07	3.7
Abbott positive	3.3	1.5	6.1	3.3
Negative patient pool	0.55	2.3	0.66	3.7
Positive patient pool	2.3	1.5	12.5	3.3

Figure Legends

Figure 1: Inpatient antibody signal. The antibody response from serial plasma samples collected from 20 inpatients with confirmed SARS-CoV-2 infection are displayed. Antibody signal was measured on both Abbott (left y-axis; blue circles) and Roche (right y-axis; green squares) platforms at each time point since the day of admission (day 0). The y-axis for Abbott ranges from 0-10 S/C whereas the y-axis for Roche ranges from 0-100 COI on all graphs. Points that fall below the cutoff for Abbott ($S/C < 1.4$) and Roche ($COI < 1.0$) are displayed as pink circles and squares, respectively. Corresponding patient symptoms at the time of admission, relevant past medical history, length of hospital stay (LOS), and discharge (D/C) destination are listed below each graph. The SARS-CoV-2 PCR status at the time of discharge is noted as positive (PCR+) or negative (PCR-) if known. The following abbreviations are used: AMS (altered mental status), C (cough), Cx (culture), D (diarrhea), F (fever), GI (gastrointestinal upset), GVHD/ECP (graft versus host disease/extracorporeal photopheresis), Hospital (affiliate regional hospital), LOA (loss of appetite), LOS (loss of smell), M (malaise), NF (neutropenic fever), N/V (nausea/vomiting), PC (pancytopenia), P (pharyngitis), and SOB (shortness of breath). Two patients received convalescent plasma (CP) during their hospital stay; the day of CP administration is denoted by a purple dashed vertical line.

