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Multiplex assessment of SARS-CoV-2 antibodies improves assay sensitivity and correlation with neutralizing antibodies

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ABSTRACT

Objectives: Detection of antibodies to multiple SARS-CoV-2 antigens in a single assay could increase diagnostic accuracy, differentiate vaccination from natural disease, and aid in retrospective exposure determination. Correlation of binding antibody assessment in clinical assays with neutralizing antibodies is needed to better understand the humoral response to SARS-CoV-2 infection and establish of correlates of protection.
Methods: A cohort of 752 samples was used to assess specificity, sensitivity, and comparison to 6 other Conformitè Europëenne serologic assays for the BioRad SARS-CoV-2 IgG multiplex assay which measures receptor binding domain IgG (RBD), spike-S1 IgG (S1), spike-S2 IgG (S2), and nucleocapsid IgG (N). A subset of serial specimens from 14 patients was also tested for neutralizing antibodies (n = 61).
Results: Specificity for RBD and S1 IgG was 99.4% (n = 170) and 100% for S2 and N IgG (n = 170) in a cohort selected for probable interference. Overall assay concordance with other assays was >93% for IgG and total antibody assays and reached 100% sensitivity for clinical concordance at >14 days as a multiplex assay. RBD and S1 binding antibody positivity demonstrated 79–95% agreement with the presence of neutralizing antibodies.
Conclusions: The BioRad SARS-CoV-2 IgG assay is comparable to existing assays, and achieved 100% sensitivity when all markers were included. The ability to measure antibodies against spike and nucleocapsid proteins simultaneously may be advantageous for complex clinical presentations, epidemiologic research, and in decisions regarding infection prevention strategies. Additional independent validations are needed to further determine binding antibody and neutralizing antibody correlations.

1. Introduction

SARS-CoV-2 causes COVID-19 which is a major cause of acute respiratory distress syndrome. The COVID-19 pandemic has necessitated emergency use authorization (EUA) of diagnostic testing in the US and similar expedited approvals worldwide, leading to testing that may be lacking sufficient data for appropriate clinical utilization. Serologic detection of SARS-CoV-2 began in early 2020 with poorly characterized assays and inappropriate claims regarding the utility and accuracy of SARS-CoV-2 serology which led to initial distrust of serologic assays [1,2]. In the months following, independent assessments demonstrated clinical utility and additional manufacturers contributed to the development of important clinical assays. Due to the continued expedited reviews of SARS-CoV-2 diagnostics, independent and well-defined validation of commercially available assays remains of critical importance.

As the COVID-19 pandemic progresses into a vaccine-available era, the utility of SARS-CoV-2 serology is also evolving. Pre-vaccine, assays were used for complex or delayed clinical presentations, infection prevention assessments, epidemiologic research, and convalescent plasma donation determination [2]. Most assays use a single antigen to detect specific antibodies in patient blood. The most common antigens of

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interest have been nucleocapsid (N) and receptor binding domain (RBD). Other antigens from SARS-CoV-2 include spike 1 (S1) and spike 2 (S2) which correspond to the N- and C-terminals of the spike protein respectively. Assays have also varied in detection of immunoglobulin classes. Before the availability of a SARS-CoV-2 vaccine, assays against RBD seemed of particular interest as RBD is required for viral entry into the cell and is the target of neutralizing antibodies [3]. The first vaccines available in the US used spike protein as antigen which is detectable in RBD and S1 and S2 serologic assays [4,5]. This situation is similar to our assessment of hepatitis B (HepB), where anti-surface antigen antibody can be present following vaccination and natural disease and other tests are required to differentiate between the two states, though primary diagnosis for SARS-CoV-2 infection is through nucleic acid testing. The need to differentiate between natural disease and vaccine response is particularly important for epidemiologic studies. Retrospective determination of natural or vaccine positive serology is also potentially useful for infection prevention, as well as research assessing long term COVID-19 health outcomes, asymptomatic cases, or vaccine efficacy. The ability to detect antibodies to multiple SARS-CoV-2 proteins in a single assay would allow for this functionality.

Quantification of antibodies against SARS-CoV-2 proteins could help to establish an immune correlate of protection as has been done for many other vaccines (eg – MMR, HepB). Further, it is critical that we assess how these binding antibody assays correlate with neutralizing antibodies to inform clinical utility of serologic testing [6,7]. We used a defined cohort of 752 samples to assess specificity, sensitivity, and comparison to other commercially available serologic assays for the BioRad SARS-CoV-2 IgG multiplex assay and further assessed a subset of 61 samples for neutralizing antibody titer.

2. Materials and methods

2.1. Specimens

Remnant sera and data from specimens received in the University of Pittsburgh Medical Center (UPMC) clinical laboratories for routine testing between 1 January 2020 and 30 November 2020 were used under the auspices of UPMC Quality Assurance for Clinical Laboratories, the University of Pittsburgh institutional review board study #20040072, and in compliance with the World Medical Association Declaration of Helsinki. These convenience samples comprised three testing groups: 1) comparison samples (n = 298), 2) routine run samples (n = 280), 3) serial samples (n = 174). Comparison samples were sera remaining from a previous study where we compared six platforms for SARS-CoV-2 antibody assays for use in our hospital system including specificity, serial samples, sensitivity samples, and clinical correlates [8]. Of the original 338 specimens, 298 had remaining volume. Comparison samples have undergone one additional freeze thaw for this work. Routine run samples are comprised of samples from patients who had SARS-CoV-2 antibody performed as part of their clinical care or for convalescent plasma donation. Where available, clinical correlates are included. Convalescent plasma donation requires medical adjudication of COVID-19 prior infection and symptom resolution >28 days previously. Finally, serial samples are from patients entering the hospital via the emergency department with positive SARS-CoV-2 RNA testing that were subsequently admitted for care. Days from symptom onset was determined by chart review. Where symptom onset was not available or could not be determined due to lack of symptoms or overlapping comorbidities (eg – COPD) the date of SARS-CoV-2 PCR positivity reported in the medical record was used as the date of onset. Per the above a case could be considered positive based on PCR testing listed in the EMR or based on medical adjudication. Two case definitions of 'positive' have been separated as 'PCR Positive' (PCR positivity only) and 'Clinical Diagnosis', which includes both PCR positivity and medical adjudication.

Lithium heparin plasma and serum samples with separator gel were centrifuged and stored at 4 °C for up to 2 weeks before storage at −20 °C. Samples were banked at −20 °C for up to 11 months before analysis.

2.2. SARS-CoV-2 antibody assays

SARS-CoV-2 antibody assays were performed in CLIA certified high-complexity clinical laboratories at UPMC. The comparison sample cohort testing is previously published [8]. All assays, abbreviations used, manufacturers, antigens, units, positivity thresholds, and antibody types detected are listed in Table 1. Assays were performed according to manufacturer's instructions. All assays use units that are generated by comparison to an internal standard, when referring to assay results collectively we refer to these units as 'index values' for simplicity. All assays are qualitative assays except the BioRad assay which is semi-quantitative with qualitative interpretation.

2.3. Other laboratory testing

Detection of SARS-CoV-2 RNA was reported in the EMR as standard clinical testing and is performed using the Cepheid GeneXpert or a lab developed test based on the CDC protocol [9]. Measurement of specificity samples for autoimmune and infectious disease serologies in the comparison cohort is described elsewhere [8].

2.4. Focus reduction neutralization test (FRNT)

FRNT assays were performed as previously described [10]. Briefly heat-inactivated samples were diluted in DMEM and then incubated with 100 FFU of SARS-CoV-2 (University of Pittsburgh clinical isolate June 2020). Following incubation, samples were inoculated onto Vero E6 cells then overlayed with carboxymethylcelluose. After 18 h incubation plates were washed, fixed, permeabilized, blocked and foci were stained using a custom anti-SARS-CoV-2 N polyclonal antibody. Bound antibody was detected using goat anti-rabbit IgG HRP followed by TMB-H Peroxidase Substrate. Foci were imaged, counted, and processed using an ImmunoSpot Counter (CTL). Foci were counted in experimental wells and compared to control wells. The dilution of serum at which 80% of foci are neutralized is reported as the $FRNT_{80}$.

2.5. Statistical analysis

Figures and statistics were performed in Prism Graph Pad (version 8.0, La Jolla, CA, USA) and tables were created in Excel (Microsoft, Seattle, WA). Where multiple assays are presented on the same graph with a positivity cutoff of 1.0, the BR values have been divided by 10, the SiemensV values have been divided by 1000. There was no change to the Euroimmun values which have a cutoff of ≥1.1 which is visually identical to 1.0 in these figures. For concordance calculations $FRNT_{80} < 20$ was considered negative, and $FRNT_{80} \geq 20$ was considered positive for neutralizing antibodies. Diagnostic sensitivity calculations were calculated on the Anaconda 3 platform using the pandas 1.0.3 library within python 3.7.7.

3. Results

3.1. Specificity of multiplex SARS-CoV-2 IgG

The specificity of the multiplex assay was tested against an existing cohort of samples selected for a high likelihood of cross-reactivity due to other co-morbidities (n = 170, Table S1). These samples were collected before SARS-CoV-2 was geographically present and have been previously described [8]. Prior testing by several platforms is described elsewhere and overall 92% of the originally tested samples were available for this study [8]. Both S2 IgG and N IgG demonstrated 100% specificity and RBD IgG and S1 IgG demonstrated cross reactivity on one sample giving a specificity of 99.4%. This cross-reactive sample was

Table 1
SARS-CoV-2 antibody assay details.

Assay	Abbreviation	Units	Positive cutoff	Antibody	Antigen	Analyzer	Manufacturer	Manufacturer information
SARS-CoV-2 IgG Panel	BR	U/mL	≥10	IgG	RBD, S1, S2, N	BioPlex 2200	Bio-Rad Laboratories, Inc	Hercules, CA, USA
Access SARS-CoV-2 IgG	Beckman	S/CO	≥1.0	IgG	RBD	UniCel DxI 800	Beckman Coulter	Brea, CA, USA
COV2T Centaur	SiemensC	Index	≥1.0	total antibody	RBD	Advia Centaur XP	Siemens Healthineers	Erlangen, Germany
COV2T Vista	SiemensV	QUAL	≥1000	total antibody	RBD	Dimension Vista 1500	Siemens Healthineers	Erlangen, Germany
Anti-SARS-CoV-2 ELISA IgG	EuroIgG	Index	≥1.1	IgG	S1[a]	Manual ELISA	Euroimmun	Lubeck, Germany
Anti-SARS-CoV-2 ELISA IgA	EuroIgA	Index	≥1.1	IgA	S1[a]	Manual ELISA	Euroimmun	Lubeck, Germany
Elecsys Anti-SARS-CoV-2	Roche	COI	≥1.0	total antibody	N	Cobas e 411	Roche Diagnostics	Basel, Switzerland

[a] May include RBD.

molecularly positive for one of the common coronaviruses and was also found to be serologically reactive by three of six previously tested platforms [8].

3.2. Sensitivity of multiplex SARS-CoV-2 IgG

To assess assay analytical sensitivity and generally evaluate semi-quantitative claims, serial samples from patients who were hospitalized and had remnant samples available for at least 3 timepoints (n = 27, Table S2) were tested. Ten of these patients were previously tested for SARS-CoV-2 antibodies by several platforms [8]. When seroconversion occurred on a day for which there was no available sample, the average of the two available samples before and after seroconversion was taken as an estimate. In cases where it was possible to see one or two markers become reactive first (n = 17) N IgG was most often the first detectable (n = 9) followed by RBD IgG (n = 6), S2 IgG (n = 4) and S1 IgG (n = 3). Notably all three S1 IgG cases were detectable the same day as RBD IgG. In some patients, RBD was detected first and significantly earlier, than other antigens with N detection occurring late. In other patients N IgG appeared significantly earlier than others (Fig. S1, Table S2). Overall, we found that patient U/mL values increased over time with a plateau in some patients as would be anticipated in an assay with semi-quantitative

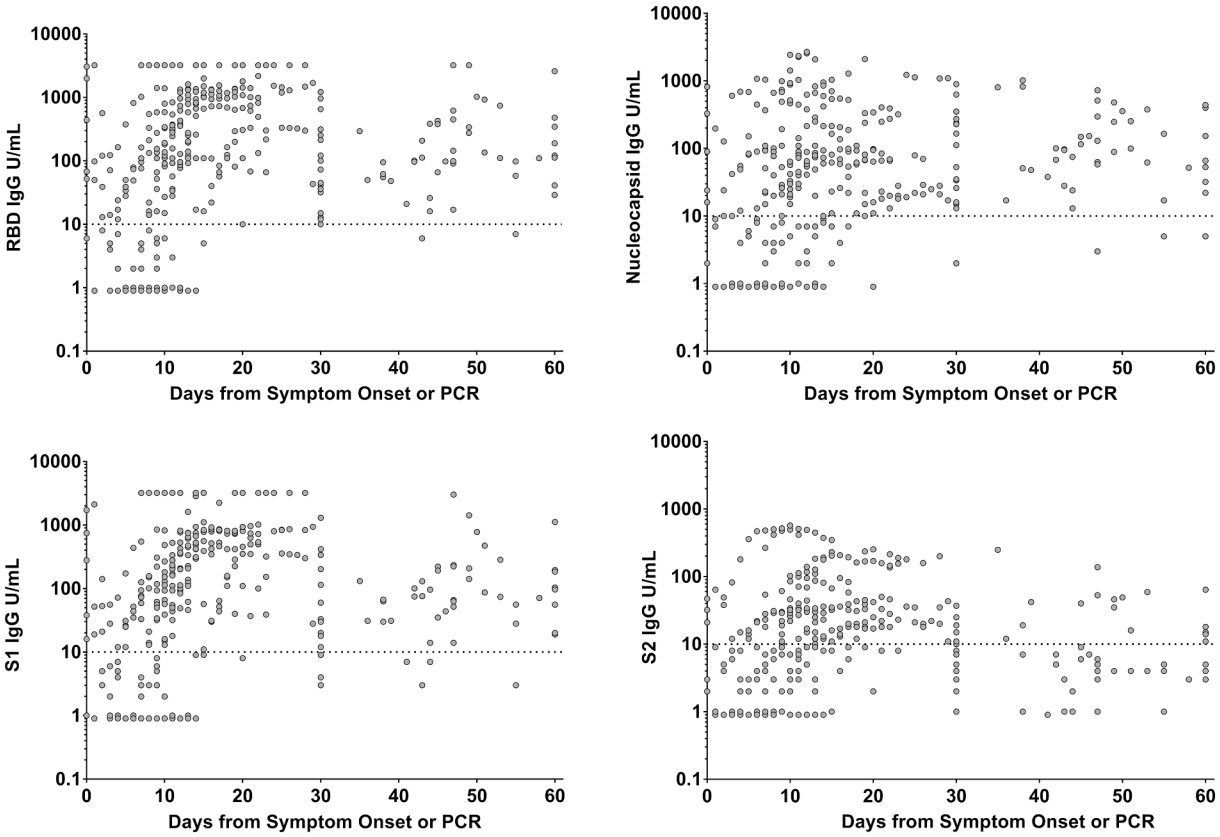

Fig. 1. Reactivity of SARS-CoV-2 IgG components compared to symptom onset. Remnant samples from patients hospitalized for SARS-CoV-2, having SARS-CoV-2 antibody testing performed as part of their medical care, or being assessed for convalescent plasma donation are plotted (n = 333). Patients being screened for convalescent plasma donation without chart notes with symptom onset information or PCR testing information were assigned days post symptom onset of 30 days, as 28 days was the minimum time required before screening for plasma donation. Patients with days from symptom onset or positive PCR>60 days were assigned days post symptom onset of 60 days for graph visualization purposes. Results < 1.0 U/mL are plotted as 0.9 U/mL. Dotted line represents assay cutoff for positivity.

values (Fig. S2).

We next assessed all patients with days from symptom onset or PCR and plotted on a logarithmic scale to better assess U/mL levels over time for all IgG components (Fig. 1). As anticipated S1 IgG often mirrors RBD IgG. S2 IgG frequently does not become detectable even at >30 days. Both RBD IgG and N IgG detect >30 days post symptom onset well, with RBD IgG being slightly better missing only two patients to four missed by N IgG. Clinical sensitivity for these samples was highest for RBD IgG individually at all timepoints (80.8%, 95% CI 76.2–84.7; n = 333), at >14 days (97.9%, 95% CI 94.1–99.3; n = 144), and at >30 days (96.8%, 95% CI 89.1–99.1; n = 63). However, the clinical sensitivity was highest when all four markers were assessed together reaching 100% for both >14 and >30 days (95% CI 97.4–100 and 94.3–100 respectively; Table S3).

3.3. Comparison of assays

Using both the comparison and routine run samples we assessed concordance between assays and clinical diagnosis (Table 2, corresponding 95% CI Table S4). We found that concordance between assays with antibodies against the same or similar antigens was high. N IgG had an overall concordance at >14 days from symptom onset of 100% (95% CI 93.4–100%; n = 54) with the Roche N assay and 98.6% for all timepoints (95% CI 96.5–99.5%; n = 289). This represented the highest concordance between any two assays, though several assays demonstrated >95% concordance. The SiemensC, SiemensV, and Beckman assays all detected antibodies against RBD and concordance for all specimens was 93.8–96.8% compared to RBD IgG. For the Beckman, SiemensC, and EuroIGG additional samples from the routine run samples allowed for a larger cohort for comparison and positive agreement at >14 days to the RBD IgG assay was 91.4, 96.7, and 98.4% (n = 58, 60, 64; 95% CI 81.4 – 96.3, 88.6–99.1, 91.7–99.7) respectively. Concordance between S1 IgG and EuroIGG and EuroIGA, the assays detecting S1 antibodies, was 97.7% and 91.9% for all specimens respectively (n = 476, 95% CI 95.9–98.7, 89.1–94.0). S2 IgG had poor concordance with all assays in all subgroups (44–85.8%), which is likely reflective of the low number of samples with detectable S2 IgG. Positive agreement with clinical diagnosis of COVID-19 or PCR positivity also demonstrated that approximately half of the cases developed S2 IgG (Table 2). Concordance between PCR positivity and serology at >14 days reached 100% when all four IgG were assessed (n = 44; 95% CI 92.0–100). 100% concordance with clinical diagnosis at >14 days was found when IgG against all four antigens were assessed (n = 75; 95% CI 95.1–100). No individual marker reached 100% positive agreement with PCR positivity or positive clinical diagnosis, but RBD IgG and N IgG both had high concordance at 96.0% and 93.3% respectively (n = 75; 95% CI 88.9–98.6, 85.3–97.1) with >14 days from symptom onset with a positive clinical diagnosis.

The multiplex assays are semi-quantitative and the other assays we compared against are qualitative but our prior work has demonstrated that the index values for these assays also appear to be semi-quantitative [8,11]. Using nine of the ten patients with serial samples from the scomparison cohort we assessed comparison between assays when the positive cutoff was normalized to an index value of 1.0 (Fig. 2). The tenth patient had only three timepoints and demonstrated a similar trend and is included in the supplementary material (Fig. S3). We found that assays detecting antibodies against RBD trended together. Trends in assays detecting antibodies to N showed more discordance in their trends, though one detected total antibodies and the other IgG specifically.

3.4. Comparison between detection of binding antibodies and neutralizing antibodies

Neutralizing antibodies have been found to correlate inversely with viral shedding and are one of the surrogate markers often used as part of disease immunity assessment [12,13]. We therefore used a subset of serial samples from 14 patients (n = 61) hospitalized and positive for COVID-19 to assess correlation between binding and neutralizing antibodies. RBD and S1 assays demonstrated higher index values with higher neutralizing antibody titers overall (Fig. 3 A, B). N and S2 assays had modest increases in index values with increasing neutralization

Table 2
Qualitative agreement between assays and diagnosis (%).

		Overall[a]	BR RBD IgG	BR Spike S1 IgG	BR Spike S2 IgG	BR Nucleocapsid IgG	Sample size
SiemensC	All specimens	96.4	96.4	94.9	82.7	92.0	336
	PCR and Reported Positive[b]	92.5	93.2	90.2	64.7	82.0	133
	>14 days[c]	93.3	96.7	90.0	53.3	90.0	60
Beckman	All specimens	96.2	96.8	97.8	84.9	91.9	371
	PCR and Reported Positive	90.2	92.4	94.7	66.7	80.3	132
	>14 days	87.9	91.4	96.6	60.3	86.2	58
EuroIGG	All specimens	96.0	98.1	97.7	79.0	92.4	476
	PCR and Reported Positive	92.2	97.4	96.6	60.3	81.0	116
	>14 days	95.3	98.4	95.3	45.3	89.1	64
EuroIGA	All specimens	89.6	91.6	91.9	78.3	87.1	479
	PCR and Reported Positive	79.2	84.2	85	59.2	70.8	120
	>14 days	78.8	83.3	83.3	53.0	75.8	66
Roche	All specimens	96.2	94.1	92.7	85.5	98.6	289
	PCR and Reported Positive	90.4	84.6	81.7	63.5	96.2	104
	>14 days	96.3	92.6	88.9	46.3	100	54
SiemensV	All specimens	93.8	93.8	95.8	85.8	91.3	289
	PCR and Reported Positive	84.6	84.6	89.4	63.5	76.9	104
	>14 days	83.3	83.3	90.7	59.3	83.3	54
Clinical	PCR Positive[d]	87.4	79.5	78.0	53.5	74.0	127
	>14 days	100	93.2	90.9	52.3	93.2	44
	Clinical Diagnosis[e]	89.3	83.0	78.6	49.1	77.4	159
	>14 days	100	96.0	88.0	44.0	93.3	75

[a] Overall: any IgG positive on BioRad assay.
[b] All cohort specimens which were tested PCR positive or recorded as positive in the electronic medical record. Note this is a subset of 'All specimens' assayed by both the listed assays.
[c] All specimens collected >14 days from tested PCR positive or recorded as positive in the electronic medical record. Note this is a subset of 'All specimens' assayed by both the listed assays.
[d] All cohort specimens which were tested PCR positive.
[e] All cohort specimens which were tested PCR positive or recorded as positive in the electronic medical record.

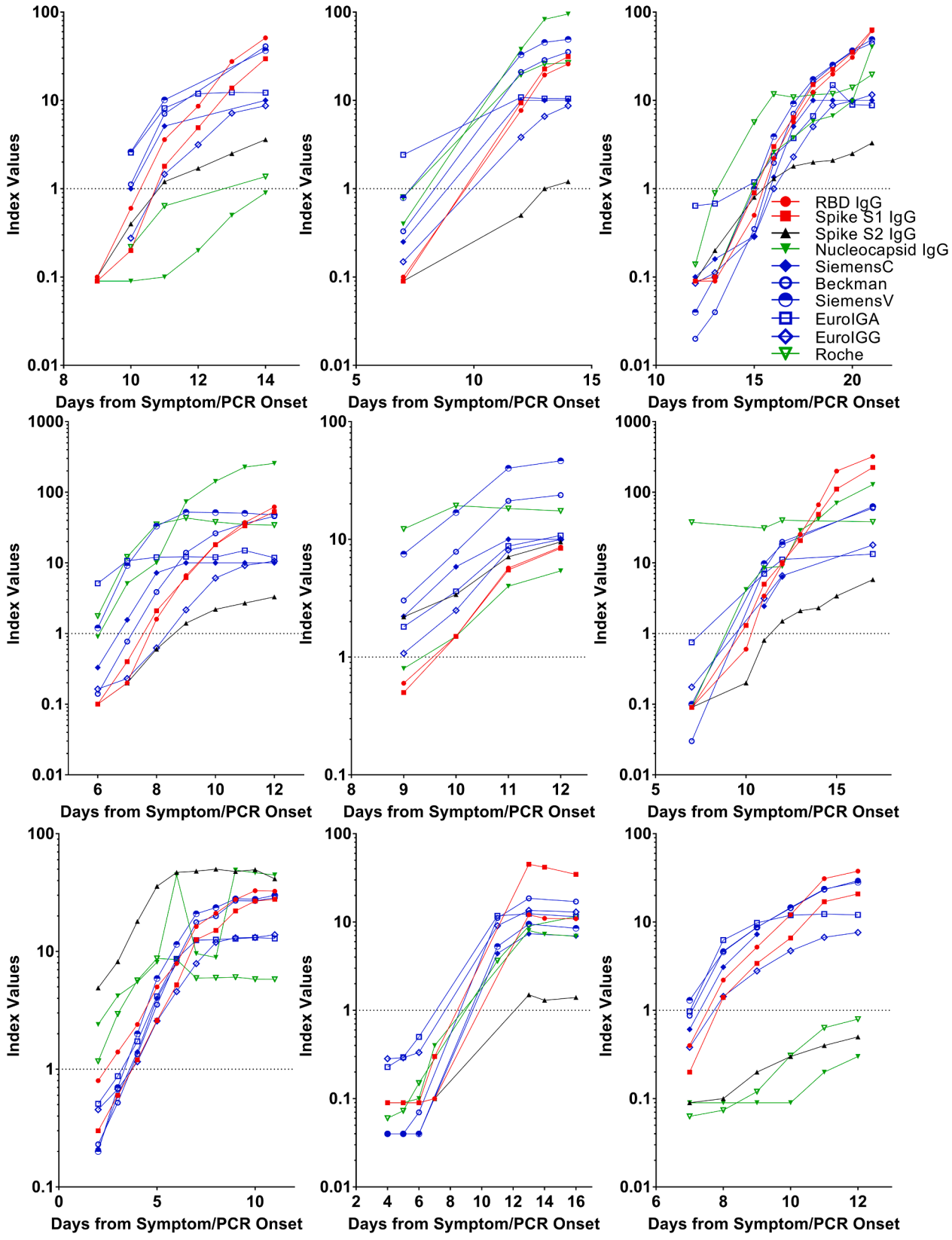

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Fig. 2. Comparison of serial SARS-CoV-2 antibody measurements between platforms. Remnant samples from patients hospitalized for SARS-CoV-2 were tested for SARS-CoV-2 antibodies at available timepoints. Days from symptom onset was obtained by chart review, where patients were asymptomatic or had chronic symptoms (eg – COPD) days from PCR positivity was used. Some assay values were normalized as follows to allow for cross assay visualization. Siemens Vista values were divided by 1000 to bring the assay positivity cutoff to 1.0. BioRad RBD, S1, S2, and N IgG U/mL were divided by 10 to bring the assay positivity cutoff to 1.0. Dotted line represents assay cutoff for positivity. EuroIGA and EuroIGG have an assay positivity cutoff of 1.1, and the dotted line approximates this. Red figures and lines represent BioRad S1 and RBD associated antibodies. Blue figures and lines represent platforms with S1 and RBD associated antibody detection. Green figures and lines represent platforms with nucleocapsid associated antibody detection. RBD: receptor binding domain, RBD IgG: BioRad SARS-CoV-2 RBD IgG, Spike S1 IgG: BioRad SARS-CoV-2 spike S1 IgG, Spike S2 IgG: BioRad SARS-CoV-2 Spike S2 IgG, Nucleocapsid IgG: BioRad SARS-CoV-2 Nucleocapsid IgG, SiemensC: Siemens Centaur Anti-SARS-CoV-2 Total, Beckman: Beckman Anti-SARS-CoV-2 IgG, SiemensV: Siemens Vista Anti-SARS-CoV-2 Total, EuroIGA: Euroimmun anti-SARS-CoV-2 IgA; EuroIGG: Euroimmun anti-SARS-CoV-2 IgG; Roche: Roche Anti-SARS-CoV-2 Total. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

titers (Fig. 3 C). Analysis by Spearman's rank correlation demonstrated statistical significance for correlation between each assay and $FRNT_{80}$ ($p < 0.01$), however correlation coefficients varied by assay and antigen. The highest correlation coefficients were for RBD based assays (RBD IgG 0.80 (95% CI 0.69–0.88), Beckman 0.88 (95% CI 0.79–0.94), SiemensC 0.89 (95% CI 0.81–0.94), SiemensV 0.86 (95% CI 0.75–0.92)) with S1 based assays demonstrating slightly less robust coefficients (S1 IgG 0.79 (95% CI 0.67–0.87), EuroIgA 0.67 (95% CI 0.46–0.80), EuroIgG 0.77 (95% CI 0.61–0.87)). N and S2 based assays had low correlation coefficients, which were still significant (S2 IgG 0.40 (95% CI 0.16–0.60), N IgG 0.48 (95% CI 0.25–0.65), Roche 0.40 (95% CI 0.11–0.64)). Qualitatively, positive agreement between neutralizing antibody titers and binding assays was highest for RBD assays, with the highest levels of positive agreement reached by the SiemensV and if the multiplex assay components were combined to generate a qualitative assessment that was positive if any one antibody was positive (Table 3, 95% CI Table S5). Negative agreement had few samples as neutralizing antibodies were found in samples that were negative by binding antibody assays, however no sample was positive for neutralizing antibodies and negative for all binding antibodies (Table S6).

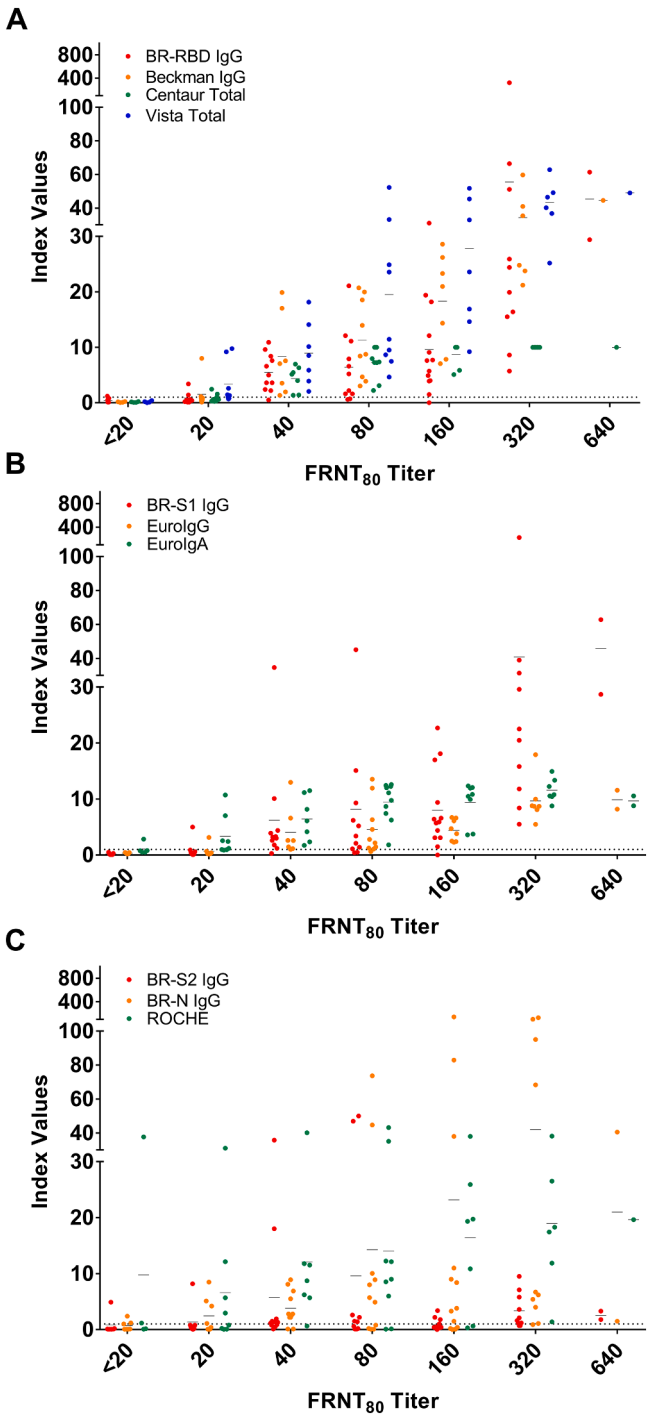
4. Discussion

We assessed the BioRad multiplex SARS-CoV-2 IgG assay to determine assay comparability to our existing methodologies and overall performance characteristics in natural disease. The introduction of SARS-CoV-2 vaccines is a needed shift in our response to this global pandemic but brings with it new challenges and opportunities for the use of serologic testing in SARS-CoV-2. Many SARS-CoV-2 vaccines use the spike antigen to elicit an immune response [5]. In assays that detect antibodies against the RBD, S1 or S2 there will likely be reactivity in vaccinees, however N reactivity should be present only in natural disease [4,5]. Assays that can provide semiquantitative results that can differentiate between natural disease and vaccine responses are desirable to aid in epidemiologic research, assess long term effects of natural disease, and assess immune response to vaccination in at risk persons with immune dysregulation. A single assay that can provide all of this information reduces the need for multiple assay maintenance and sample volume. Though it should be noted that the use of widespread serologic testing after vaccination is not currently recommended and there are limitations in our understanding of all of the immune correlates of protection after vaccination [14].

We found that RBD, S1, S2, and N IgG all had excellent specificity which was similar to that of other current EUA and CE assays [8,15,16]. We observed possible cross-reactivity in one sample for which the patient had a nasopharyngeal swab positive for one of the common coronaviruses in the RBD and S1 IgG. Our prior work with this sample found that it demonstrated SARS-CoV-2 antibody cross-reactivity in three of six platforms [8]. Due to the timing of this sample we cannot exclude true SARS-CoV-2 infection from consideration as it was drawn before SARS-CoV-2 was geographically present but not before December 2019. The patient history demonstrated no travel or contact concerns, but SARS-CoV-2 testing was not yet available to definitively exclude infection. Some antibody cross-reactivity in conserved epitopes between the common coronaviruses and SARS-CoV-2 has been found in general serology studies, though these epitopes are located in S2 and N where we saw no cross-reactivity [17].

In assessing timing of seroconversion by days from symptom onset we found that N IgG was most often the first to become detectable but also had significantly delayed responses in some patients. This heterogeneity in serologic conversion for different antigens may be an effect of disease severity, or other correlates for which we do not have information and is an area of future interest. Despite the significant delays in detection of N IgG during the seroconversion window, it appears to have strong clinical sensitivity in detecting prior infection at >14 days and misses very few samples at >30 days. These findings are in keeping with other work [8,12,15,16,18–20]. This makes both RBD IgG and N IgG helpful in convalescent plasma donation screening and determination of natural disease history in the setting of vaccination with spike protein specific vaccines. Some clinical assays have poorer detection of prior SARS-CoV-2 infection at >30 days [8] and we found that S2 IgG had poor detection overall.

We found overall concordance between several existing CE and EUA SARS-CoV-2 serologic assays using the same antigens as this multiplex assay to be clinically acceptable. Using all four multiplex assays together yielded 100% concordance with both PCR positivity and Clinical Diagnosis at >14 days. In our prior work and others with both RBD and N serologic assays, no assay achieved 100% concordance with clinical markers [8,11,15,16,20]. Agreement between assays with the same antigen was higher than we anticipated and adjusting all assays for a positivity cutoff of approximately 1.0 index value demonstrated similar trending during seroconversion. The seroconversion U/mL longitudinal increase in the multiplex assay reinforced the semi-quantitative nature of this assay, which corresponded with index values for existing qualitative assays. Due to the highly infectious nature of SARS-CoV-2 and the extended time period for which RNA testing can remain positive, antibody testing has emerged as a useful tool in infection prevention [12,13]. Determination of removal of airborne precautions for inpatients or acceptability of transfer to skilled nursing facilities is a difficult decision resting with infection preventionists. Limited work to date indicates that the presence of neutralizing antibodies correlates with a decrease in ability to culture SARS-CoV-2 from nasal swabs, thus a threshold that reflects the presence of neutralizing antibodies may also be an important step for improving our control of viral spread in medical care settings [12,13]. Most clinical serology assays assess patient samples for binding antibodies and data demonstrating how well binding antibodies correlate with neutralizing antibodies both during seroconversion and over time continues to accrue [12,21–23]. Specifically, most neutralizing antibody literature uses pseudoviral methodologies rather than the gold standard plaque and focus reduction neutralization tests, though additional work utilizing FRNT is emerging with mixed findings [24–26]. We found that positive agreement between $FRNT_{80}$ neutralizing assay and antibodies to RBD ranged from 83 to 95% across binding assays. S1 also demonstrated high positive agreement between neutralization and binding assays ranging from 79 to 82%. Positive agreement was highest between neutralizing antibodies and the SiemensV at 95% (95% CI 82.7–98.5) and second by the full multiplex assay qualitative result at 91% (95% CI 80.1–96.0), though 95%



(caption on next column)

Fig. 3. Comparison of binding assays and neutralization titers. Serial remnant specimens from 14 patients were assessed for binding and neutralizing antibodies. Binding antibodies are plotted as index values on the y-axis, neutralization assays are plotted as end titer ($FRNT_{80}$) on the x-axis. Some binding assay values were normalized as follows to allow for cross assay visualization. Siemens Vista values were divided by 1000 to bring the assay positivity cutoff to 1.0. BioRad RBD, Spike S1, Spike S2, and Nucleocapsid IgG U/mL were divided by 10 to bring the assay positivity cutoff to 1.0. Dotted line represents assay cutoff for positivity. EuroIGA and EuroIGG have an assay positivity cutoff of 1.1, and the dotted line approximates this. (A) Binding assays against the receptor binding domain are plotted. (B) Binding assays against the S1 protein are plotted. (C) Binding assays against nucleocapsid and S2 protein are plotted. RBD: receptor binding domain, BR-RBD IgG: BioRad SARS-CoV-2 RBD IgG, BR-S1 IgG: BioRad SARS-CoV-2 spike S1 IgG, BR-S2 IgG: BioRad SARS-CoV-2 Spike S2 IgG, BR-N IgG: BioRad SARS-CoV-2 Nucleocapsid IgG, Centaur Total: Siemens Centaur Anti-SARS-CoV-2 Total, Beckman IgG: Beckman Anti-SARS-CoV-2 IgG, Vista Total: Siemens Vista Anti-SARS-CoV-2 Total, EuroIGA: Euroimmun anti-SARS-CoV-2 IgA; EuroIGG: Euroimmun anti-SARS-CoV-2 IgG; Roche: Roche Anti-SARS-CoV-2 Total.

confidence intervals overlapped for all assays. For discrepancies between neutralizing and binding antibodies it was most common that neutralizing antibodies were detected while binding antibody assays were not yet reactive. We expect RBD antibodies, rather than S1, S2 or N, to correlate better with neutralizing antibody assays as these are thought to be the principal drivers in virus neutralization [3]. Recent work by other groups has found reasonable correlation between FRNT and commercial binding antibody assays with various antigen targets, $FRNT_{50}$ rather than $FRNT_{80}$ has been used for those correlates [25,26]. Though some groups have reported insufficient correlation to consider binding antibody assays reflective of functional antibody response [24]. This has important implications for determining immune correlates of protection for binding antibodies for both natural disease and vaccine induced immunity.

Overall, we found the BioRad SARS-CoV-2 IgG assay to be acceptable for clinical use and comparable to existing CE and EUA serologic assays. This work has several limitations including: additional freeze–thaw cycle for samples assayed by the BR and neutralization assays; relatively small cohort size, particularly for FRNT; lack of demographic and co-morbidity information. The ability to measure multiple analytes simultaneously may be advantageous for complex clinical presentations, epidemiologic research to differentiate between natural disease and some vaccines, and in some decisions regarding infection prevention strategies. Additional independent validations are needed to further determine RBD binding antibody and neutralizing antibody correlations and corroborate these findings.

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Author contributions

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Competing interests

This work was partially funded by BioRad Laboratories, Inc. AA and NC are employees of BioRad Laboratories, Inc. Other authors state no conflict of interest.

Ethical approval

This study was performed with the approval of the UPMC Quality Assurance for Clinical Laboratories, the University of Pittsburgh institutional review board study #20040072, and in compliance with the World Medical Association Declaration of Helsinki

Table 3
Qualitative Agreement Between Binding and Neutralizing Assays (%).

	RBD IgG	Spike S1 IgG	Spike S2 IgG	Nucleocapsid IgG	BR combined		Sample size
Pos Agreement	83.3	81.5	51.9	74.1	90.7		54
Neg Agreement	85.7	100	85.7	57.1	57.1		7
Overall Agreement	83.6	83.6	55.7	72.1	86.9		61
	Beckman	SiemensC	SiemensV	Roche	EUROIGA	EUROIGG	Sample size
Pos Agreement	86.8	86.8	94.7	76.3	84.2	78.9	38
Neg Agreement	100	100	100	50.0	100	100	4
Overall Agreement	88.1	88.1	95.2	73.8	85.7	81.0	42

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinbiochem.2021.08.006.

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