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Evaluation of a multiplexed coronavirus antigen array for detection of SARS-CoV-2 specific IgG in COVID-19 convalescent plasma

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ABSTRACT

Mitigation of the COVID-19 pandemic requires an understanding of the antibody response to SARS-CoV-2. However, throughout the development of SARS-CoV-2 IgG antibody assays during the past year, cross-reactivity to other coronaviruses remained a question. To address these issues, we evaluated IgG in COVID-19 convalescent plasma samples for reactivity against three SARS-CoV-2 antigens including full-length spike, receptor binding domain, and the proximal extracellular fusion domain, and spike antigens from other coronaviruses (SARS-CoV, MERS-CoV, hCoV-HKU1, hCoV-OC43, hCoV-229E and hCoV-NL63) using the VaxArray Coronavirus SeroAssay which is a multiplexed antigen assay developed by InDevR Inc. These results were compared to two commercial SARS-CoV-2 IgG ELISAs targeting either the SARS-CoV-2 nucleocapsid or spike antigens and a live virus focus reduction neutralizing antibody test (FRNT). The VaxArray platform showed high specificity for detection of SARS-CoV-2 IgG, evident from lack of reactivity to SARS-CoV-2 antigens despite significant reactivity to endemic coronavirus antigens in pre-pandemic samples. SARS-CoV-2 IgG positive samples reacted weakly to SARS-CoV spike but not to MERS-CoV. While the VaxArray platform had overall comparable results to the spike and nucleocapsid IgG ELISAs, results were more similar to the spike antigen ELISA and the platform displayed a higher sensitivity and specificity than both ELISAs. Samples with FRNT titers below 1/23 reported negative on VaxArray, while positive samples on VaxArray had significantly higher neutralizing antibody titers. These results suggest that the VaxArray Coronavirus SeroAssay performs with high sensitivity and specificity for the detection of SARS-CoV-2 IgG, and positive results on the platform indicate SARS-CoV-2 neutralizing activity.

1. Introduction

A plethora of antibody assays have been developed in response to the novel Coronavirus Disease-19 (COVID-19) pandemic that continues to spread across the globe. Analysis of SARS-CoV-2 antibodies has utility for understanding the prevalence of infection within communities

(Angulo et al., 2021; Bajema et al., 2020; Koopmans and Haagmans, 2020; Tanne, 2020), providing evidence of past infection or recent infection when viral detection by PCR is negative (Anderson et al., 2020; Jia et al., 2021), and for the analysis of convalescent plasma that has been used therapeutically to treat COVID-19 (DomBourian et al., 2020; Shen et al., 2020). Additionally, with the implementation of COVID-19

Abbreviations: COVID-19, Coronavirus Disease 2019; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus-2; CoV, Coronavirus; S, Spike protein; RBD, Receptor Binding Domain; ELISA, Enzyme Linked Immunosorbent Assay; FDA, Food and Drug Administration; EUA, Emergency Use Authorization; CCP, COVID-19 Convalescent Plasma; RPP, Respiratory Pathogen Panel; EDI, Epitope Diagnostic Inc; HRP, Horseradish peroxidase; TMB, Tetramethyl Benzidine; FRNT, Focus Reduction Neutralizing Antibody Test; OD, Optical Density.

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vaccination, analysis of the antibody response to these vaccines is necessary in order to understand both the short-term immune response and the longevity of the immune response.

Initially, the development of antibody assays was fraught with concerns of cross-reactivity to pre-existing antibodies to the non-SARS-CoV-2 human coronaviruses (hCoVs) hCoV-HKU1, hCoV-OC43, hCoV-229E and hCoV-NL63 and therefore, lower specificity of antibody detection. Over the course of the past several months, antibody assays with high specificity and sensitivity (depending on the timing of sample collection post infection) have been developed and implemented in clinical laboratories (Abbasi, 2020; National S-C-SAEG, 2020). However, few have incorporated antigens from related hCoVs to address the question of antibody specificity.

The VaxArray Coronavirus SeroAssay incorporates the S1 domain from the spike (S) protein of SARS-CoV, MERS-COV, and either the S1 domain or the full-length S protein of four human endemic CoVs, hCoV-HKU1, hCoV-OC43, hCoV-229E and hCoV-NL63 along with the full-length S protein, the receptor binding domain (RBD), and the proximal extracellular fusion domain (S2) of the SARS-CoV-2 S antigen in a single assay (Dawson et al., 2021). Each of these antigens is spotted, in nine replicates, onto a functionalized glass slide in a 9×9 grid. When incubated with human serum followed by anti-human IgG conjugated to a fluorescent molecule, reactivity to each antigen appear as distinct fluorescent spots that can be quantified as relative fluorescent units. Inclusion of the S antigen from related CoVs enables evaluation of the specificity of the antibody response to SARS-CoV-2.

Here we compare the performance of the VaxArray Coronavirus SeroAssay with a CE-marked and clinically validated ELISA and an FDA Emergency Use Authorization (EUA) approved assay for analysis of SARS-CoV-2 IgG antibodies. Additionally, we compare the VaxArray Coronavirus SeroAssay with a functional assay for SARS-CoV-2 neutralizing antibodies.

2. Materials and methods

2.1. Donors

Children's Hospital Colorado became eligible to collect COVID-19 Convalescent Plasma (CCP) after registering with the FDA on March 31, 2020. According to the FDA's requirements, individuals were eligible to participate in the CCP donor program if they had a confirmed PCR positive test for SARS-CoV-2 and were symptom-free for at least 14 days prior to plasma donation (Annen et al., 2021).

2.2. Samples

Three sets of samples were included in this study: (a) Ninety-six deidentified plasma or serum samples collected from SARS-CoV-2 PCR positive donors from Children's Hospital Colorado CCP donor program (PCR positive samples); (b) Twenty-four deidentified samples that were collected prior to November 2019 (pre-pandemic samples); and (c) six deidentified residual samples that tested positive on the respiratory pathogen panel (RPP) (BioFire FilmArray® Respiratory Panel (RP), Salt Lake City, UT) for hCoV-NL63, hCoV-OC43, hCoV-229E, or hCoV-HKU1 or adenovirus and tested negative on a SARS-CoV-2 PCR test (RPP-positive samples).

2.3. SARS-CoV-2 IgG ELISA

Two commercial ELISAs, the CE-marked Epitope Diagnostics Inc. (EDI) ELISA, (San Diego, CA), (#KT-1032) and the CE-marked and FDA EUA approved EUROIMMUN ELISA, (Lubeck, Germany) (#2606) were evaluated in this study. For both assays, samples were diluted, tested and analyzed according to the kit instructions for detection of SARS-CoV-2 IgG. The EDI ELISA utilizes recombinant SARS-CoV-2 nucleocapsid antigen and the EUROIMMUN ELISA utilizes the recombinant SARS-CoV-2

S1 domain, which includes the RBD. Briefly, samples were diluted with sample diluent either 1:100 for EDI or 1:101 for EUROIMMUN, and plates were incubated and washed. Anti-human IgG horse radish peroxidase (HRP)-conjugated detection antibody was added, followed by an incubation, wash, and the addition of the substrate, tetramethylbenzidine (TMB). After a brief incubation, colour development was halted with 0.5 M sulfuric acid, and plates were read at 450 nm within 10 min of stopping the reaction.

2.4. VaxArray Coronavirus SeroAssay

Multiplexed fluorescence imaging platform, InDevR VaxArray Coronavirus SeroAssay, (Boulder, CO, USA) analyzes antibody response to multiple CoVs (Table 1). The manufacturer's published characteristics for the assay include 98.5% sensitivity, 100% specificity and an average precision of 11% CV (Dawson et al., 2021). Plasma and serum samples, including human pooled AB serum as a negative control, were diluted 1:100 in kit-provided protein blocking buffer and added to the microarray slide. The slide was then incubated for an hour in a humidity chamber at room temperature on an orbital shaker set to 80 rpm. Following incubation, the slide was washed with kit specific wash buffer and incubated under the same conditions as previously stated for five minutes. After removal of the wash buffer, slides were incubated for thirty minutes with detection solution containing fluorescently labeled IgG antibody under the same conditions as previously stated. Slides were then washed with kit specific wash buffer, followed by 70% ethanol and deionized water. Once dry, slides were analyzed with the VaxArray Imaging System (InDevR). For each CoV antigen the median relative fluorescent unit of the nine replicates was divided by the median background signal to give a signal to background ratio that was used for the analysis of the SeroAssay.

2.5. Focus reduction neutralization assay (FRNT)

Vero E6 cells (ATCC, Manassas, VA, USA) were seeded in 96-well plates. Serum samples were heat inactivated and serially diluted (2-fold, starting at 1:10) in DMEM (ThermoFisher, Pittsburgh, PA, USA) plus 1% FBS in 96-well plates. Approximately 100 focus-forming units (FFU) of SARS-CoV-2 USA-WA1/2020 (Harcourt et al., 2020) (deposited by the Centers for Disease Control and Prevention and obtained through BEI Resources, NIAID, NIH) was added to each well and the serum plus virus mixture was incubated for 1 h at 37 °C. At the end of 1 h, medium was removed from cells and the serum sample plus virus mixture was added for 1 h at 37 °C. After 1 h, samples were removed and cells overlaid with 1% methylcellulose (MilliporeSigma, St. Louis, MO, USA) in MEM (ThermoFisher, Pittsburgh, PA, USA) /2% FBS and incubated 30 h at 37 °C. Cells were fixed with 4% paraformaldehyde (Acros Organics, Pittsburgh, PA, USA) and probed with 1 µg/mL of an anti-SARS-CoV S monoclonal antibody (CR3022, Absolute Antibody, Boston, MA, USA) in Perm Wash (1 × PBS/0.1% saponin/0.1% bovine serum albumin [BSA]) for 2 h at RT. After washing, cells were incubated with horseradish peroxidase (HRP)-conjugated goat anti-human IgG (Southern Biotech, Birmingham, AL, USA, 1:1,000) for 1.5 h at RT. After washing, SARS-CoV-2-positive foci were visualized with TrueBlue substrate (ThermoFisher, Pittsburgh, PA, USA) and counted using a CTL Biospot analyzer and Biospot software (Cellular Technology Ltd., Shaker Heights, OH, USA).

2.6. Interpretation of results

2.6.1. For the EDI assay, positive, negative, and borderline results were calculated based on the average triplicate optical density (OD₄₅₀) value for the negative control for the specific assay. The positive and negative cut-off values were calculated using the formula: positive cut-off = $1.1 \times (x_{NC} + 0.18)$ and negative cut-off = $0.9 \times (x_{NC} + 0.18)$, where x_{NC} is the average OD₄₅₀ of triplicate negative control OD₄₅₀

Table 1
Coronavirus antigens present on the VaxArray Coronavirus SeroAssay.

	Label	Protein	Source	Coronavirus
<p>Viral Protein Map</p>	nCoV(i)	Full length spike (S1, S2)	Mammalian	SARS-CoV-2
	nCoV(ii)	RBD	Mammalian	SARS-CoV-2
	nCoV(iii)	S2, ECD	Insect	SARS-CoV-2
	SARS(i)	S1	Mammalian	SARS-CoV-1
	MERS(i)	S1	Mammalian	MERS
	HKU1(i)	S1	Mammalian	Endemic hCoV-HKU1
	OC43(i)	Full length spike (S1, S2)	Insect	Endemic hCoV-OC43
	229E(i)	S1	Mammalian	Endemic hCoV-229E
	NL63(i)	S1	Mammalian	Endemic hCoV-NL63

Each antigen is coated on a separate location of the multiplexed slide arranged in a 9×9 grid. Within this grid each antigen is spotted on to the slide in a 3×3 grid. S represents the full spike protein; S1, the first subunit of the spike protein; S2, the second subunit including the extracellular domain (ECD); and RBD, the receptor binding domain.

values. Samples that had OD_{450} values that fell between positive and negative cut-off values were reported as borderline.

2.6.2. The EUROIMMUN assay was interpreted based on the ratio of the sample OD_{450} to the calibrator OD_{450} . Samples with a ratio of less than 0.8 were deemed negative, samples with a ratio of greater than 1.1 were positive, and ratios between 0.8 and 1.1 were reported as borderline.

2.6.3. The VaxArray assay results were evaluated for SARS-CoV-2 full-length S (nCoV(i)), RBD (nCoV(ii)) or S2 (nCoV(iii)) reactivity by calculating the ratio of the median antigen-specific fluorescence signal to the median background signal. Samples were considered positive for SARS-CoV-2 antibodies if they met two criteria: (a) The signal to background ratio for nCoV(i) was greater than 1.5, and (b) the sum of the signal to background ratio for nCoV(i), nCoV(ii), and nCoV(iii) was greater than 6.18. If either criterion was not met, then the sample was considered negative.

2.6.4. The FRNT₅₀ titer was calculated relative to a virus only control (no serum) set at 100%, using GraphPad Prism 9 (San Diego, CA, USA) default nonlinear curve fit constrained between 0 and 100%.

2.7. Data analysis

Analysis of data was performed with GraphPad Prism 9 (San Diego, CA, USA) and Microsoft Excel 2016 (Microsoft, Redmond, WA, USA).

3. Results

3.1. Characterization of pre-pandemic samples and PCR positive samples on the VaxArray CoV SeroAssay

We evaluated the VaxArray Coronavirus SeroAssay by testing serum or plasma samples from SARS-CoV-2 PCR positive individuals, pre-pandemic samples, as well as SARS-CoV-2 PCR negative but respiratory pathogen panel (RPP)-positive samples. We also compared a randomly selected subset of the samples using commercial ELISAs for SARS-CoV-2 IgG detection, and a FRNT₅₀ assay for neutralizing antibodies to SARS-CoV-2. While the non-SARS-CoV-2 hCoVs do not have established positive and negative criteria, a sample was considered positive for SARS-CoV-2 antibodies on the VaxArray platform if it met the two criteria described in the methods.

The VaxArray is distinct from the other platforms in that in addition to analyzing IgG antibodies for reactivity against SARS-CoV-2 S antigens, it also simultaneously evaluates IgG antibodies for reactivity against the S1 subunit or full-length S protein from other hCoVs.

Representative analyses of serum from SARS-CoV-2 PCR positive individuals and pre-pandemic serum samples are shown in Fig. 1A–C. Serum from individuals with confirmed SARS-CoV-2 infection reacted to full-length S, RBD of the S antigen and to a lesser extent, the extracellular S2 domain of the S antigen as well as antigens from hCoVs associated with the common cold (Fig. 1C). Pre-pandemic sera also displayed reactivity to hCoVs associated with the common cold but showed little to no reactivity with SARS-CoV-2 antigens (Fig. 1B).

A set of ninety-six serum or plasma samples from SARS-CoV-2 PCR positive individuals, and thirty SARS-CoV-2 presumed negative samples (including twenty-four pre-pandemic samples, and six RPP-positive samples) was analyzed. The normalized fluorescence signals for individual S antigens are presented as a heat map with the SARS-CoV-2 antigens separated from the other hCoV antigens (Fig. 2A–D). Of the ninety-six PCR positive donors, seven were negative for SARS-CoV-2 antibodies based on the VaxArray criteria (Fig. 2A and E). Weak reactivity to SARS-CoV, as defined by a signal to background ratio greater than 2.00 but less than 10.00, was detected in thirteen PCR positive samples and no reactivity was seen with MERS-COV S protein (Fig. 2B). In contrast, while all but one of the thirty presumed SARS-CoV-2 negative serum samples had antibodies to one or more of the endemic CoVs, none of these samples were positive for SARS-CoV-2 full-length S (nCoV(i)) and only one sample was weakly positive for RBD (nCoV(ii)) antibodies (Fig. 2C and D). Weak reactivity to the extracellular S2 antigen, nCoV(iii), was detected in seven of the samples (Fig. 2C). Despite weak reactivity to S2 or RBD, none of the thirty presumed SARS-CoV-2 negative samples fulfilled criteria for a positive signal for SARS-CoV-2 antibodies. Notably, all of the SARS-CoV-2 PCR positive and all but one of the presumed negative samples had varying levels of reactivity to the four endemic hCoVs (Fig. 2B and D).

3.2. Comparison of IgG ELISAs to VaxArray Coronavirus SeroAssay

A smaller sample set consisting of seventy-four of the SARS-CoV-2 PCR-positive samples and twenty-nine presumed negative samples (twenty-four pre-pandemic samples and five RPP-positive samples) were

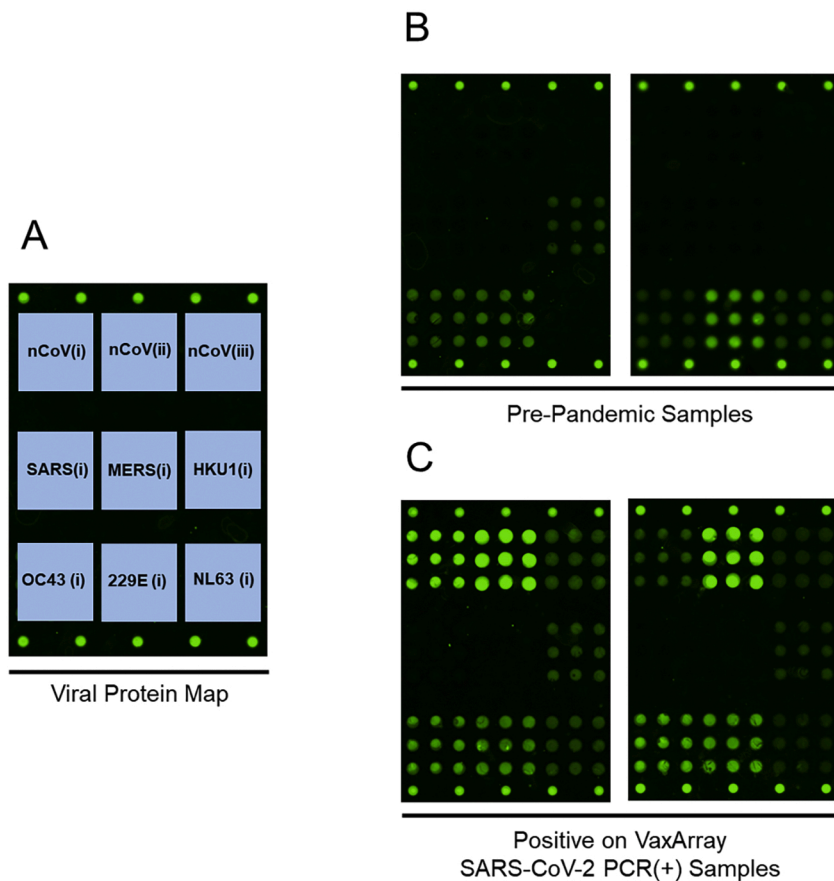


Fig. 1. Representative slide samples for the VaxArray Coronavirus SeroAssay. The spike antigens of SARS-CoV, MERS-COV, hCoV-HKU1, hCoV-OC43, hCoV-229E and hCoV-NL63, and the full spike (S, nCoV(i)), receptor binding domain (RBD, nCoV(ii)) and extracellular domain of S (S2, nCoV(iii)) are spotted onto a glass slide (A). Comprised in the slide's 9×9 grid, each antigen is represented in a 3×3 grid, yielding 9 replicate sets of data for each of the 9 antigens. Representative pre-pandemic sample slides (B) and representative VaxArray positive SARS-CoV-2 PCR positive sample slides (C).

compared among the two ELISAs, EDI and EUROIMMUN, and the VaxArray Coronavirus SeroAssay. Excluding borderline results from the EDI and EUROIMMUN assays, positive and negative results based on assay-specific cut-off values were used to determine assay sensitivity and specificity. The EDI ELISA had 78.9% sensitivity and 96.3% specificity (compared to the manufacturer's claim of 98.4% sensitivity and 99.8% specificity), EUROIMMUN had 87.1% sensitivity and 100% specificity (manufacturer's claim: 93.6% sensitivity and 100% specificity), and VaxArray had 93.2% sensitivity and 100% specificity (manufacturer's claim: 98.5% sensitivity and 100% specificity (Dawson et al., 2021)) (Table 2).

3.3. Qualitative results compared to neutralizing antibody titer

Qualitative results of thirty randomly selected SARS-CoV-2 PCR-positive samples analyzed by EDI, EUROIMMUN, and VaxArray assays were compared to the reciprocal FRNT50 titer (Fig. 3). For each assay the negative cut-off for the FRNT50 titer was determined by the mean plus one standard deviation of the samples characterized as negative by the respective antigen-binding assay. The EDI ELISA produced a clear distinction between positive and negative samples at a neutralizing antibody titer of 1/43. The one borderline result, however, had neutralizing activity above 1/100. Positive and negative samples appeared to segregate at neutralizing antibody titer of 1/40 in the case of the EUROIMMUN ELISA; however, due to a negative sample with a neutralizing activity of 1/85, the cut-off was less precise at a titer of 1/80 (the mean + 1SD of negative samples). Samples that had borderline results with the EUROIMMUN assay had variable neutralizing activity, ranging from 1/10–1/300. Samples tested on the VaxArray clearly segregated into positive and negative groups at a neutralizing antibody titer of 1/23. The three samples categorized as negative on VaxArray are highlighted in green across the three assays. While these samples were

all categorized as negative by the EDI assay, one sample was categorized borderline by EUROIMMUN.

4. Discussion

A significant number of SARS-CoV-2 antibody assays have entered the market since early 2020, with several having received EUA from the FDA (<https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/eua-authorized-serology-test-performance>, n.d.). The majority of antibody assays currently implemented for clinical use utilize one of the two major antigens, the nucleocapsid or S protein of SARS-CoV-2 (with many assays using only a portion of the S protein, such as the S1 subunit or just the RBD). During the early days of SARS-CoV-2 antibody assay development, there was considerable concern regarding the possibility of pre-existing, and potentially cross-reacting antibodies to the four endemic hCoVs that could affect the specificity of SARS-CoV-2 antibody detection. Laboratories developing and validating SARS-CoV-2 antibody assays have generally relied on evaluation of pre-pandemic serum or plasma samples to evaluate specificity of antibody assays, with the assumption that these samples should be SARS-CoV-2 naïve. The VaxArray Coronavirus SeroAssay incorporates into a single assay the ability to assess specificity of the response to SARS-CoV-2 by the inclusion of S antigens from related CoVs – SARS-CoV, MERS-COV, hCoV-HKU1, hCoV-OC43, hCoV-NL63 and hCoV-229E, thereby enabling us to address the question of specificity of the SARS-CoV-2 antibody response.

Analysis of samples from individuals who were known to be PCR positive for SARS-CoV-2 revealed IgG response to SARS-CoV-2 full-length S antigen, RBD and the S2 extracellular fusion domain of the S antigen. In general, reactivity to S2 was lower than reactivity to the full S and RBD. Low level cross reactivity was noted with S1 subunit from the SARS-CoV S protein in some of the samples. This is not surprising given

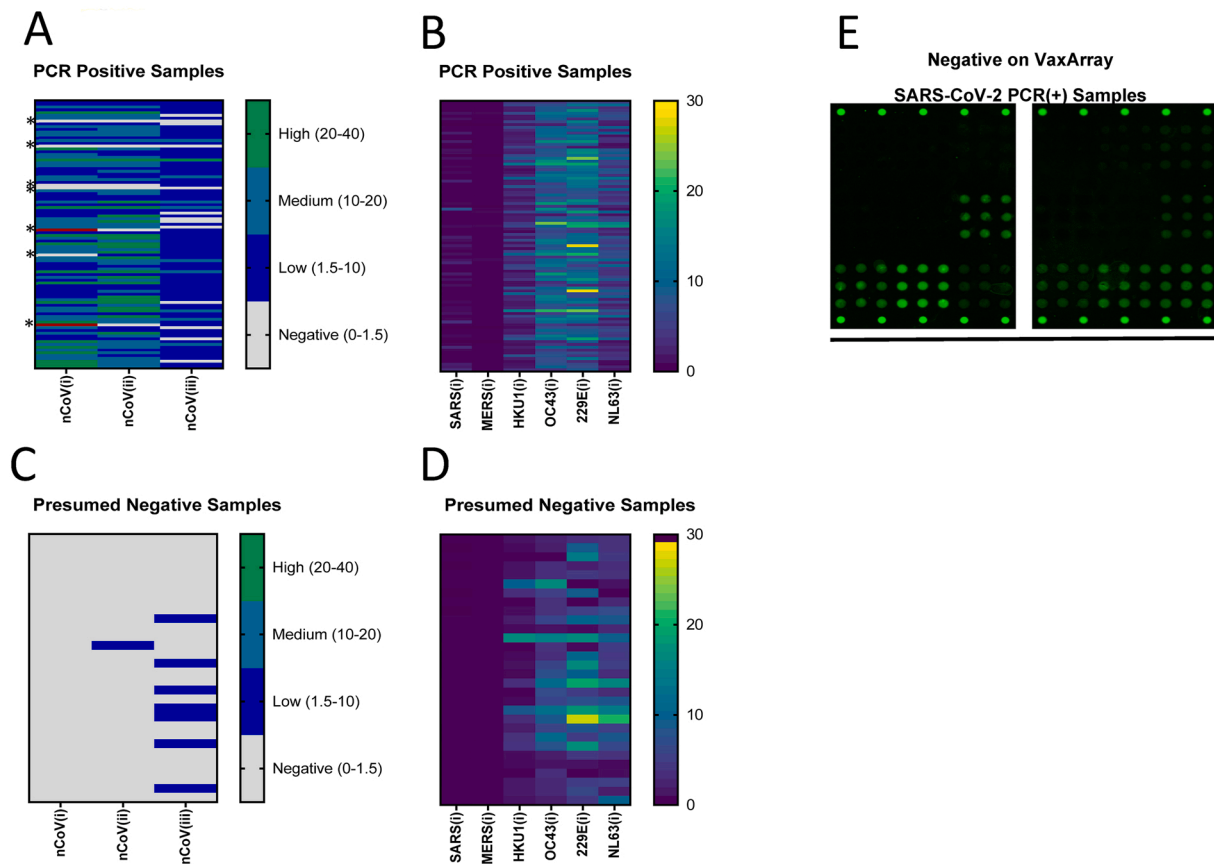


Fig. 2. Antibody response to coronavirus antigens on the VaxArray Coronavirus SeroAssay. Ninety-six SARS-CoV-2 PCR positive samples and 30 presumed negative samples were evaluated for IgG antibody response to SARS-CoV-2 antigens (A and C, respectively) and to other hCoVs (B and D, respectively). The normalized fluorescence signals are displayed in two different heat maps; panels A and C with the SARS-CoV-2 antigens displaying a negative, low, medium and high response and panels B and D with the other hCoVs displaying a spectrum with blue indicating a low response and yellow representing a high response. In panel A, an asterisk beside the sample indicates a VaxArray negative sample and the samples highlighted in red indicate that while the nCoV(i) signal was greater than 1.5, the sum of the three antigens was less than 6.18 so the sample was categorized as negative. Representative slides of the SARS-CoV-2 PCR positive samples that were negative on the VaxArray platform (E). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Sensitivity and specificity of EDI, EUROIMMUN, and VaxArray assays.

	EDI	EUROIMMUN	VaxArray
Presumed Negative Samples			
Total Samples	29	29	29
Negative	26	29	29
Positive	1	0	0
Borderline	2	0	0
Specificity	96.3	100	100
SARS-CoV-2 PCR Positive Samples			
Total Samples	74	74	74
Negative	15	9	5
Positive	56	61	69
Borderline	3	4	0
Sensitivity	78.9	87.1	93.2

Cut-off values were kit specific and borderline results were excluded.

that SARS-CoV and SARS-CoV-2 share close to 76% homology for full-length S protein (Jaimes et al., 2020). None of the PCR-positive samples cross-reacted with MERS-CoV S antigen, which again is not surprising since SARS-CoV-2 and MERS-CoV belong to different clades of the *Betacoronavirus* genus (Chen et al., 2020). Inclusion of S antigens of the four endemic hCoVs (hCoV-HKU1, hCoV-OC43, hCoV-229E and hCoV-NL63) enabled us to examine potential cross reactivity of pre-existing antibodies to these viruses with SARS-CoV-2 S antigen. The thirty presumed negative samples showed no cross reactivity with SARS-

CoV-2 full-length S and RBD and no reactivity to SARS-CoV and MERS-CoV S antigens, despite having significant reactivity to one or more of the endemic CoVs. These results are consistent with data showing that while the betacoronaviruses hCoV-OC43 and hCoV-HKU1 share 30–40% S antigen sequence homology with SARS-CoV-2, there is significant lack of similarity in the N-terminal region of the S that includes the RBD (Hicks et al., 2021). Our data are in agreement with those of Hicks J *et al* who showed that serum samples collected from healthy individuals prior to 2019 had robust IgG reactivity with hCoV-OC43 and hCoV-HKU1 S protein, but did not react to the S protein of SARS-CoV, SARS-CoV-2 and MERS-CoV (Hicks et al., 2021). Seven of the thirty presumed negative samples showed marginal cross reactivity with the SARS-CoV-2 S2, in line with data showing that S2 is more conserved across hCoVs (Liu et al., 2004).

Seven of the SARS-CoV-2 PCR positive samples tested negative for SARS-CoV-2 IgG on the VaxArray Coronavirus SeroAssay. As limited clinical information was available for these individuals, it is possible that their initial viral load may have been low, therefore leading to a suboptimal antibody response (Wang et al., 2020), or that perhaps the PCR result was a false positive. All seven samples were negative on the EDI assay and out of the five samples tested on the EUROIMMUN assay, four were negative and one was borderline. The neutralizing titer of the three negative samples tested for neutralizing antibodies was $<1/23$, demonstrating very little antibody neutralizing activity.

When compared with the EDI and EUROIMMUN assays, the performance of VaxArray was more sensitive than both assays, was superior to

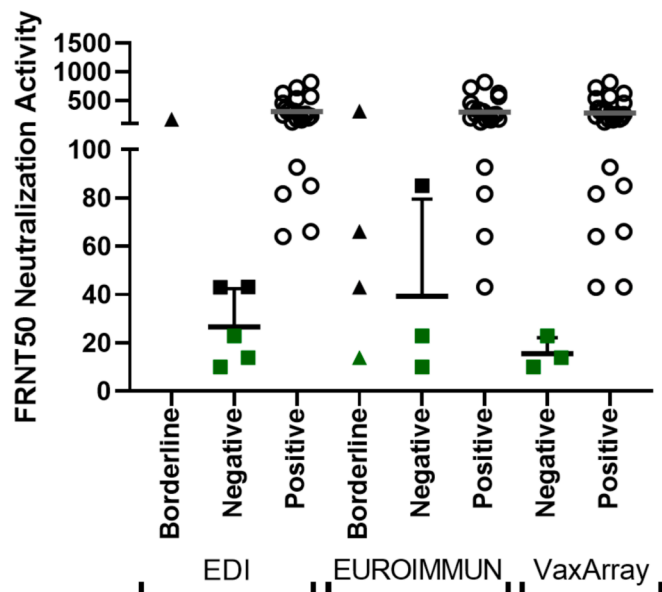


Fig. 3. Comparison of qualitative results from EDI, EUROIMMUN, and VaxArray to FRNT neutralizing results. Thirty SARS-CoV-2 PCR positive samples were evaluated on EDI, EUROIMMUN, and VaxArray assays. Qualitative results, based on kit-specific cut-off values were compared to FRNT50 titer. The mean FRNT50 titer for VaxArray-classified positive and negative samples is shown as a horizontal bar and the error bars on the negative samples represent one standard deviation. The mean FRNT50 titer plus one standard deviation was used as the negative cut-off for each assay. The three negative samples on the VaxArray platform are highlighted in green across each assay. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

EDI in specificity, and was similar in specificity to EUROIMMUN, at least for this sample set. The similarity with the EUROIMMUN ELISA is not surprising, given that both assays utilize the S antigen. The VaxArray qualitative results produced clear segregation between positive and negative samples based on anti-SARS-CoV-2 neutralizing antibody titer with the 3 negative samples exhibiting titers of $<1/23$, and positive samples with titers of $>1/23$. This distinction was less clear with the EUROIMMUN assay that also uses the S antigen as its target. Correlation of neutralizing antibody titer with the qualitative results of the EDI assay was less consistent and perhaps not surprising, given that anti-N antibodies do not play a major role in virus neutralization. Selection of antibody assays that correlate well with functional analysis is important when considering the utility of such assays in triaging COVID-19 convalescent plasma units for suitability for therapy (Annen et al., 2021; Bradfute et al., 2020) as well as when evaluating protection from reinfection.

Given that the vaccines currently being deployed in the US and several other countries are based on the S antigen, analysis of serological responses to these vaccines is important, not just in the short term but for an understanding of long term immunity to SARS-CoV-2. While not evaluated in this study, with the addition of a standard, the VaxArray platform can perform quantitative analysis, thereby giving more insight into changes in serological responses (Dawson et al., 2021). Given the flexibility of the VaxArray platform, incorporation of the SARS-CoV-2 nucleocapsid antigen will be useful to discriminate between vaccination and infection, evaluate population-wide serological responses either to infection or vaccination, and support continued vaccine development and clinical trials. Additionally, as new variants that

include mutations in the S antigen emerge, an assay platform that can evolve to evaluate serological responses to different strains of the virus is important. With the constantly changing response to the SARS-CoV-2 pandemic, the ability to efficiently and accurately compare and contrast IgG antibody response to multiple CoVs as well as potentially compare multiple variants will allow us to gain a more complete understanding of the immune response to SARS-CoV-2.

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Declaration of Competing Interest

Kathy L. Rowlen and Erica D. Dawson are stockholders of InDevR, Inc. All other authors declare that they have no conflicts of interest.

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