Research paper

# Analysis of COVID-19 convalescent plasma for SARS-CoV-2 IgG using two commercial immunoassays

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## A B S T R A C T

Coronavirus Disease 2019 (COVID-19) convalescent plasma (CCP) was approved by the FDA for use in severe cases of COVID-19 under an emergency Investigational New Drug (IND) protocol. Eligibility criteria for CCP donors includes documentation of evidence of COVID-19 either by viral RNA detection at the time of illness or positive SARS-CoV-2 IgG after recovery if diagnostic testing for COVID-19 was not performed at the time of illness. In addition to analysis of CCP, analysis of SARS-CoV-2 IgG provides information for possible past exposure and may support diagnosis when SARS-CoV-2 PCR is negative and clinical suspicion for COVID-19 is high. Furthermore, assays with high sensitivity and specificity for SARS-CoV-2 IgG are critical for understanding community exposure rates to SARS-CoV-2. Currently, there are several assays that test for antibodies to SARS-CoV-2 using a variety of methods, including point-of-care lateral flow-based devices, high throughput immunoassay analyzers, and manual methods such as ELISA. These assays target a number of SARS-CoV-2 antigens, including the nucleocapsid protein (N), full length spike protein (S), S1 subunit, or receptor binding domain (RBD) of the S protein. Given the heterogeneity among methods for, and antigenic targets used in SARS-CoV-2 antibody assays, it is necessary for careful evaluation of these assays prior to implementation for clinical use. We compared two assays that had received the CE mark of regulatory approval and that used either the N antigen or S1-RBD antigen as the target for analysis of a large set of CCP samples. Our data indicates that sensitivity and specificity vary between these assays and that more than one antigenic target may be required to improve the sensitivity and specificity of IgG detection to SARS-CoV-2.

## 1. Introduction

Coronavirus disease 2019 (COVID-19), caused by the RNA virus Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has rapidly spread worldwide since its discovery in December 2019. (Zhu et al., 2020) The World Health Organization (WHO) declared COVID-19 a pandemic in March 2020, and currently there are over 6 million cases and greater than 350,000 deaths globally. (World Health Organization, 2020) Early in the pandemic, our facility established a collection program for the experimental COVID-19 intravenous therapy known as COVID-19 Convalescent Plasma (CCP), which is derived from individuals who have recovered from COVID-19. Plasma antibodies from a recovered COVID-19 patient might shorten illness duration, reduce morbidity, and potentially prevent death when administered to a COVID-19 patient with active infection. (Duan et al., 2020) In addition,

convalescent plasma has been used in previous viral epidemics such as those caused by SARS-CoV-1, Ebola, and Influenza A virus type H1N1. (Cheng et al., 2005; Zhou et al., 2007; World Health Organization, 2014; Hung et al., 2011)

The United States Food and Drug Administration(FDA) authorized CCP for compassionate use on March 27, 2020 and provided guidance for CCP collection. The fundamental eligibility requirement is a confirmed positive SARS-CoV-2 PCR or serology test, with the most recent guidance mandating at least 14-days of COVID-19 recovery prior to donation. (U.S. Food and Drug Administration, 2020) Aside from assisting in eligibility determination, serological testing informs CCP donor management decisions related to continual eligibility as detectable antibody in donors would be of benefit to potential recipients. In addition to the analysis of CCP, SARS-CoV-2 antibody testing provides information related to past exposure that would be useful in

epidemiological studies to understand disease seroprevalence as well as confirm vaccine response during clinical trials. In the patient care setting, as with many antibody tests for infectious disease, SARS-CoV-2 antibody testing is not used for primary diagnosis. However, it may serve to support diagnosis in SARS-CoV-2 PCR-negative cases if the patients' viral loads are below the limit of detection in PCR testing, but there is a high clinical suspicion for COVID-19.

SARS-CoV-2 antibody analysis is evolving at a rapid pace with several assays coming to market, some having received CE-mark (European Economic Area) approval, and a few, more recently, having received FDA Emergency Use Authorization (EUA). (New York State Department of Health, 2020; Infectious Disease Society of America, 2020) However, assay analytical evaluation and robust comparative data among assays is lacking. Currently, there are a number of methodologies including ELISA, automated immunoassay, and lateral flow-based point of care devices. These assays generally target one of the virus's four main structural proteins including the spike (S), small envelope (E), membrane (M) and nucleocapsid (N) glycoproteins. (Ahmed et al., 2020) The S protein has additional antigenic targets in the S1 domain and receptor binding domain (RBD). (Lan et al., 2020) Here, we evaluated the analytical performance of two commercially available ELISA-based SARS-CoV-2 serological assays targeting different viral antigens utilizing a relatively large CCP donor sample set.

## 2. Materials and methods

### 2.1. Donors

Children's Hospital Colorado's CCP donor program was registered with the FDA as eligible to collect CCP on March 31, 2020. Eligible individuals for the CCP donor program were confirmed PCR-positive for SARS-CoV-2 and were symptom-free for at least 14 days prior to plasma donation, and met all standard blood donation criteria per FDA requirements.

### 2.2. Samples

Three sets of samples were included in this study: (a) de-identified plasma or serum samples collected from SARS-CoV-2 PCR-positive donors from the Children's Hospital Colorado CCP donor program; (b) respiratory pathogen panel (RPP)-positive samples, which were de-identified residual samples from patients who had tested positive for one of the respiratory viral pathogens (adenovirus; human metapneumovirus [HMPV]; influenza virus A hemagglutinin

[H] subtypes H1, H3, and 2009 H1N1; influenza virus B; respiratory syncytial virus; coronaviruses NL63, OC43, 229E, and HKU1; human rhinovirus/enterovirus; parainfluenza types 1–4; *Bordetella pertussis*; *Mycoplasma pneumonia*; and *Chlamydophilia pneumonia*) by BioFire FilmArray® Respiratory Panel (RP), (Salt Lake City, UT) and who were confirmed to be PCR-negative for SARS-CoV-2 (Table 1); and (c) de-identified samples that were collected prior to November 2019 (prepandemic samples).

### 2.3. SARS-CoV-2 IgG ELISA

Two commercial ELISAs, Epitope Diagnostics Inc. (EDI) (San Diego, CA) that is CE-marked and Euroimmun ELISA (Lubeck, Germany), that is both CE-marked and FDA EUA approved were compared in this study. The manufacturer's claims for sensitivity and specificity of these assays are shown in Table 2. For this study, the assays were used per the manufacturers' specifications.

The EDI ELISA utilizes the SARS-CoV-2 recombinant nucleocapsid antigen. Positive and negative assay controls, and samples diluted 1:100 with the kit-specific COVID-19 IgG sample diluent were added to the wells. Following a 30-min incubation at room temperature, the plates were washed 5 times using the kit-specific wash buffer and anti-

**Table 1**
Respiratory viral pathogen PCR positive and COVID-19 PCR-negative samples.

| Sample ID | Viral pathogen detected | EPITOPE | EUROIMMUN |
|---|---|---|---|
| RPP001 | Adenovirus | Neg | Neg |
| RPP007 | Adenovirus, human metapneumovirus, rhinovirus/enterovirus | Neg | Pos |
| RPP008 | Adenovirus, mycoplasma pneumoniae, rhinovirus/enterovirus | Neg | Neg |
| RPP009 | Coronavirus 229E, rhinovirus/enterovirus | Neg | Neg |
| RPP010 | Coronavirus HKU1 | Neg | Neg |
| RPP011 | Coronavirus NL63 | Neg | Neg |
| RPP012 | Coronavirus NL63 | Neg | Neg |
| RPP013 | Parainfluenza virus type 1 | Neg | Neg |
| RPP014 | Coronavirus NL63 | Neg | Neg |
| RPP015 | Coronavirus NL63 | Neg | Neg |
| RPP017 | Coronavirus NL63 | Neg | Neg |
| RPP021 | Human metapneumovirus | Neg | Neg |
| RPP025 | Human metapneumovirus, rhinovirus/enterovirus | Neg | Neg |
| RPP028 | Parainfluenza virus, type 1 | Neg | Neg |
| RPP029 | Parainfluenza virus, type 4 | Neg | Neg |

**Table 2**
Performance characteristics of Euroimmun and EDI assays established by the manufacturer.

| | | Estimate of performance (manufacturer's claim) | |
|---|---|---|---|
| | | Performance characteristic | 95% Confidence intervals |
| Euroimmun[a] | Sensitivity/ PPA* | 90.0% | 73.5%–97.9% |
| | Specificity/ PNA** | 100% | 95.5%–100% |
| EDI[b] | Sensitivity/PPA | 98.4% | 95.4%–0.995% |
| | Specificity/PNA | 99.8% | 99.1%–99.97% |

[a] Sample cohort consisted of 30 SARS-CoV-2 antibody-positive serum samples from individuals confirmed with a nucleic acid amplification test (NAAT) and 80 antibody-negative serum and plasma samples, collected prior to 2020 (Euroimmun Anti-SARS-CoV-2 ELISA (IgG) Instructions For Use (IFU)).

[b] Sample cohort consisted of normal healthy patients with samples collected prior to the COVID-19 outbreak ($N = 624$) and RT-PCR confirmed positive patients ($N = 187$) (EDI™ Novel Coronavirus COVID-19 IgG ELISA kit insert).

* PPA – Percent positive agreement.

** NPA- Percent negative agreement.

human IgG horseradish peroxidase (HRP)-conjugated detection antibody was added. The plate was incubated for 30 min at room temperature, followed by 5 washes, and addition of the substrate tetramethylbenzidine (TMB). The reaction was stopped with 0.5 M sulfuric acid after 20 min, and the plate was read at 450 nm within 10 min of halting the reaction.

The Euroimmun ELISA assay utilizes the S1 domain, including the receptor binding domain (RBD) of the SARS-CoV-2 spike protein. A kit-specific calibrator, positive and negative controls, and samples diluted 1:101 with the kit-specific dilution buffer were added to pre-coated wells. Following a 1-h incubation at 37 °C, the plates were washed 3 times with wash buffer. Anti-human IgG-HRP conjugated detection antibody was added and the plates incubated for another 30 min at 37 °C. Plates were then washed 3 times, and substrate TMB was added. Color development was halted after 30 min at room temperature with 0.5 M sulfuric acid, and the plate was read at 450 nm within 10 min of halting the reaction.

### 2.4. Interpretation of results

For the EDI assay, positive, negative and borderline results were calculated based on the average optical density ($OD_{450}$) value for the

negative control assayed in triplicate for the specific assay. The positive and negative cut-off values were calculated using the formula: positive cut-off = 1.1 x (xNC + 0.18) and negative cut-off = 0.9 x (xNC + 0.18), where xNC is the average $OD_{450}$ of triplicate negative control OD values. Samples that had $OD_{450}$ values that fell between positive and negative cut-off values were reported as borderline.

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 $OD_{450}$ values between 0.8 and 1.1 were reported as borderline.

### 2.5. Statistical analysis

Based on the qualitative results, receiver operating characteristic (ROC) curves were plotted for each of the assay using GraphPad Prism (San Diego, CA).

### 3. Results

Of the 102 PCR-positive donor samples tested, 84 tested positive, 3 tested borderline, and 15 tested negative by the EDI ELISA. In comparison, the same set of samples tested by the Euroimmun ELISA yielded 90 positive, 4 borderline and 8 negative results. Six samples tested negative by both assays (Fig. 1).

Specificity of the assays for SARS-CoV-2 compared with other respiratory viral pathogens, including the four non-SARS-2 human coronaviruses, was greater than 95%. While the EDI assay had 100% specificity for SARS-CoV-2 in this set of 20 samples, the Euroimmun assay had one false positive for a sample that was positive for multiple viral pathogens (adenovirus, HMPV, and human rhinovirus/enterovirus). Notably, samples from patients who had tested positive for one of the four non-SARS-2 human coronaviruses were negative by both assays (Fig. 2).

Of the 106 pre-pandemic serum samples tested, the EDI assay had one false positive and 3 borderline-positive samples, while the Euroimmun assay had 3 false positives and 2 borderline-positive samples. Only one sample tested borderline in both assays (Fig. 2). The EDI

positive sample and remaining two borderline samples were negative in the Euroimmun assay and the Euroimmun positives and remaining borderline sample were negative in the EDI assay.

ROC curves were generated for the EDI and Euroimmun assays based on positive (SARS-CoV-2 PCR-positive) and negative results (2019 pre-pandemic samples, and viral pathogen panel-positive/ SARS-CoV-2 PCR-negative samples) (Fig. 3). With the defined assay cutoffs, sensitivity of the EDI assay was 84.8% and specificity was 99.1% (compared with the manufacturer's claims of 94.8% sensitivity and 99.8% specificity), whereas sensitivity of the Euroimmun assay was 91.8% and specificity was 96.8% (compared with 90% sensitivity and 100% specificity).

### 4. Discussion

Given the diversity among serological methods used in SARS-CoV-2 antibody testing assays, a careful evaluation of these assays prior to implementation is warranted. Being one of the first facilities in the United States to collect CCP enabled us to have a relatively large donor sample set to compare two ELISA-based assays that used different SARS-CoV-2 antigenic targets and had received regulatory approval either by European Union standards or the FDA. Our study design, employing pre-pandemic specimens and SARS-CoV-2 PCR negative samples that were PCR-confirmed for other seasonal coronaviruses (e.g.; NL63, OC43, 229E, and HKU1) provides an ideal assessment of assay specificity and allows for confidence in the lack of significant cross-reactivity observed. Further, using a larger dataset of samples collected from SARS-CoV-2 -PCR-positive individuals and 2019 pre-pandemic serum enabled us to further examine the manufacturers' claims of sensitivity and specificity of these assays for SARS-CoV-2 antibodies.

As seen in Fig. 1, both assays performed similarly with SARS-CoV-2 PCR-positive samples with over 93% positive result agreement. Interestingly, samples that were discrepant between the two assays tested either borderline- or low-positive in one of the assays and negative in the other. While the majority of moderately to strongly positive samples were positive for both N and S1 antigens, a small subset of samples was positive for either N or for S. These samples tend to be low positive or borderline. Of note, since borderline samples cannot be reliably categorized as positive or negative, we chose to drop borderline results from subsequent calculations for sensitivity and specificity of the assays in this study.

Our data show that the EDI assay has higher specificity (99%) at the manufacturer defined cutoff compared to the specificity of the Euroimmun assay (96.8%). However, EDI displayed a lower sensitivity, as it missed low-positive or borderline antibody responses that were detected in the Euroimmun assay. These findings suggest that the performance characteristics of a SARS-CoV-2 antibody assay may depend on specific antigen used, and may therefore influence the context in which the assay is used – for example, when used for community surveillance, an assay with lower sensitivity may lead to under-representation of seroconversion rates in population studies.

This serologic validation was limited by host related variability in antibody response to SARS-CoV-2 infection, with the timing of specimen collection likely influencing assay performance, and the limited number of non-SARS-CoV-2 coronavirus PCR positive samples. It is also likely that sensitivity of these assays may be different in a clinical setting with non-recovered patients during the acute phase of infection.

### 5. Conclusion

Based on our comparison findings, a combination of two or more antigenic targets may be required to achieve greater sensitivity for SARS-CoV-2 antibody detection. In our study, when positive and negative results from both assays were combined, sensitivity for a positive result in SARS-CoV-2 PCR-positive samples increased to 93.2%;
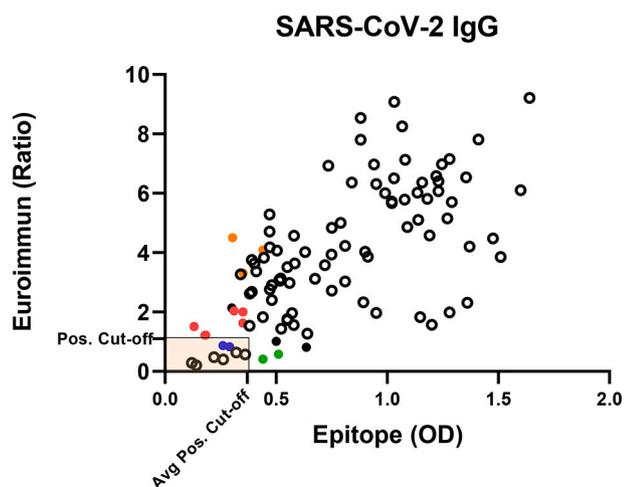


**Fig. 1.** Comparison of SARS-CoV-2 IgG in SARS-CoV-2 PCR-positive samples analyzed by the Epitope and Euroimmun ELISA. Red symbols: negative by Epitope and positive by Euroimmun; Green symbols: positive by Epitope and negative by Euroimmun; Orange symbols: borderline by Epitope and positive by Euroimmun; Black symbols: borderline by Euroimmun and positive by Epitope; Blue symbols: borderline by Euroimmun and negative by Epitope. Open circles indicate correlative results between the two assays. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
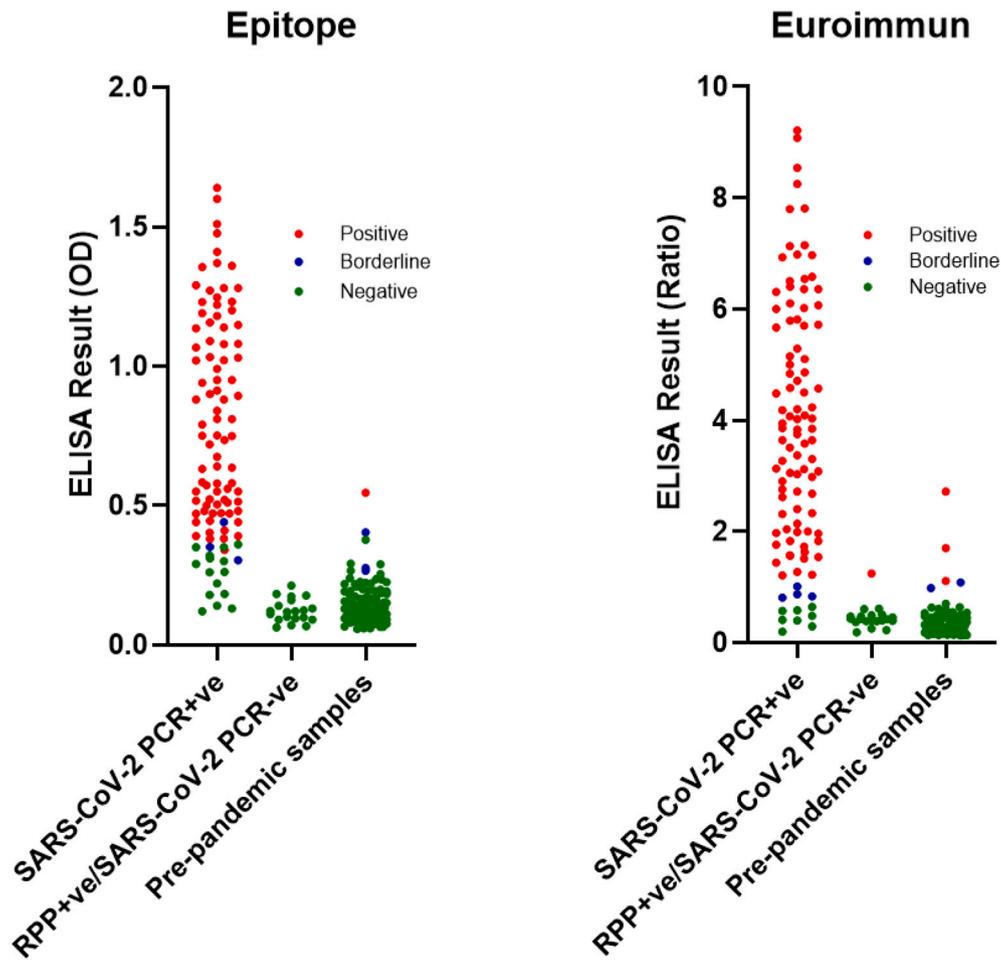
**Fig. 2.** Analysis of SARS-CoV-2 PCR-positive samples, pre-pandemic samples and SARS-CoV-2 PCR-negative/respiratory viral panel-positive samples (RPP) by the Epitope and Euroimmun ELISA.
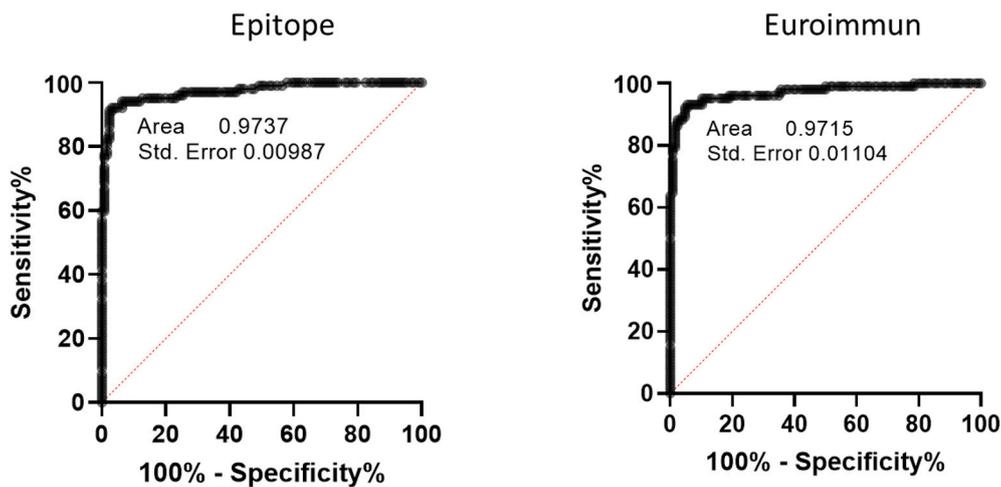


**Fig. 3.** Receiver Operating Characteristics curves for the Epitope (OD) and Euroimmun (Ratio) ELISA based on the manufacturer's recommended calculations for positive and negative cut-off values.

however, specificity dropped to 96.0%. Therefore, this approach must be balanced with maintaining high specificity, as false-positives may be erroneously interpreted as possible protective immunity, despite lack of evidence that such positive results are protective and may lead to potential neglect of personal safety measures against SARS-CoV-2 exposure. Additionally, a false-positive result could lead to a patient receiving treatment with convalescent plasma that is ineffective.

Regardless of adequate analytical performance among assays, the interpretation of serological findings remains a challenge with knowledge in this arena evolving as this pandemic continues. While samples from initial donations were used in this study, many of our CCP donor cohort have donated multiple times. This will allow for future information on duration of antibody presence in this population.

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## Declaration of Competing Interest

All authors declare that they have no conflicts of interest.

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