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SHORT COMMUNICATION

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Correlation between a quantitative anti-SARS-CoV-2 IgG ELISA and neutralization activity

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Abstract

In the current COVID-19 pandemic, a better understanding of the relationship between merely binding and functionally neutralizing antibodies is necessary to characterize protective antiviral immunity following infection or vaccination. This study analyzes the level of correlation between the novel quantitative EUROIMMUN Anti-SARS-CoV-2 QuantiVac ELISA (IgG) and a microneutralization assay. A panel of 123 plasma samples from a COVID-19 outbreak study population, preselected by semi-quantitative anti-SARS-CoV-2 IgG testing, was used to assess the relationship between the novel quantitative ELISA (IgG) and a microneutralization assay. Binding IgG targeting the S1 antigen was detected in 106 (86.2%) samples using the QuantiVac ELISA, while 89 (72.4%) samples showed neutralizing antibody activity. Spearman´s correlation analysis demonstrated a strong positive relationship between anti-S1 IgG levels and neutralizing antibody titers ($r_s = 0.819$, $p < 0.0001$). High and low anti-S1 IgG levels were associated with a positive predictive value of 72.0% for high-titer neutralizing antibodies and a negative predictive value of 90.8% for low-titer neutralizing antibodies, respectively. These results substantiate the implementation of the QuantiVac ELISA to assess protective immunity following infection or vaccination.

KEYWORDS

COVID-19, ELISA, microneutralization, SARS-CoV-2

1 | INTRODUCTION

In the current COVID-19 pandemic, the development of validated, standardized serological assays that quantitatively assess the antibody response against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is of crucial importance. These assays serve multiple purposes, including the quantification of antibodies against SARS-CoV-2, the collection of data for epidemiological surveillance and control, postvaccination monitoring, or the screening of recovered COVID-19 patients for convalescent plasma therapy.[1–3]

The formation of SARS-CoV-2-specific antibodies that effectively reduce virulence/pathogenicity is likely to be crucial for the development of population immunity, which in turn is a major prerequisite to halt the COVID-19 pandemic.[4,5] For the determination of neutralizing activity in

patient sera, neutralization assays based on live viral particles serve as the reference gold standard, assessing the presence of antibodies that inhibit infection of cultured cells (e.g., plaque-reduction neutralization test or microneutralization assay). These test systems, however, are time- and labor-intensive, restricted to biosafety level 3 laboratories, difficult to standardize, not automatable, and their implementation on a large scale is logistically impracticable.[3] These limitations might be overcome by using standardized commercially available serological tests that are essentially based on recombinant SARS-CoV-2 antigens, focusing on the highly immunogenic spike (S) protein, including the S1 domain and receptor-binding domain (RBD), or the nucleocapsid protein.[6,7] It is, however, still unknown what threshold titer of neutralizing antibodies confers protective immunity and whether results from commercial assays are capable of predicting such immunity. One prerequisite would be a strong correlation with neutralization activity, but pertinent data are limited and indicate that the accuracy in predicting levels of neutralizing antibodies can differ considerably between the assays.[8–12]

The present study investigated the level of correlation between a novel standardized enzyme-linked immunosorbent assay (ELISA) for the quantitative detection of anti-SARS-CoV-2 S1 IgG and a microneutralization assay.

2 | METHODS

2.1 | Plasma samples

Plasma samples originated from a cross-sectional seroepidemiological study conducted between March 31, 2020, and April 6, 2020, in the community of Gangelt (Heinsberg district, North Rhine-Westphalia, Germany), where a carnival celebration on February 15, 2020, led to SARS-CoV-2 super-spreading. The original dataset[13] was filtered for participants that had been categorized as seropositive based on IgG reactivity in the semiquantitative anti-SARS-CoV-2 ELISA IgG (EUROIMMUN Medizinische Labordiagnostika AG). A total of 123 plasma samples were available for further testing in the present study, including 106 anti-S1 IgG positive (ratio ≥ 1.1) and 17 borderline (ratio 0.8–1.1) samples according to semiquantitative pre-characterization. These samples were from individuals (45.5% male, mean age 49.6 years, range 3–87 years), of whom 29 (23.6%) were asymptomatic and 94 (76.4%) oligosymptomatic. For more details about the demographics of the study cohort, see Streeck et al.[13] EDTA plasma was stored at −80°C until analysis.

The study was approved by the Ethics Committee of the Medical Faculty of the University of Bonn (approval number 085/20) and has been registered at the German Clinical Trials Register (https://www.drks.de, identification number DRKS00021306, study arm 1).

2.2 | Enzyme-linked immunosorbent assay

The EUROIMMUN Anti-SARS-CoV-2 QuantiVac ELISA (IgG), hereafter referred to as QuantiVac ELISA, was processed on the EUROIMMUN Analyzer I platform according to the manufacturer's instructions. The ELISA is based on 96-well microplates coated with the SARS-CoV-2 S1 domain (including RBD) expressed recombinantly in the human cell line HEK293 (ATCC). The data sheet reports no cross-reactivities with samples from patients infected with SARS-CoV-1, HCoV-229E, or HCoV-OC43.[14] Quantification of S1-specific IgG was performed using a 6-point calibration curve covering a range from 1 to 120 relative units (RU)/ml. Samples yielding results above this analytical range were re-evaluated at a higher dilution. Positive and negative controls were included in each test run. By multiplication with factor 3.2, results in RU/ml were converted into standardized binding antibody units (BAU)/ml. Results <25.6 were considered negative, ≥25.6 to <35.2 borderline, and ≥35.2 positive.

2.3 | Virus microneutralization assay (CPE reduction NT assay)

Microneutralization assays were performed as previously described[13] using a SARS-CoV-2 strain (SARS-CoV-2/human/Germany/Heinsberg-01/2020, lineage B.3) that had been isolated from a throat swab of an infected patient at the University of Bonn, Germany in March 2020. In brief, plasma samples were heat-inactivated at 56°C for 30 min. Twofold serial plasma dilutions (starting from 1:2) were prepared in triplicate on a 96-well plate in Dulbecco´s modified Eagle medium (+3% fetal bovine serum; Gibco). The dilutions were incubated with an equal volume of 100 TCID50 SARS-CoV-2 solutions for 1 h at 37°C. A suspension containing 2×10^4 Vero E6 cells was added to each well and plates were incubated at 37°C (5% CO_2) for 2 days. The cytopathic effect (CPE) was evaluated via microscopy. Neutralizing antibody titers were calculated using the Spearman–Kaerber formula[15] and indicate the reciprocals of the highest plasma dilution protecting 50% of the wells. A neutralization titer of 2.8 corresponded to CPE suppression in all three replicates of the 1:2 dilution. Samples with a neutralization titer ≥2.8 were considered positive. Samples showing CPE suppression in one or two wells of the 1:2 dilution were assigned a neutralization titer of 1.7 or 2.2, respectively, indicating borderline results. Samples showing a CPE equal or similar to the negative control (titer < 1.7) were considered negative.

2.4 | Statistics

Confidence intervals (95% CI) were calculated according to the modified Wald method. Interassay concordance was assessed using the percentage of agreement and Kappa statistics, considering borderline results as positive. Cohen's kappa coefficient was calculated using the formula $\text{Kappa}(\kappa) = (P_0 - P_e)/(1 - P_e)$, where P_0 is the relative observed agreement, and P_e is the hypothetical probability of random agreement. Kappa values of 0–0.20, 0.21–0.40, 0.41–0.60, 0.61–0.80, and 0.81–1.00 indicate poor, fair, moderate, good, and very good agreement, respectively.[16] Spearman rank-order correlation analysis was performed to evaluate the relationship between two assays. The unpaired

TABLE 1 Agreement between quantitative ELISA and microneutralization assay in 123 plasma samples obtained from inhabitants of a German community after a local SARS-CoV-2 super-spreading event

		CPE reduction NT assay			Agreement of assays[a]	
		Positive *n* (%)	Borderline *n* (%)	Negative *n* (%)	Positive agreement (95% CI)	100% (95.0%–100%)
Anti-SARS-CoV-2 QuantiVac ELISA (IgG)	Positive	68 (72.4%)	18 (14.6%)	10 (8.1%)	Negative agreement (95% CI)	50.0% (34.1%–65.9%)
	Borderline	0 (0%)	3 (2.4%)	7 (5.7%)	Overall agreement (95% CI)	86.2% (78.9%–91.3%)
	Negative	0 (0%)	0 (0%)	17 (13.8%)	Kappa value (95% CI)	0.591% (0.427%–0.756%)

[a]Borderline results were considered positive.

Student's *t* test was used to identify significant differences between the means of the two groups. *p* values of less than 0.05 were considered statistically significant. Statistical analyses were conducted using GraphPad Prism version 6, GraphPad QuickCalcs (GraphPad Software, Inc.), and SigmaPlot 13.0 (SSI).

3 | RESULTS

In 123 plasma samples that had been precharacterized as SARS-CoV-2 seropositive by semiquantitative IgG testing, anti-SARS-CoV-2 S1 IgG levels were measured quantitatively using the QuantiVac ELISA. This resulted in values between <3.2 and 9881.6 BAU/ml with a mean ± standard deviation (SD) of 432.8 ± 1058.2 BAU/ml (median, 125.9 BAU/ml; interquartile range [IQR], 47.6–315.7 BAU/ml). Titers of neutralizing antibodies were determined by CPE reduction NT assay, ranging between <1.7 and 286 with a mean ± SD of 11.4 ± 33.6 (median, 3.5; IQR, 0–9.0).

Using the QuantiVac ELISA, 96 out of 123 samples were classified as positive, 10 as borderline, and 17 as negative. The CPE reduction NT assay yielded positive, borderline, and negative results in 68, 21, and 34 out of 123 cases, respectively (Table 1). Thus, if borderline results were considered positive, the sensitivity of the QuantiVac ELISA amounted to 86.2% (106/123) and that of the CPE reduction NT assay to 72.4% (89/123).

All 89 samples testing positive or borderline by CPE reduction NT assay were also anti-S1 IgG positive or borderline by QuantiVac ELISA, corresponding to a positive agreement rate of 100% (89/89). Among 34 neutralization-negative samples, 17 were negative for anti-S1 IgG by QuantiVac ELISA, resulting in a negative agreement rate of 50%. Accordingly, the overall agreement between the two assays amounted to 86.2% based on the correlation of positive and negative results in 106/123 cases. To correct the agreement for the probability of random coincidence, the Kappa coefficient was calculated (κ = 0.591) and indicated moderate agreement (Table 1).

Spearman rank-order correlation analysis revealed a strong positive, statistically significant association between the quantitative levels of anti-SARS-CoV-2 S1 IgG and the titers of neutralizing antibodies (r_s = 0.819, $p < 0.0001$; Figure 1).

To substantiate the calculated correlation, samples were assigned to two groups: high anti-SARS-CoV-2 S1 IgG (≥480 BAU/ml, *n* = 25) and low anti-SARS-CoV-2 S1 IgG (<480 BAU/ml, *n* = 98).

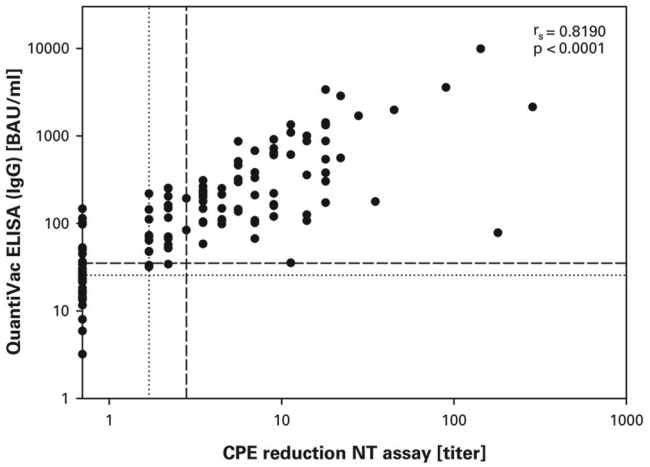

FIGURE 1 Correlation between quantitative ELISA and microneutralization assay. Binding anti-SARS-CoV-2 S1 IgG was determined quantitatively using the QuantiVac ELISA and titers of neutralizing antibodies were determined using the CPE reduction NT assay (*n* = 123). Neutralization titers correspond to reciprocal plasma dilutions protecting 50% of the wells at incubation with 100 $TCID_{50}$ of SARS-CoV-2. Samples with a cytopathic effect (CPE) equal or similar to the negative control are depicted on the *y*-axis. Dotted and dashed lines indicate borderline and positivity cut-offs, respectively. r_s, Spearman rank-order correlation coefficient

Comparison of the two groups revealed a significant difference between the mean neutralization titers (34.4 vs. 5.5, $p < 0.0001$). Median titers were 18.0 (IQR, 9.0–22.0) and 2.2 (IQR, 0–4.5) in the high and low groups, respectively. The positive predictive value (PPV) of high anti-S1 IgG for the presence of high-titer (≥10) neutralizing antibodies was 72.0% (52.2%–85.9%), whereas low anti-S1 IgG levels were associated with a negative predictive value (NPV) of 90.8% (83.3%–95.3% for low-titer (<10) neutralizing antibodies.

4 | DISCUSSION

Conventional neutralization assays are unsuitable for large-scale routine testing in the current COVID-19 pandemic. This has raised the question as to whether there are standardized and scalable serological assays that show a degree of correlation allowing estimates on neutralizing activity to assess immunity to reinfection and to support vaccination programs or antibody-based therapeutic trials.

To the best of our knowledge, this is the second study presenting the level of correlation between quantitative anti-S1 IgG levels determined by the EUROIMMUN QuantiVac ELISA and titers of neutralizing antibodies measured by a microneutralization assay. The positive, negative, and overall qualitative agreement between both assays were 100%, 50%, and 86.2%, respectively, while the kappa value indicated moderate agreement (κ = 0.591). In addition, the quantitative results revealed a strong correlation (r_s = 0.819), which was confirmed by high PPV (72.0%) and NPV (90.8%) results.

Rubio-Acero et al.[17] were first to report the performance of two quantitative anti-S1 assays, namely the QuantiVac ELISA (EUROIMMUN) and the Elecsys Anti-SARS-CoV-2 S (Roche) using 362 samples. Among others, their results showed a high, statistically significant correlation of the QuantiVac ELISA with the primary semiquantitative EUROIMMUN Anti-SARS-CoV-2 ELISA IgG (r = 0.55), quantitative Elecsys assay (r = 0.50), cPass surrogate neutralization test (GenScript; r = 0.41), and *recom*Line SARS-CoV-2 IgG MG-RBD (Mikrogen; r = 0.46). The QuantiVac ELISA results increased significantly between the dilution categories of a microneutralization assay, with ELISA values ≥49.8 U/ml predicting neutralization titers >1:5 in 95% of cases. Similar performance was shown for the quantitative Elecsys assay, although this one provided a clearer positive-negative separation and 3% less nonspecific reactivity. The results of Rubio-Acero et al.[17] correspond to and go beyond the correlation data of the present study.

The primary semiquantitative EUROIMMUN Anti-SARS-CoV-2 ELISA IgG was also analyzed by other groups, indicating a high correlation with neutralization activity (r_s, 0.75-0.88).[9,11,18] In this semiquantitative ELISA, the same S1 antigen is coated on the microplate wells as in the QuantiVac ELISA, giving a possible explanation for the similarly high degree of correlation observed in the present study.

The strong correlation of the QuantiVac ELISA with neutralization testing suggests a high potential to quantitatively predict neutralizing antibody titers. However, the QuantiVac ELISA results are not a 100% correlate of the CPE reduction NT assay, and it has to be considered that a part of neutralization potency seems to be mediated by S-specific IgM antibodies.[19–21] Therefore, the implementation of the QuantiVac ELISA should be evaluated for the different contexts of use where thresholds in anti-S1 IgG levels are accepted to predict neutralizing activity. In addition, it adds great value as a pre-screening tool for neutralization assessment, as it would strongly reduce the number of samples needing to undergo labor-intensive cell-based neutralization assays.

According to validation data on the clinical performance, the diagnostic sensitivity of the QuantiVac ELISA ranges between 90.3% (>10 days post symptom onset [dpso], n = 165) and 93.2% (>20 dpso, n = 46), at a specificity of 99.8% based on measurements in 1458 samples from healthy and disease controls.[14]

The QuantiVac ELISA is one of the first SARS-CoV-2 serological assays that allow reporting of quantitative results in standardized, WHO-approved binding antibody units (BAU/ml) which numerically correspond to international units (IU)/ml. Optionally, the QuantiVac ELISA can be processed on fully automated equipment, enabling high throughput in the diagnostic workup.

This study has limitations, however. First, the study panel was limited in the number of samples, necessitating the confirmation of results in further studies. Second, as the assays were not performed simultaneously, differences in storage time and freeze-thaw cycles may have affected the measurement of antibody levels.

In summary, the QuantiVac ELISA provides quantitative levels of anti-S1 IgG, allowing confirmation and monitoring of recent and past SARS-CoV-2 infections. Strong correlation with neutralization testing substantiates its implementation in clinical diagnostics and vaccination monitoring.

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CONFLICT OF INTERESTS

Sandra Saschenbrecker and Katja Steinhagen are employed by EUROIMMUN Medizinische Labordiagnostika AG, a manufacturer of diagnostic reagents and co-owner of a patent application pertaining to the detection of antibodies to the SARS-CoV-2 S1 antigen. Katja Steinhagen is designated as an inventor. The other authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Gunther Hartmann and Katja Steinhagen contributed to the conception and design of the study. Hendrik Streeck, Ramona Dolscheid-Pommerich, Marcel Renn, Beate M. Kümmerer, Bianca Schulte, Eva Bartok, Gunther Hartmann, and Katja Steinhagen were involved in sample collection and performed acquisition and analysis of data. The manuscript was written by S. Saschenbrecker, Gunther Hartmann, and Katja Steinhagen. All authors had access to the data, revised the manuscript, and approved the final version.

DATA AVAILABILITY STATEMENT

Data are available on request from the authors.

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