

Independent side-by-side validation and comparison of four serological platforms for SARS-CoV-2 antibody testing

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Summary

Four broadly used serological testing platforms for the detection of antibodies against SARS-CoV-2 were evaluated regarding their suitability for the screening of health care staff and potential convalescent plasma donors or other persons with mild to moderate courses of COVID-19.

Abstract

Highly sensitive and specific platforms for the detection of anti-SARS-CoV-2 antibodies are becoming increasingly important for (1) evaluating potential SARS-CoV-2 convalescent plasma donors, (2) studying the spread of SARS-CoV-2 infections and (3) identifying individuals with seroconversion. This study provides a comparative validation of four anti-SARS-CoV-2 platforms. Unique feature of this study is the use of a representative cohort of COVID-19-convalescent patients with mild-to-moderate disease course. All platforms showed significant correlations with a SARS-CoV-2 plaque-reduction-neutralization test, with highest sensitivities for the Euroimmun and the Roche platforms, suggesting their preferential use for screening of persons at increased risk of SARS-CoV-2 infections.

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Introduction

In the context of the current pandemic with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the development and provisioning of serological test systems for the determination and observation of seroprevalence and seropositivity is crucial for both public health aspects as well as for specific protection of health care professionals [1]. Due to the initial lack of sufficient numbers of pharyngeal swab NAT tests, the number of confirmed SARS-CoV-2 infections in Germany and other countries has long been considered to be largely underestimated [2]. Nevertheless, a recent seroprevalence survey from Switzerland demonstrated that most of the population of Geneva remained uninfected until the end of the first SARS-CoV-2 wave [3].

Interpretation of serological test results is complicated by the fact that the majority of the initial reports on COVID-19 naturally included hospitalized patients with more severe disease courses, associated with different seroconversion behavior and antibody titers than patients with asymptomatic to moderate COVID-19 courses [4, 5]. Caution must therefore be applied concerning the validation of commercial test platforms, since the companies' availability of representative validation cohorts, particularly of positive control groups may have been limited or biased at the time tests were developed. In April 2020 we started screening patients who recovered from mild to moderate COVID-19 to evaluate their suitability as convalescent plasma donors within a prospective randomized clinical trial (CAPSID, 2020-001310-38, EudraCT No: 2020-001310-38, ClinicalTrials.gov-Identifier: NCT04433910). The cohort of these donors was found to be ideal to represent the general population including health care workers with regard to SARS-CoV-2 infection.

The present study is the first providing a side-by-side validation of four commercially available serological platforms (Euroimmun, Snibe/Medac, Roche and Abbott) for the detection of anti-SARS-CoV-2 antibodies by use of the above-mentioned COVID-19-convalescent cohort. Based on this cohort, we calculated assay performance indicators including sensitivity, specificity, positive and negative predictive values, and the concordances between the platforms. Moreover, our study correlates all serological results with a wild-type SARS-CoV-2 neutralisation assay, allowing predications on the predictive value of serology for a potential therapeutic efficacy of immune plasma from COVID-19-convalescent donors.

Methods

For Materials and Methods see online supplement (Supplementary Materials and Methods).

Results

Characteristics of validation groups.

Serum samples for the positive validation group (COVID-19⁺) were collected from individuals who presented to our institute for assessment as potential convalescent plasma donors for a planned randomized prospective trial of convalescent plasma for treatment of patients with severe COVID-19 (CAPSID, EudraCT 2020-001310-38 and ClinicalTrials.gov-Identifier: NCT04433910). Donors had a history of SARS-CoV-2 infection confirmed by a positive pharyngeal swab SARS-CoV-2-PCR. They were characterized by mild to moderate symptoms including loss of taste and/or olfaction, limb pain/headache, fever up to 40.0 °C, dry cough and fatigue. With one exception, more severe symptoms, which would have required oxygen supplementation or hospitalization, were absent in this group. Gender distribution and frequency of symptoms for the cohort of 119 convalescent plasma donors are summarized in **Table 1A**. The average age was 41 years (20 – 61 years), median duration between symptom onset and serology was 48 days (18 – 170 days), median duration of the symptomatic period was 12 days (0 – 154 days), median duration from documented positive pharyngeal swab SARS-CoV-2-NAT to serology was 47 days (10 – 165 days) and median duration from symptom convalescence to serology was 35 days (0 – 143 days).

The control cohort included 110 healthy individuals from the pool of health care workers at our institute and their dependents. Median age was 52 years (14 to 82 years), 27.3% were males, 72.7% were females. Selection criteria were either an absent history of COVID-19-typical symptoms and the absence of risk contacts, or a negative pharyngeal swab SARS-CoV-2 NAT in the suspected presence of either risk contacts or symptoms.

Validation and assay performance indicators of serologic platforms.

A total of 229 serum validation samples (119 COVID-19⁺ and 110 COVID-19⁻ samples) were independently tested on the Euroimmun, the Roche and the Abbott platforms. For the Snibe/Medac platform only 58 COVID-19⁺ and 72 COVID-19⁻ samples could be tested. The seven analytes tested are detailed in the Supplementary Materials and Methods Section. As expected, quantitative analysis confirmed that the serological results for all analytes were significantly higher in the positive validation group as compared to the negative validation group (not shown).

More importantly, qualitative analysis of the serological results allowed us to calculate assay performance indicators including sensitivity, specificity, positive and negative predictive values (**Table 1B & Supplementary Table 1**) as well as false-positive and false-negative result rates (**Supplementary Table 2**). The highest analytical sensitivity with 97.5% was reached when the three analytes from the Euroimmun ELISA platform were combined. Sensitivities of the single analytes from the Euroimmun platform were close to the values provided by the manufacturer (**Supplementary Table 1**). Sensitivity of the Roche platform was 95.0%, sensitivity of the Abbott platform 81.5%. To our surprise, the Snibe/Medac system failed to provide a sufficient sensitivity (60.3%) to reliably detect COVID-19⁺ individuals from our validation cohort.

Analytical specificity on the other hand was highest with the Roche and the Abbott platforms (100% each), followed by a specificity of 97.2% with the Snibe/Medac and 90.9% with the Euroimmun platforms. Of note, combining the anti-nucleocapsid IgG analyte from Roche with the anti-spike IgG analyte from Euroimmun enabled us to increase the combined test sensitivity to 96.6%, while keeping specificity at 100% (**Table 1B**).

Correlation and concordance between serologic platforms.

When correlating the analytic results from the different platforms, strong correlations were found between the Euroimmun analytes anti-spike IgG, anti-spike IgA and anti-nucleocapsid IgG among each other (not shown), as well as with the analytes from the other three manufacturers (**Figure 1A, B**). Of note, the analytic results from the Roche platform for the COVID-19⁻ validation group form a very flat scatter-plot, which reflects our finding that more than 99% of the COI results from this group range between 0 and 0.2. This again illustrates the high specificity of the Roche platform.

When considering individual concordances between the serologic platforms, Euroimmun and Roche stood out from the other platforms with an overall concordance rate of > 96% (**Supplementary Table 3A**). Several samples from the COVID-19⁺ validation group were identified as positive by the Euroimmun, but not by the other platforms including the Roche and the Abbott platforms, although the COI values for some of these sample were very close to the cutoff values (**Figure 1A, B**, red dots in the left lower quadrants). On the other hand, multiple samples from the COVID-19⁺ group were correctly identified by the Roche and the Abbott platforms (**Supplementary Table 3A & B, upper panels**), but were missed by the Euroimmun platform, when anti-spike IgG was included only. In contrast, when all three Euroimmun analytes were included, all samples were correctly identified (**Supplementary Table 3A & B, lower panels**), however at the cost of a lower specificity, which was reflected by six false positive Euroimmun results. A very high overall concordance rate was also found between the Roche and the Abbott platforms, which was expected since both platforms detect anti-nucleocapsid IgG (**Supplementary Table 3C**). Rather low overall concordance rates were found between the Snibe/Medac platform and the other three platforms (**Supplementary Tables 3D, E, F**), which is in line with the particularly low sensitivity of the Snibe/Medac system (**Table 2 & Supplementary Table 2**).

Correlation of serologic platforms with a wild-type SARS-CoV-2 neutralization assay.

Particularly for therapeutic use of plasma from COVID-19-reconvalescent donors it is paramount to know in advance the potential of the harvested plasma products to inhibit SARS-CoV-2. Since viral plaque inhibition assays are laborious and time-consuming, serological platforms may allow a more rapid prediction of the potential neutralization capacity of a plasma product. We therefore correlated the results from all four serological platforms with the results from a wild-type SARS-CoV-2 neutralization assay [6, 7]. The strongest correlations were found for the three analytes from the Euroimmun platform (**Figure 1C**). In contrast, the weakest correlations were found with the analytes from Roche and Snibe/Medac, whereas the Abbott analyte showed an intermediate correlation (**Figure 1D**).

Discussion

Meanwhile several reports on SARS-CoV-2 antibody testing platforms have been published [8-10]. The major results of these reports are confirmed by our present study. More importantly however, our study represents the first and most comprehensive side-by-side comparison of four independent and commercially available serological platforms in this regard. The need of serological SARS-CoV-2 antibody test methods for screening of the general population and for the establishment of so-called “immunity passports” is currently matter of debate in many countries, unfortunately very often strongly influenced by political and economical considerations. Independently of this discussion, health care professionals, who are naturally at a higher risk of being infected with SARS-CoV-2, will definitely benefit from serological tests with utmost sensitivity and specificity. In addition, the recruitment of donors for convalescent plasma requires screening tests to identify those candidates who mounted a strong humoral immune response.

Our comparative analysis demonstrated that the serological tests currently available on the market show striking differences regarding sensitivity, specificity, as well as positive and negative predictive values. Our data suggest that particularly the platforms from Euroimmun and from Roche provide excellent sensitivities, allowing the screening of health care professionals with frequent contact to COVID-19 patients. Besides, the Euroimmun platform appears to be particularly suitable to test potential convalescent plasma donors, since its analytes showed the strongest correlations with a wild-type SARS-CoV-2 neutralization assay. On the other hand, specificity was highest with the Roche and the Abbott platforms, suggesting both tests may be used for testing broader populations with a comparably low risk of having recently been infected with SARS-CoV-2. Of note, the widely used Snibe/Medac platform failed to detect a significant portion of COVID-19 patients with mild to moderate disease courses, so that our data rather discouraged its use for the above-mentioned indications in its current form. Importantly, we were recently informed by Snibe/Medac about efforts to improve their assays. A limited number of our validation samples was tested with novel kits from the company and indeed produced results that appeared to be much closer to the results from the other platforms. Nevertheless, due to limited time and material resources, the analysis of a higher number of samples with the optimized assays could not be performed.

In summary, we present here an independent and comprehensive validation and comparative analysis of the serological anti-SARS-CoV-2 platforms from Euroimmun, Roche, Abbott and Snibe/Medac. The unique feature of our study is the use of a representative cohort of 119 COVID-19-convalescent patients with a mild to moderate disease course, instead of hospitalized COVID-19 patients. Based on this cohort, the highest sensitivities were found for the Euroimmun and the Roche platforms, whereas the highest specificity was obtained with the Roche and the Abbott platforms. The Snibe/Medac platform had an extraordinarily low sensitivity in our COVID-19⁺ cohort, so that we excluded it from further use for the broad screening of health care staff and for our convalescent plasma donor program. Of note, a combination of the Euroimmun and the Roche platforms resulted in a combined test sensitivity of 96.6%, while keeping specificity at 100%. Based on these results, antibody screening of persons at increased risk of SARS-CoV-2 infections (e.g. health care professionals) can be performed with the Roche platform for initial testing and, in cases with increased risk or borderline COI values in the Roche test, the additional use of the Euroimmun platform. Moreover, the Euroimmun platform can be used for the screening of potential convalescent plasma donors (e.g. anti-spike IgG, cutoff 1.1). In case of a reactive result, this screening can be followed by the PRNT assay as described. If PRNT50 OR PRNT90 titers are > 1:20, the donor may be accepted for convalescent plasma donor programs and may be invited for plasma donation. In case of non-reactivity in the initial screening with the Euroimmun assay, or in case of PRNT50 AND PRNT90 titers < 1:40 the donor appears rather unlikely to be suitable as convalescent plasma donor.

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Author contributions

B.J. screened patients for the COVID-19⁺ cohort, supervised the analytics and generated hypotheses; J.K., C.L. and T.S. performed analytics; B.J., J.K. and C.L. conducted data analyses and prepared figures, V.M.C., S.K., M.R., R.L., C.W., C.D., E.S., T.ST., H.J.G and H.S. provided key research tools, S.K., E.S. and H.S. developed the CAPSID protocol, and B.J. and H.S. wrote the manuscript.

Conflict of interest disclosure

Dr. Victor Max Corman is named together with Euroimmun on a patent application filed recently regarding the diagnostic of SARS-CoV-2 by IgA testing. All other authors declare no commercial affiliations and no competing financial conflicts of interest.

Abbreviations

ABEI	N-(4-Aminobutyl)-N-ethylisoluminol
AU	Arbitrary Units
CLIA	Chemiluminescence immunoassay
CMIA	Chemiluminescent microparticle immunoassay
COI	Cutoff Index
COVID-19	Corona Virus Disease 2019
ECLIA	Electrochemiluminescence immunoassay
ELISA	Enzyme-linked immunosorbent assay
IgA/G/M	Immunoglobulin A/G/M
NAT	Nucleic Acid Testing
NCP	Nucleocapsid Protein
OD	Optical density
PRNT50	≥ 50% Plaque Reduction Neutralization Test/Titer
PRNT90	≥ 90% Plaque Reduction Neutralization Test/Titer
RLU	Relative light units
S	Spike protein
SARS-CoV-2	Severe Acute Respiratory Syndrome Corona Virus 2
S/C	Calculated Index of sample result and calibrator result
SEM	Standard Error of Means

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Figure and table legends

Figure 1. Correlation between serologic anti-SARS-CoV-2 analytes and wild-type SARS-CoV-2 neutralisation titers.

Serum samples from up to 119 (58 for the Snibe/Medac platform) convalescent plasma donors after mild to moderate COVID-19 disease as documented by positive pharyngeal swab NAT testing (red circles) were collected and tested using the serological platforms from Euroimmun, Snibe/Medac, Roche, Abbott as well as a wild-type SARS-CoV-2 neutralisation assay. The control group (black circles) consisted of serum samples from 110 (72 for the Snibe/Medac platform) healthy subjects with either no COVID-19 symptoms and no risk contacts, or with a negative pharyngeal swab NAT testing. **(A, B)** The results from the Euroimmun analytes (anti-spike IgG, anti-spike IgA and anti-nucleocapsid IgG) were correlated with **(A)** the Roche and **(B)** the Abbott analytes (both anti-nucleocapsid IgG). Analytic results are given in OD ratios for the Euroimmune analytes with a cutoff value of 0.8, a cutoff index (COI) for the Roche analyte with a cutoff value of 1.0 and a cutoff index (COI) for the Abbott analyte with a cutoff value of 1.4. Cutoff values are indicated by horizontal and vertical dotted lines. Note that the red dots in the left lower quadrants represent false-negative results, and the black dots in all other quadrants represent false-positive results. Also note that > 99% of the Roche COI results from the negative control group samples range between 0 and 0.2, illustrating the high specificity of the Roche compared to the other platforms (arrows in **(A)**).

(C, D) The results from the wild-type neutralisation assay were correlated with **(C)** the three analytes from the Euroimmun platform as well as **(B)** the analytes from the Snibe (anti-spike IgG and IgM), the Roche and the Abbott platforms. Serologic results on the y-axes are given in OD ratios for the Euroimmune analytes, in AU/ml for the Snibe analytes, and as cutoff indices (COI) for the Roche and the Abbott analytes. Virus neutralisation efficiency (x-axes) is shown as the titer required for 50% plaque inhibition (PRNT50). Error bars indicate SEM, p values <0.0001 indicate highly significant Pearson's correlations.

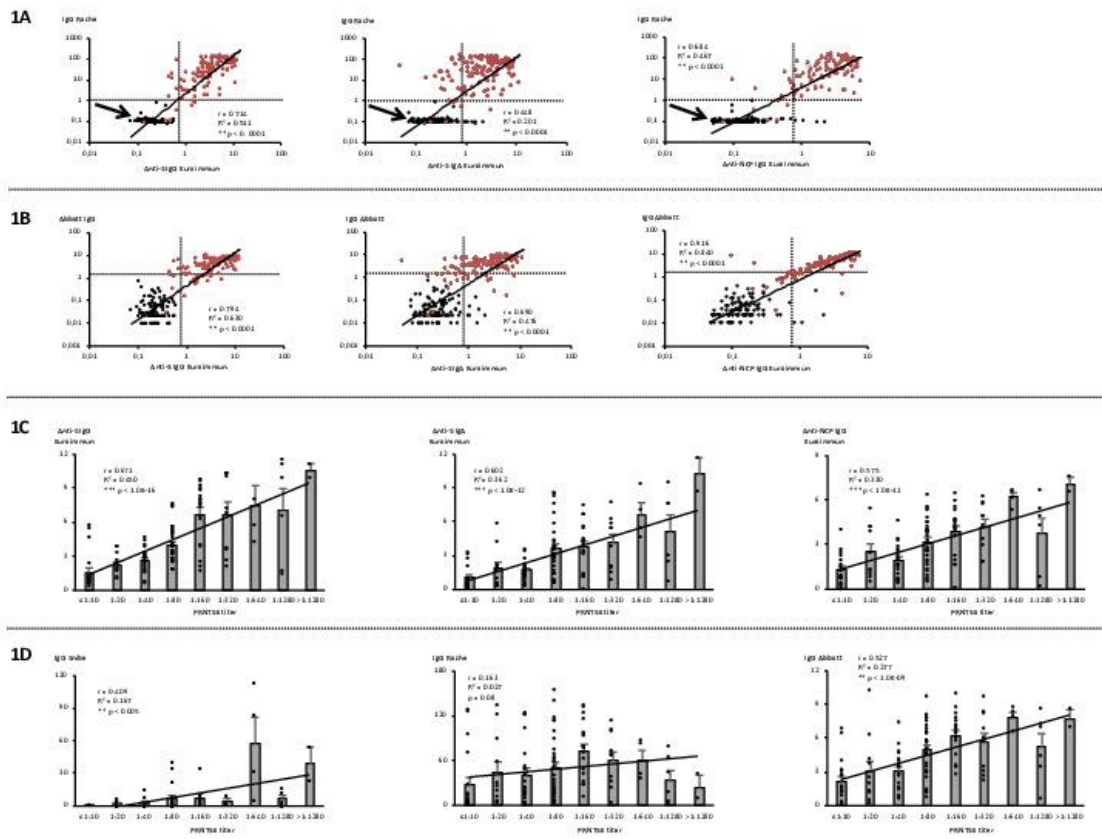
Table 1A. Symptoms of convalescent plasma donors.

Information on symptoms was available from 104 plasma donors after convalescence from mild to moderate COVID-19 disease as documented by positive pharyngeal swab NAT testing and is summarized in the present table.

Table 1B. Calculation of assay performance indicators for four different serological anti-SARS-CoV-2 antibody platforms.

Serum samples from 119 (58 for the Snibe/Medac platform) plasma donors after convalescence from mild to moderate COVID-19 disease as documented by positive pharyngeal swab NAT testing (COVID-19⁺) and 110 (72 for the Snibe/Medac platform) healthy subjects with either no history of COVID-19-typical symptoms and no risk contacts, or negative pharyngeal swab SARS-CoV-2-NAT in the suspected presence of risk contacts or symptoms (COVID-19⁻), were collected and tested for anti-SARS-CoV-2 antibodies using various serological platforms including the Euroimmun ELISA platform (detecting anti-spike IgG + anti-spike IgA + anti-nucleocapsid IgG), the Roche ECLIA platform (detecting anti-nucleocapsid IgG + further unspecified antibodies), the Snibe/Medac CLIA platform (detecting anti-spike IgG + IgM) and the Abbot CMIA platform (detecting anti-nucleocapsid IgG). Analytic results were obtained as OD ratios for the Euroimmune analytes, in AU/ml for the Snibe analytes, and as cutoff indices (COI) for both the Roche and the Abbott analytes. Note that the combination of the analytical results from the Roche (anti-nucleocapsid IgG) and the Euroimmun (anti-spike IgG) platforms resulted in improved overall assay performance indicators based on our validation cohorts (blue).

Figure 1



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Table 1A

Symptoms	Fever	Chills	Limb pain / headache	Loss of taste / olfaction	Congested nose	Sore throat	Dry cough	Shortness of breath	Loss of appetite	Diarrhea	Fatigue	No symptoms	Hospitalization / Oxygen support
Total (n=104)	52	11	65	69	24	25	52	11	10	13	43	5	1
Frequency (%)	48.1%	10.2%	60.2%	63.9%	22.2%	23.1%	48.2%	10.2%	9.3%	12.0%	39.8%	4.6%	0.9%
Male (n=54)	31	6	35	34	10	7	27	4	5	5	19	2	0
Frequency (%)	56.4%	10.9%	63.6%	61.8%	18.2%	12.7%	49.3%	7.3%	9.1%	9.1%	34.5%	3.6%	0.0%
Female (n=50)	21	5	30	35	14	18	25	7	5	8	24	3	1
Frequency (%)	39.6%	9.4%	56.6%	66.0%	26.4%	34.0%	47.2%	13.2%	9.4%	15.2%	45.3%	5.7%	19%

Table 1B

Platforms Validation groups	Euroimmun (S-IgG + S-IgA + NCP-IgG)	COVID-19 ⁺	COVID-19 ⁻	Roche	COVID-19 ⁺	COVID-19 ⁻	Sinbe/Modac (IgG + IgM)	COVID-19 ⁺	COVID-19 ⁻	Abbott	COVID-19 ⁺	COVID-19 ⁻	Roche & Euroimmun (S-IgG only)	COVID-19 ⁺	COVID-19 ⁻
Cutoff values		0.8	0.8		1.0	1.0		1.0	1.0		1.4	1.4		1.0	1.0
Positive results (true false)		116	10		113	0		35	2		97	0		115	0
Negative results (false true)		3	100		6	110		23	70		22	110		4	110
Sensitivity (%)		97.5			95.0			60.3			81.5			96.6	
Specificity (%)		90.9			100.0			97.2			100.0			100.0	
Positive predictive value (%)		92.1			100.0			94.6			100.0			100.0	
Negative predictive value (%)		97.1			94.8			75.3			83.3			96.5	