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Clinical performance of different SARS-CoV-2 IgG antibody tests

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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) serological assays are
urgently needed for rapid diagnosis, contact tracing, and for epidemiological studies. So
far, there is limited data on how commercially available tests perform with real patient
samples, and if positive tested samples show neutralizing abilities. Focusing on IgG
antibodies, we demonstrate the performance of two enzyme-linked immunosorbent
assay (ELISA) assays (Euroimmun SARS-CoV-2 IgG and Vircell COVID-19 ELISA IgG) in
comparison to one lateral flow assay (FaStep COVID-19 IgG/IgM Rapid Test Device)
and two in-house developed assays (immunofluorescence assay [IFA] and plaque
reduction neutralization test [PRNT]). We tested follow up serum/plasma samples
of individuals polymerase chain reaction-diagnosed with COVID-19. Most of the
SARS-CoV-2 samples were from individuals with moderate to the severe clinical
course, who required an in-patient hospital stay. For all examined assays, the sensi-
tivity ranged from 58.8 to 76.5% for the early phase of infection (days 5-9) and from
93.8% to 100% for the later period (days 10-18).

KEYWORDS
antibody tests, ELISA, IFA, IgG, PRNT, SARS-CoV-2

1 | INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a
new coronavirus, belonging to the group of β-coronaviruses, which
emerged in December 2019 in Wuhan, China. It is the causative agent
of an acute respiratory disease known as coronavirus disease 2019
(COVID-19). The spectrum of clinical signs can be very broad and
asymptomatic infections are reported. The virus has rapidly spread
globally. On 11 March 2020 the World Health Organization declared
COVID-19 as a pandemic. Nucleic acid amplification testing is the
method of choice in the early phase of infection.[1] However, to acquire
knowledge about the seroprevalence of SARS-CoV-2 and to test for
(potential) individual immunity, there is an increasing demand in the

detection of antibodies—especially of IgG antibodies. Convalescent
plasma may be used for therapeutic or prophylactic approaches as
vaccines and other drugs are under development.[2] For all these pur-
poses, sensitive and especially highly specific antibody assays are
needed. The spike (S) protein of SARS-CoV-2 has shown to be highly
immunogenic and is the main target for neutralizing antibodies.[3] Cur-
rently, there are many S protein-based commercially or in-house de-
veloped assays available, but there is limited data on how these tests
perform with clinical samples, and if the detected IgG antibodies pro-
vide protective immunity. This study aims to provide a quick overview
on some of these assays (two commercially available enzyme-linked
immunosorbent assay [ELISA] assays, one lateral flow assay and two in-
house developed assays [immunofluorescence assay, IFA and plaque

reduction neutralization test, PRNT]), focusing on the detection and neutralization capacity of IgG antibodies in follow up serum or plasma samples of individuals with polymerase chain reaction (PCR)-diagnosed infections with SARS-CoV-2. To assess potential cross-reactivity, we examined defined follow-up samples of individuals infected with endemic coronaviruses and other infectious diseases.

2 | MATERIALS AND METHODS

2.1 | Serum and plasma samples

We collected follow up serum or plasma samples (in the following simply stated as samples) from individuals with PCR-diagnosed infections with SARS-CoV-2 (n = 33) at different time points (Table 1). Most of these individuals had a moderate to severe clinical course and required an in-patient hospital stay in the intensive care unit. Additionally, follow up samples of recent PCR-diagnosed infections with SARS-CoV (three patients from the 2003 outbreak), HCoV-OC43 (n = 4), HCoV-HKU1 (n = 1), HCoV-NL63 (n = 2), HCoV-229E (n = 4) and recent serological/PCR-diagnosed infections with acute Epstein Barr virus (EBV) (n = 4, three serologically EBV-VCA-IgM positive and one PCR- and serologically EBV-VCA-IgM positive) and acute cytomegalovirus (CMV) (n = 3) (all serologically IgM and PCR-positive) were collected. The samples of individuals infected with endemic human coronavirus, CMV, and EBV were used to assess potential cross-reactivity and the risk of potential false-positive results.

2.2 | Lateral flow assay

The FaStep (COVID-19 IgG/IgM) rapid test cassettes (COV-W32M, Assure Tech [Hangzhou] Co., Ltd, China) were used according to the

manufacturer's recommendation. We have no details on the used antigen component. About 10 μL serum and two drops of sample buffer were applied to the sample well. Test results were visually evaluated after 10 minutes.

2.3 | Enzyme-linked immunosorbent assay

The CE certified versions of the Euroimmun SARS-CoV-2 IgG ELISA (Euroimmun, Lübeck, Germany) and Vircell COVID-19 ELISA IgG (Vircell Spain S.L.U., Granada, Spain) were used, in an identical manner, according to the manufacturer's recommendation. Both ELISA assays use SARS-CoV-2 recombinant antigen from spike glycoprotein (S protein) and the Vircell ELISA additionally Nucleocapsid (N protein). Samples were diluted 1:101 or 1:20, respectively, in sample buffer and incubated at 37°C for 60 minutes in a 96-well microtiter plate followed by each protocol washing and incubation cycles, including controls and required reagents. Optical density was measured for both assays at 450 nm using a Virclia microplate reader (Vircell Spain S.L.U., Granada, Spain). The titers were calculated and results interpreted according to each manufacturer's protocol.

2.4 | Immunofluorescence assay

For an immunofluorescence assay Vero cells (African green monkey, ATCC CCL-81; American Type Culture Collection, Manassas, VA) were infected with SARS-CoV-2 and harvested 2 days postinfection. Briefly, cells were trypsinized and washed once with PBS before transferred onto a 10-well diagnostic microscope slide. After drying, cells were fixated with 100% ethanol for 10 minutes. Patient samples were diluted in sample buffer (Euroimmun AG, Lübeck, Germany) in a dilution of 1:50 and 30 μL applied per well. The slides were incubated

TABLE 1 Sensitivity and specificity of the examined SARS-CoV-2 IgG assays from days 5-9 and days 10-18

| Company | Days after confirmed SARS-CoV-2 PCR | | | |
| | 5-9 (d) | 10-18 (d) | | |
	Sensitivity (%)		Specificity (%)	Specificity (%) incl. SARS-CoV (2003)[a]
Euroimmun (ELISA)	58.8 (10/17)	93.8 (15/16)	95.7 (22[b]/23)	96.2 (25/26)
Vircell (ELISA)	70.6 (12/17)	100 (16/16)	95.2 (20/21)	83.3 (20/24)
IFA (in-house)	76.5 (13/17)	100 (16/16)	100 (19/19)[c]	86.4 (19/22)
Assure Tech (Rapid test)	62.5 (10/16)	93.8 (15/16)	100 (13/13)	...
PRNT (in-house)	76.5 (13/17)	100 (16/16)	...	...

Note: Details on tested samples see Tables S1 and S2.
Abbreviations: ELISA, enzyme-linked immunosorbent assay; IFA, immunofluorescence assay; PCR, polymerase chain reaction; PRNT, plaque reduction neutralization test; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
[a]Including follow up samples of SARS-CoV (2003 outbreak), which is closely related to SARS-CoV-2.
[b]One "borderline" result.
[c]One unspecific result was excluded-, not examined.

at 37°C for 1 hour and washed three times with phosphate-buffered saline (PBS)-Tween (0.1%) for 5 minutes. 25 μL of goat anti-human fluorescein-labeled IgG conjugate was used as a secondary antibody. The slides then were incubated for 30 minutes and washed three times with PBS-Tween for 5 minutes. The microscopic analysis was performed by 200-fold magnification using a Leica DMLS fluorescence microscope (Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany).

2.5 | Plaque reduction neutralization test

To test for the neutralizing capacity of SARS-CoV-2 specific antibodies, Caco-2 cells (human colon carcinoma cells, ATCC DSMZ ACC-169 (American Type Culture Collection) were seeded on a 96-well plate 3 to 5 days prior infection. Twofold dilutions of the test sera beginning with a 1:10 dilution (1:10; 1:20; 1:40; 1:80; 1:160; 1:320; 1:640, and 1:1280) were made in culture medium (Minimum essential medium; Gibco, Dublin, Ireland) before mixed 1:1 with 100 tissue culture infectious dosis 50 of reference virus (SARS-CoV-2 FFM1 isolate). FFM1 was isolated from a patient at University Hospital Frankfurt who was tested positive for SARS-CoV-2 by PCR. The Virus-serum mixture was incubated for 1 hour at 37°C and transferred onto the cell monolayer. Virus related cytopathic effects (CPE) were determined microscopically 48 to 72 hours postinfection. To determine a potential neutralizing ability of patient serum or plasma, a CPE at a sample dilution of 1:10 is defined as nonprotective while a CPE at a dilution of more than equal to 1:20, is defined as protective.

3 | RESULTS

In the early phase of infection, from days 5 to 9 after PCR-confirmed infection with SARS-CoV-2, the in-house developed IFA and PRNT showed a sensitivity of 76.5% (13/17), the Vircell ELISA a sensitivity of 70.6% (12/17), the Assure Tech Rapid Test sensitivity of 62.5% (10/16) and the Euroimmun ELISA a sensitivity of 58.8% (10/17). For the later period from days 10 to 18, the Euroimmun ELISA and Assure Tech Rapid Test showed a sensitivity 93.8% (15/16), the Vircell ELISA, IFA, and PRNT of 100% (16/16) (Table 1). For selected samples (SARS-CoV samples from the 2003 outbreak excluded; Table S2), the Euroimmun ELISA showed a specificity of 95.7%, generating a borderline result for the HCoV-OC43 sample, the Vircell ELISA of 95.2%, generating a positive result for HCoV-229E sample and the in-house developed IFA of 100% (an unspecific result for one EBV sample was excluded). Including the three SARS-CoV samples from the 2003 outbreak, the Euroimmun ELISA showed a specificity of 96.2% (not generating any cross-reactive results for the SARS-CoV samples), the IFA of 86.4% and the Vircell ELISA of 83.3% (both assays generating positive results for all three SARS-CoV samples). The Assure Tech Rapid Test did not generate any false-positive results for the tested samples. None of the other tested samples cross-reacted in terms of generating borderline or false-positive results.

The titers of the Euroimmun and Vircell ELISA and the corresponding PRNT titers for the tested SARS-CoV-2 follow-up samples are shown in Figure 1. In samples 3, 10, and 11, none of the examined assays (including the IFA and Assure Tech Rapid Test), detected SARS-CoV-2 antibodies. In sample 1, only the Vircell ELISA, in samples 4 and 19 only the Vircell ELISA and PRNT (including the IFA and for sample 4 additionally the Assure Tech Rapid Test) detected antibodies. In samples 12 and 16, only the PRNT (and IFA) detected antibodies (in sample 16 with a titer of 1:10). With the exception of sample 1, all with the ELISA positive tested samples were also positively tested with the IFA. In the detection of antibodies, the IFA performed like the PRNT on all examined samples. All with the commercially available assays positive tested samples (except of sample 1) showed neutralizing properties in the PRNT (titer ≥1:20).

4 | DISCUSSION

In terms of sensitivity, our data are consistent with previously published data. In a study from Liu et al,[4] using an rS-based ELISA assay, the group found SARS-CoV-2 IgG antibodies in less than 60% of the samples from days 6 to 10 after disease onset. The sensitivity increased to more than 90% in samples from days 16 to 20[4] and from more than equal to 15 days in a study from Cassaniti et al using a rapid test.[5] In follow-up samples (median 7 days (interquartile range, 4-11) after the first COVID-19 positive result) of 30 hospitalized patients in Italy, a lateral flow assay detected IgM and IgG antibodies in 63.3% of the cases.[6] In a study from Wölfel et al,[7] using an in-house developed IFA, the group found seroconversion in all examined follow-up serum samples of COVID-19 patients by day 14 after the onset of symptoms. The samples were further analyzed via PRNT, all showed neutralization activity against SARS-CoV-2.[7]

An important finding of our study is, that (with the exception of sample 1) all detected SARS-CoV-2 IgG antibodies in the analyzed cohort, using the commercially available assays examined, demonstrated neutralizing (potentially protective) properties in the PRNT. The screening for SARS-CoV-2 IgG antibodies [especially for potential protective IgG antibodies against the S protein[8] using ELISA or lateral flow assays is more convenient and practicable than using the hands-on- and time-intensive IFA or PRNT, which can only be performed by experienced personnel, and the PRNT, only in a BSL-3 laboratory. ELISA-based assays can be automated and used for larger sample sizes. Lateral flow assays can be used by less experienced personnel in a point-of-care setting, generating results in a short time. Some samples, however, were only detected with the IFA and PRNT as the gold standard. The titer needed for potential protective immunity is not yet (officially) defined. In one study, it is reported, that an individual cleared SARS-CoV-2 without developing antibodies up to 46 days after illness.[9] The mechanism of immunity, especially of protective immunity (if applicable) and how long it will last, needs to be further investigated. Besides a humoral-mediated immune response, there is evidence that T-cell mediated immunity plays a role.[10] Most of the SARS-CoV-2 samples analyzed in this study were from
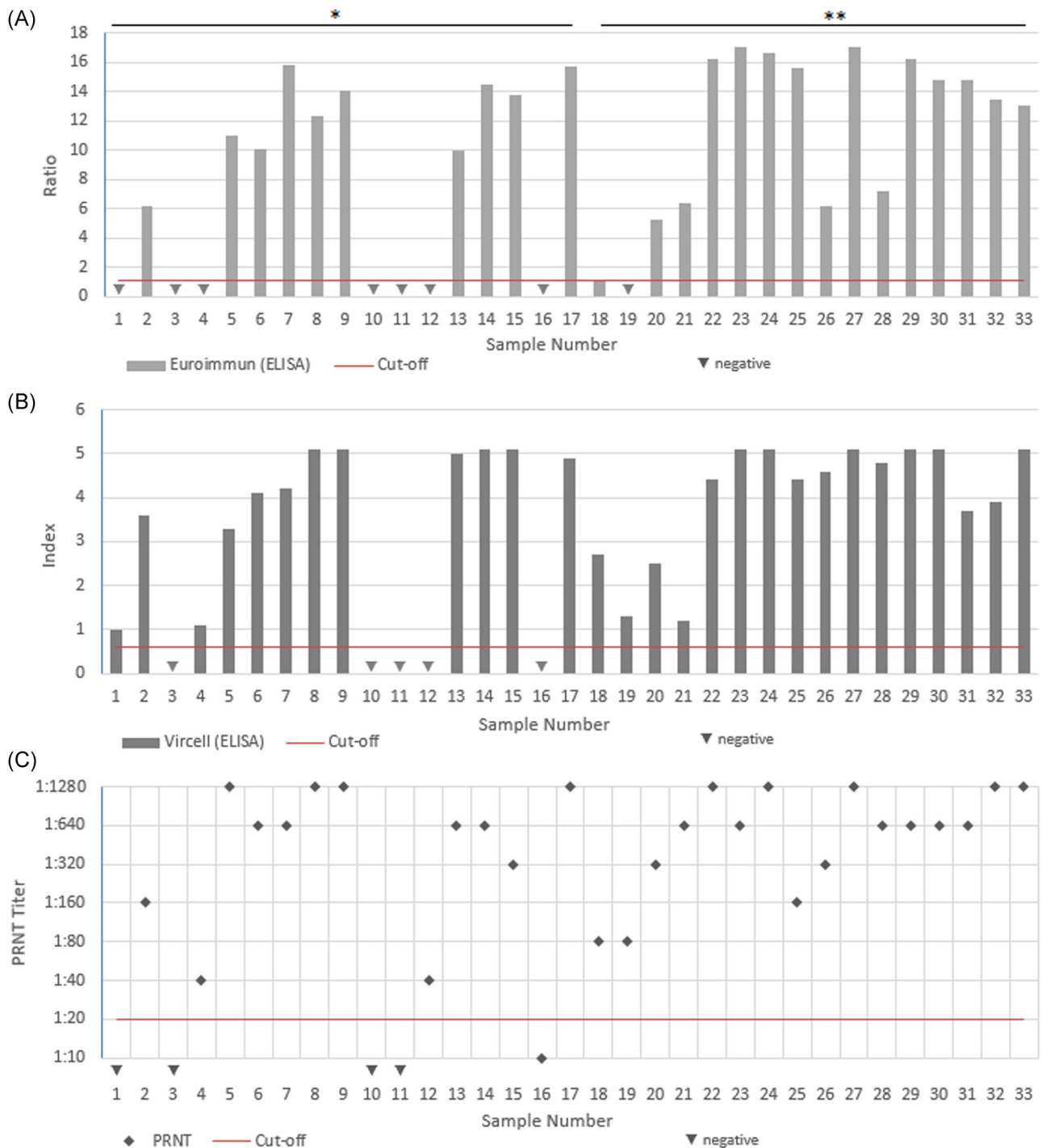
(A)

(B)

(C)

FIGURE 1 Results of the for sensitivity tested samples in the ELISA assays and PRNT; A, Euroimmun ELISA ratios of tested samples; B, Vircell ELISA Indexes for tested samples; C, PRNT Titer for tested samples. *Days 5 to 9 /**Days 10 to 18 after confirmed SARS-CoV-2 PCR. ELISA, enzyme-linked immunosorbent assay; PRNT, plaque reduction neutralization test

individuals with moderate to severe clinical course, who required an in-patient hospital stay. In a Chinese study, male and female patients showed a higher IgG titer (female higher than male) in severe clinical status than in mild clinical status.[11] We have also tested follow-up samples of individuals PCR-diagnosed with COVID-19 with mild or no symptoms at all, IgG antibodies could only be detected after 6 weeks (data not shown). In terms of specificity,

cross-reacting antibodies of endemic coronavirus infected individuals or of individuals with other active infectious diseases (eg, EBV or CMV) is a known phenomenon.[12] The examined assays in our study demonstrated a good specificity. Only the Vircell ELISA generated one positive result for one HCoV-229E sample, whereas the Euroimmun ELISA generated only one borderline result for the HCoV-OC43 sample and the IFA an unspecific signal in one EBV sample.

For the Assure Tech Rapid Test, no cross-reactions were observed, however, a larger sample size would be needed to get a clearer picture. The cross-reactivity of the SARS-CoV samples from the outbreak of 2003 in the Vircell ELISA and IFA is of less importance as the virus is known to be eradicated. Nonetheless, as a false positive result might give a false sense of security, efforts should be made to further improve the specificity of the available assays. All in our study, examined assays are eligible for the detection of SARS-CoV-2 IgG antibodies. Ideally, to get the maximum sensitivity, testing should be performed in the later phase of infection (≥10 days) after PCR-confirmation or disease onset of COVID-19. The Vircell ELISA, IFA, and PRNT demonstrated the highest sensitivity throughout our study. At the moment, however, the PRNT is still the method of choice in detecting potential neutralizing antibodies.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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