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Title

Comparison of four new commercial serologic assays for determination of SARS-CoV-2 IgG

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Highlights

- **-4 new SARS-CoV-2 IgG assays can identify individuals with past SARS-CoV-2 infection**
- **-Combination of two different assays may increase sensitivity and specificity**

Abstract

Background: Facing the ongoing pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), there is an urgent need for serological assays identifying individuals with past coronavirus disease 2019 (COVID-19).

Study Design: Our study is the first to compare four new commercially available assays using 75 sera from patients tested positive or negative by SARS-CoV-2 PCR: the anti SARS-CoV-2 ELISA (IgG) (Euroimmun, Germany), the EDI New Coronavirus COVID-19 IgG ELISA, (Epitope diagnostics (EDI), USA), the recomWell SARS-CoV-2 IgG ELISA (Mikrogen, Germany), and the SARS-CoV-2 Virachip IgG (Viramed, Germany).

Results: We found a sensitivity of 86.4%, 100%, 86.4%, and 77.3% and a specificity of 96,2%, 88,7%, 100%, and 100% for the Euroimmun assay, the EDI assay, the Mikrogen assay, and the Viramed assay, respectively.

Conclusions: Commercially available SARS-CoV-2 IgG assays have a sufficient specificity and sensitivity for identifying individuals with past SARS-CoV-2 infection.

Keywords: COVID-19; ELISA; SARS-CoV-2

1. Background

In December 2019 the new coronavirus SARS-CoV-2 causing coronavirus disease 2019 (COVID-19) emerged in China leading to an ongoing pandemic [1] [2]. Reverse transcriptase real-time polymerase chain reaction (RT-PCR) of respiratory specimens represents the gold standard for identifying patients with acute SARS-CoV-2 infection as well as asymptomatic carriers. Its timely development allowed containment of the pandemic in many countries. Successful future management of the disease's spread will require the serological detection of past infection to determine immunity [3]. Especially in healthcare workers, this is of outmost importance to identify immune personnel that will treat vulnerable patient groups and for the planning and management of infection preventive measures. It also allows the identification of plasma donors for therapeutic interventions.

2. Objective

Lately, several commercial assays for the determination of SARS-CoV-2 IgG became available. In this study, we compared four assays in respect to their sensitivity and specificity.

3. Study design

75 sera of 56 patients hospitalized in the University Hospital RWTH Aachen, Germany, were included into this study. 25 sera were collected from 25 patients with a negative SARS-CoV-2 PCR result in respiratory specimens. 50 sera were collected from 31 patients with a positive SARS-CoV-2 PCR result in respiratory specimens. Sample and data acquisition were approved by the Medical Ethics Committee of the University Hospital RWTH Aachen (EK 093/20). Three semiquantitative ELISAs (the anti-SARS-CoV-

2 ELISA IgG, Euroimmun, Germany, the EDI New Coronavirus COVID-19 IgG ELISA, Epitope Diagnostics (EDI), USA, and the recomWell SARS-CoV-2 IgG ELISA, Mikrogen, Germany), as well as one qualitative immunoblot based on a set of immunoassays in a microarray format (the SARS-CoV-2 Virachip IgG immunoblot, Viramed, Germany) were compared in this study for the detection of SARS-CoV-2-specific IgG antibody titers.

In analogy to a previous study [4] the SARS-CoV-2 IgG status of the sera was defined as follows: A serum was regarded as SARS-CoV-2 IgG negative if at least three of the four assays compared here had a negative test result applying the manufacturer's interpretation criteria. On the other hand, a serum was regarded as SARS-CoV-2 IgG positive if at least two of the four assays had a positive test result.

Comparison of the kinetic of the SARS-CoV-2 IgG titer was done using the three semiquantitative ELISAs and sera of 2 COVID-19 patients of whom several consecutive sera were available. To allow comparison of the semiquantitative values between assays, the values were divided by the assay-specific cut off value for normalization. Normalized values of >=1 represented a positive test result.

4. Results

3.1. Determination of sensitivity and specificity

75 sera were included in this study, 25 of which were collected from patients with a negative SARS-CoV-2 PCR result and 50 from patients with a positive SARS-CoV-2 PCR result in respiratory specimens. The sera of patients with negative SARS-CoV-2 PCR result were drawn on the day of PCR examination. The sera of the 31 patients with positive SARS-CoV-2 PCR were collected 11.9 days (± 5.0 days) post onset of symptoms. Each serum was tested in parallel with four assays for the presence of SARS-CoV-2 IgG antibodies as recommended by the manufacturers: three ELISAs

(from Euroimmun, Epitope Diagostics (EDI), and Mikrogen) as well as one immunoblot (from Viramed). The SARS-CoV-2 IgG status of a serum was determined as described in Study design.

Using the Euroimmun assay, 21 sera were classified as SARS-CoV-2 IgG positive, 54 were classified as IgG negative. With the EDI assay 25 sera had a positive result, three an intermediate result, and 47 a negative result. The Mikrogen assay and the Viramed assay revealed 19 and 17 positive test results as well as 56 and 54 negative results, respectively.

Applying the criteria described in Study design, 22 sera were considered SARS-CoV-2 IgG positive and 53 sera were regarded as IgG negative. All sera collected from patients with a negative SARS-CoV-2 PCR were SARS-CoV-2 IgG negative. 28 out of 50 patients with a positive SARS-CoV-2 PCR exhibited a negative antibody test.

The rate of correct positive and the rate of correct negative test results of the four assays is displayed in Table 1. They result in a sensitivity of 86.4%, 100%, 86.4%, and 77.3% for the Euroimmun assay, the EDI assay, the Mikrogen assay, and the Viramed assay, respectively. The corresponding results for the specificity are 96,2%, 88,7%, 100%, and 100%.

3.2. Kinetics of SARS-CoV-2 IgG antibody titers

The kinetics of the SARS-CoV-2 IgG antibody titers could only be compared for the ELISA assays resulting in semiquantitative values. For comparison the semiquantitative test results were normalized as described in the Study design section. Values of 1 and above represent positive test results.

We found that the kinetics of the SARS-CoV-2 IgG antibody titers differed for each assay; moreover the time point of seroconversion of each assays differed for both patients. In one patient the EDI assay was the first to give a positive test (8 days after onset of symptoms) followed by the assays of Euroimmun (9 days) and Mikrogen (10

days), whereas in the second patient the Mikrogen assay was the first to give a positive test result (8 days after onset of symptoms) followed by the assays of EDI (9 days) and Euroimmun (11 days). Thus, it took at least 10 days after onset of symptoms to obtain positive test results in all three assays.

We also noticed that the EDI assay has a smaller range of linearity compared to both of the other tests preventing the proportional detection of a further increase of antibody titers with time.

5. Discussion

SARS-CoV-2 specific antibodies are usually only detected more than one week after onset of symptoms [5], limiting the role of serology for identification of acute infection. However, serologic assays are urgently needed to supplement the diagnostic repertoire in identifying patients with past SARS-CoV-2 infection. This is important for (i) the prognosis of the further course of the pandemic, (ii) the identification of presumably immune health care workers who can work with vulnerable groups of patients, and (iii) the identification of potential plasma donors for therapeutic transfusion. Furthermore, serologic assays might allow for the detection of patients presenting during a later stage of the disease when viral clearance may precede the disappearance of symptoms.

As today, the ELISA of Euroimmun is the only validated commercial ELISA available in Germany. According to a recent publication [3], this commercial IgG specific ELISA exhibited lower specificity and sensitivity compared to *in house* assays.

Recently, additional antibody test assays became commercially available. The aim of this study was to compare four commercially available serological assays for SARS-CoV-2 IgG, the Euroimmun assay, and 3 new IgG assays.

The strength of the EDI assay is its high sensitivity; however, we found a comparatively low specificity and narrow linear range. On the other hand, the Viramed assay is highly specific but showed the lowest sensitivity. Both, the Euroimmun assay and the Mikrogen assay had a medium sensitivity. Regarding specificity the Mikrogen assay reached a higher level than the Euroimmun assay in our test setting.

In respect to the identification of SARS-CoV-2 IgG positive health care workers and plasma donors with high levels of SARS-CoV-2 IgG specific antibodies, specificity and linear range are the most important aspects of the assays. Thus, the Euroimmun assay and the Mikrogen assay appear to be most suited to fulfill both requirements, with a somewhat better performance of the Mikrogen assay in our experimental setting.

In conclusion, the four tested SARS-CoV-2 IgG assays showed sufficient specificity and sensitivity for identifying individuals with past SARS-CoV-2 infection. Moreover combination of two different assays may further increase sensitivity and specificity, especially at early time points after onset of symptoms (<10 days). However, more studies are necessary to fully assess the performance of these assays.

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Author contributions

Alexander Krüttgen: writing, investigations

Christian G. Cornelissen: Supply of samples, review, editing

Michael Dreher: Supply of samples, review, editing

Mathias Hornef: review & editing

Matthias Imöhl: Resources, review & editing

Michael Kleines: Conceptualization, investigations, writing

Declaration of interest statement/Conflict of interest statement

None

References

[1] Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579:270-3.
[2] Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. The New England journal of medicine. 2020;382:727-33.
[3] Okba NMA, Muller MA, Li W, Wang C, GeurtsvanKessel CH, Corman VM, et al. Severe Acute Respiratory Syndrome Coronavirus 2-Specific Antibody Responses in Coronavirus Disease 2019 Patients. Emerging infectious diseases. 2020;26.
[4] de Ory F, Guisasola ME, Balfagon P, Sanz JC. Comparison of commercial methods of immunoblot, ELISA, and chemiluminescent immunoassay for detecting type-specific herpes simplex viruses-1 and -2 IgG. Journal of clinical laboratory analysis. 2018;32.
[5] Haveri A, Smura T, Kuivanen S, Osterlund P, Hepojoki J, Ikonen N, et al. Serological and molecular findings during SARS-CoV-2 infection: the first case study in Finland, January to February 2020. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2020;25.

Table 1:

Sensitivity and specificity of four SARS-CoV-2 IgG assays

Assay	Manufacturer	Test results		Sensitivity	Specificity
		Rate of correct positive test results	Rate of correct negative test results		
	Euroimmun	19/22	51/53	86.4 %	96.2 %

	EDI	22/22	47/53	100 %	88.7 %
	Mikrogen	19/22	53/53	86.4 %	100%
	Viramed	17/22	53/53	77.3 %	100%