Monitoring antibody response following SARS-CoV-2 infection: diagnostic efficiency of 4 automated immunoassays

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ABSTRACT

Introduction: SARS-CoV-2 seroconversion is important for epidemiological studies as well as contact tracing.
Material and methods: The antibody response against SARS-CoV-2 was examined in 111 patients with a positive qRT-PCR. Seroconversion was assessed using the Elecsys from Roche, the Liaison S1/S2 IgG from Diasorin, the IgG and IgA from Euroimmun, as well as the VIDAS IgG and IgM. Specificity was estimated based on the measurement of SARS-CoV-2 antibodies in 96 residual samples collected during a non-pandemic period.
Results: The highest overall sensitivity for detecting seroconversion was obtained using the Elecsys (81.1%), the Euroimmun with a combined detection of IgG/IgA (86.5%), and the VIDAS with a simultaneous measurement of IgG/IgM (78.4%).The Elecsys and the VIDAS IgG/IgM demonstrated a specificity as well as a positive predictive value of 100%.
Conclusions: The Elecsys and the VIDAS methods with a combination of IgG/IgM measurement demonstrated a high sensitivity with no false positive results.

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1. Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for the coronavirus infectious disease (COVID-19), recently declared by the World Health Organization (WHO) as a global pandemic (European Centre for disease Prevention and Control (ECDC), 2019). Besides the molecular diagnosis using nucleic acid amplification, the serology is a complementary method potentially filling the gap of false negative ribonucleic acid (RNA) and allowing the rapid screening of individuals at a larger scale. Both techniques are essential to guide antiviral treatment, epidemiological measures, vaccination when available and consequently to control the disease by applying appropriate lockdown exit strategies (Gilbert et al., 2020; To et al., 2020).

Different commercial serological tests are available for monitoring the antibody response following a SARS-CoV-2 infection. Enzyme linked immunosorbent assays (ELISA), chemiluminescent immunoassays (CLIA) as well as enzyme linked fluorescence assays (ELFA) are the main methodologies available for automated analytical platforms (Lippi et al., 2020; Montesinos et al., 2020; Padoan et al., 2020). Potential antigens for serodiagnosis of COVID-19 differ; some of them are based on a recombinant SARS-CoV-2 nucleocapsid protein, while others use a recombinant spike protein. Furthermore, IgG, IgA, IgM, or a combination of different classes of immunoglobulins (i.e. IgG /IgM) can be measured.

In this study, the diagnostic efficiency of four anti-SARS-CoV-2 automated immunoassays detecting different classes of immunoglobulins was evaluated: Elecsys Anti-SARS CoV-2 (Roche Diagnostics, Vilvoorde, Belgium), Liaison SARS-CoV-2 S1/S2 IgG (Diasorin, Saluggia, Italy), Euroimmun Anti-SARS CoV-2 ELISA IgG and IgA assays (Euroimmun, Luebeck, Germany), and VIDAS Anti-SARS CoV-2 IgG and IgM (BioMérieux, Marcy-l'Etoile, France). Seroconversion was assessed on 111 COVID-19 patient samples collected at different time points post disease onset for symptomatic patients or post positive quantitative real-time reverse transcriptional polymerase chain reaction (qRT-PCR) for asymptomatic cases. The assessment of immunoassays specificity was performed by analyzing 96 residual serum

Abbreviations: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, coronavirus infectious disease; WHO, World Health Organization; RNA, ribonucleic acid; ELISA, enzyme linked immunosorbent assay; CLIA, chemiluminescent immunoassay; ELFA, enzyme linked fluorescence assay; qRT-PCR, quantitative real-time reverse transcriptional polymerase chain reaction; ICU, intensive care unit; RFV, relative fluorescence value; ROC, receiver operator curve; AUC, area under the curve; PPV, positive predictive value; NPV, negative predictive value; CI 95%, Confidence interval 95%.
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samples collected during a non-pandemic period. The performances of these commercial immunoassays along with other characteristics including the principle of detection, the recombinant antigen used, the sample throughput and the time of first result were also kept into account in order to guide different types of laboratories to choose the best immunoassay as well as strategy for monitoring SARS-CoV-2 antibodies.

2. Material and methods

2.1. Patients and samples

A total of 111 samples from symptomatic ($n = 87$) and asymptomatic ($n = 24$) COVID-19 patients confirmed by qRT-PCR were tested. Clinical data about the number of days since the onset of first symptoms and severity of the disease were extracted from electronic medical records for symptomatic patients. The most frequent clinical symptoms encountered for mild to moderate symptomatic patients were fever, headache, cough, and myalgia. Severe disease was defined as the need for oxygen supplementation, respiratory failure requiring mechanical ventilation, admission to the intensive care unit (ICU) or death (To et al., 2020). The number of days post positive SARS-CoV-2 qRT-PCR were collected for asymptomatic patients. The qRT-PCR was performed using the RealStar® SARS-CoV-2 RT-PCR kit 1.0 (Altona Diagnostics, Hambourg, Germany) according to the manufacturer instructions. Asymptomatic patients were defined as individuals without any symptoms who were screened positive for SARS-CoV-2 nucleic acid due to close contacts with COVID-19 patients.

Based on the number of days post disease onset for symptomatic patients or the number of days post SARS-CoV-2 positive qRT-PCR for asymptomatic patients to serum collection, patients were divided in three groups; 0 to 7 days, 8 to 14 days, and >15 days. The assay specificity was assessed by testing residual serum samples non-SARS-CoV-2 ($n = 96$) collected before the pandemic COVID-19 from January to February 2019.

Consecutive samples were collected at different time points (at least 3) for either mild-moderate symptomatic ($n = 4$) or severe symptomatic patients ($n = 2$). Serum remnant was retrieved from blood samples taken for routine biochemical testing and stored at -20°C. The study was performed according to the advice (AK/10-06-41/3907) of the ethical board of CHU Saint-Pierre.

2.2. Serological assays

2.2.1. Elecsys Anti-SARS CoV-2

The Elecsys Anti-SARS CoV-2 assay was performed on a Cobas e801 analyzer (Roche Diagnostics, Vilvoorde, Belgium). This sandwich assay uses a SARS-CoV-2 specific recombinant antigen representing the nucleocapsid protein. Briefly, the sample is incubated with the biotinylated recombinant antigen and the recombinant antigen labeled with ruthenium. The separation of immune complexes is performed after adding streptavidin-coated particles that are then magnetically attracted onto an electrode where a voltage is applied, generating a chemiluminescent emission. The electrochemiluminescent signal produced is compared to the cut-off signal value previously obtained with two calibrators. Results are expressed either as negative (cut-off index; COI <1) or positive (COI ≥1) for anti-SARS CoV-2 antibodies.

2.2.2. Liaison SARS-CoV-2 S1/S2 IgG

The Liaison SARS-CoV-2 kit, an indirect CLIA, was assayed on a Liaison XL analyzer (Diasorin, Saluggia, Italy). The sample is first incubated with magnetic microbeads coated with recombinant spike S1/S2 antigen. Mouse monoclonal antibodies directed against human IgG are then added. The chemiluminescence signal produced is measured and the concentration of IgG anti S1/S2 is reported in arbitrary units (AU/mL). Results are interpreted as follows: <12 AU/mL = negative, ≥12 to <15 =

borderline, ≥15 = positive. Borderline data were considered positive for the statistical analyses.

2.2.3. Euroimmun Anti-SARS CoV2 IgG and IgA ELISA

The Euroimmun Anti-SARS CoV-2 ELISA IgG and IgA assays (Euroimmun, Luebeck, Germany) were performed on the ETI-MAX 3000 (DiaSorin, Saluggia, Italy). These assays allow a determination of IgG and IgA against the SARS-CoV-2. The microplate wells are coated with recombinant S1 structural protein. The results are evaluated by calculation of a ratio of the extinction of samples over the extinction of the calibrator. The ratio interpretation was as follows: <0.8 = negative, ≥0.8 to <1.1 = borderline, ≥1.1 = positive. Borderline data were considered positive for the statistical analyses.

2.2.4. VIDAS Anti-SARS CoV-2 IgG and IgM

The VIDAS Anti-SARS CoV-2 is a two-step sandwich ELFA performed on a VIDAS analyzer (BioMérieux, Marcy-l'Etoile, France). The IgG and IgM in the sample are captured by a recombinant SARS-CoV-2 sub domain spike antigen coated on a solid phase, then an anti-human IgG or IgM labeled with alkaline phosphatase is added. The intensity of the fluorescence produced by the substrate hydrolysis is measured at 450 nm and is proportional to the antibody level. An index is calculated as the ratio between the relative fluorescence value (RFV) measured in the sample and the RFV obtained for the calibrator (humanized recombinant anti-SARS CoV-2 IgG or IgM) and interpreted as negative (index <1) or positive (index ≥1).

The principle of antibody detection, the recombinant antigen used, the immunoglobulin classes recognized, the kit format, the samples throughput as well as the time to the first result of the different commercial immunoassays for monitoring antibodies production are described in Table 1.

2.3. Statistical analyses

Results were analyzed using the Graph Pad Prism software version 5.0 (La Jolla, USA). Median and range were used for describing continuous variables. Receiver operator characteristic (ROC) curve was calculated and the area under the curve (AUC) obtained was reported for each assay. The Youden index was calculated to select the optimal threshold value giving the best combination of sensitivity and specificity.

Categorical variables were compared using the Fisher's exact test. Sensitivity, specificity, positive (PPV) and negative predictive (NPV) values were reported. Differences (positive rate, antibody titer, number of days post positive qRT-PCR versus number of days post disease onset) between asymptomatic, mild to moderate symptomatic, and severe symptomatic patients were evaluated using the Kruskal–Wallis test with a Dunn's post hoc test. A P-value lower than 0.05 was considered statistically significant.

3. Results

Eighty-seven symptomatic patients (median age: 60 years old, range: 21–88 years old, 36 women, 51 men) and 24 asymptomatic patients (median age: 61 years old, range: 20–85 years old, 11 women, 13 men) were included in the present study. Among the 87 symptomatic patients, 40 were considered as presenting a severe disease. The median number of days post disease onset (symptomatic patients) or post positive qRT-PCR (asymptomatic patients) was 12 days (range 0–54 days).

Borderline data were found for four samples analyzed using the Liaison IgG, two samples with the Euroimmun IgG as well as the Euroimmun IgA.

Sensitivity, specificity, PPV, and NPV values are shown in Table 2. By analyzing results according to the time of collection, 35 samples were collected from 0 to 7 days, 31 samples from 8 to 14 days and 45 samples

Table 1
Characteristics of the evaluated immunoassays including the principle of detection, the recombinant antigen used, the immunoglobulin (Ig) classes, the kit format, the sample throughput and the time of first result.

Immunoassays	Principle of detection	Recombinant antigen	Ig classes	Kit format	Sample throughput	Time of first result
Elecsys Anti-SARS CoV2	CLIA	Nucleocapsid	IgM/IgG	300 tests*	200 tests/h	18 min
Liaison SARS CoV2 S1/S2 IgG	CLIA	Spike S1/S2	IgG	100 tests	170 tests/h	35 min
Euroimmun Anti-SARS CoV2 IgG	ELISA	Spike S1	IgG	96 tests	90 tests/3h	3 h
Euroimmun Anti-SARS CoV2 IgA	ELISA	Spike S1	IgA	96 tests	90 tests/3h	3 h
VIDAS Anti-SARS CoV-2 IgG	ELFA	Spike (Sub domain)	IgG	Unit test (2x30)	30 tests/h	27 min
VIDAS Anti-SARS CoV-2 IgM	ELFA	Spike (Sub domain)	IgM	Unit test (2x30)	30 tests/h	27 min

* for the Cobas e801

>15 days post disease onset or post positive qRT-PCR. The positive detection rate of the four assays investigated was the lowest during the early stage of the disease (i.e. 0–7 days) and increases progressively to reach a maximum value after Day 15. The only exception was encountered for the VIDAS Anti-SARS CoV-2 IgM for which the highest sensitivity was obtained between 8 and 14 days (i.e. 80.7%). From 0–7 to >Day 15, the positive rates increase from 68.6% to 88.9% for Elecsys anti-SARS CoV-2, from 51.4% to 87.7% for the Liaison IgG, from 60% to 91.1% for the Euroimmun IgG, from 71.4% to 93.3% for the Euroimmun IgA, from 57.1% to 86.7% for the VIDAS Anti-SARS CoV-2 IgG, and from 40% to 73.3% for the VIDAS Anti-SARS CoV-2 IgM. The overall sensitivity estimated for the 111 patients samples collected from 0 to >15 days was 81.1% for the Elecsys, 70.3% for the Liaison IgG, 75.7% for the Euroimmun IgG, 82.9% for the Euroimmun IgA, 73% for the VIDAS Anti-SARS CoV-2 IgG, and 64.9% for the VIDAS Anti-SARS CoV-2 IgM.

To assess the specificity of all immunoassays detecting anti-SARS-CoV-2, 96 samples from non COVID-19 patients (median age: 38, range: 0 to 87 years old, 62 women, 38 men) were analyzed. No positive result was observed with the Elecsys anti-SARS CoV-2 assay, the VIDAS Anti-SARS CoV-2 IgG, as well as IgM, with a specificity and a positive predictive value of 100%. The Liaison IgG, the Euroimmun IgG and IgA demonstrated 2, 4, and 4 false positive results with a respective specificity of 97.9%, 95.8%, and 95.8%.

When combining the results of the different immunoglobulin classes detected with the same analytical platform, an improvement of the sensitivity (86.5%, CI 95%: 78.7–92.2%) was obtained for the IgG/IgA Euroimmun while the specificity decreased (92.7%, IC 95%: 85.6–97%). A rise of the positive detection rate was also underlined when IgG and

IgM were simultaneous measured using the VIDAS Anti-SARS CoV-2 (sensitivity: 78.4%, CI 95%: 69.6–85.6%) with a preservation of the specificity as well the positive predictive value at 100%.

The AUC obtained for different immunoassays were 0.921 for the Elecsys (95 CI%: 0.880–0.963), 0.855 for the Liaison IgG (95 CI%: 0.801–0.910), 0.936 for the Euroimmun IgG (95% CI: 0.905–0.967), 0.938 for the Euroimmun IgA (95% CI: 0.903–0.973), 0.911 for the VIDAS Anti-SARS CoV-2 IgG (95% CI: 0.868–0.955), and 0.893 for the VIDAS Anti-SARS CoV-2 IgM (0.844–0.942). The optimal threshold value was estimated using the Youden index. The best cut-off value calculated for the Elecsys (COI >0.97) was completely in agreement with those established by the manufacturer (COI ≥1) with a sensitivity of 81.1% and a specificity of 100%. The optimal cut-off value calculated for the Liaison IgG was also in line with those estimated by the manufacturer (>12.1 AU/mL versus ≥12 AU/mL for borderline data) with a sensitivity and a specificity of 70.3% and 97.9% respectively. The calculated threshold value for the ratio measured using the Euroimmun IgG/ IgA as well as the VIDAS IgG/IgM were lower compared with those established by the manufacturer. At a cut-off value higher than 0.67 for the ratio measured using the Euroimmun IgG, the best combination for sensitivity (77.5%) and specificity (94.8%) were reached. A sensitivity of 85.6% and a specificity of 95.8% were obtained for Euroimmun IgA using a ratio higher than 0.66. For the VIDAS, the Youden index was calculated at >0.3 for IgG (sensitivity: 81.1%, specificity: 99%) and >0.41 for IgM (sensitivity: 74.8%, specificity: 96.9%).

The rate of seroconversion and the antibody titer were evaluated in patients according to the clinical severity of the disease (Table 3). The positive rates obtained for the 24 asymptomatic patients were

Table 2
Sensitivity (CI 95%) calculated for different immunoassays (Elecsys Anti-SARS CoV-2, Liaison SARS-CoV-2 S1/S2 IgG, Euroimmun Anti-SARS-CoV-2 IgG and IgA, VIDAS Anti-SARS-CoV-2 IgG and IgM) at different time points: 0–7 days (n:35), 8–14 days (n:31) and >15 days (n: 45) post disease onset (symptomatic) or post positive qRT-PCR (asymptomatic patients). The overall sensitivity (CI 95%), specificity (CI 95%), positive predictive value as well as negative predictive value (CI 95%) for assessing SARS CoV2 seroconversion is reported for each immunoassay.

Diagnostic efficiency	Elecsys Anti-SARS CoV-2	Liaison SARS CoV-2 S1/S2 IgG	Euroimmun Anti-SARS CoV-2 IgG	Euroimmun Anti-SARS CoV-2 IgA	VIDAS Anti-SARS CoV-2 IgG	VIDAS Anti-SARS CoV-2 IgM
Sensitivity 0–7 days post symptoms or post + PCR	24/35 68.6% (50.7–83.2%)	18/35 51.4% (34–68.6%)	21/35 60% (42.1–76.1%)	25/35 71.4% (53.7–85.4%)	20/35 57.1% (39.4–73.7%)	14/35 40% (23.9–57.9%)
Sensitivity 8–14 days post symptoms or post + PCR	26/31 83.9% (66.3–94.6%)	21/31 67.7% (48.6–83.3%)	22/31 71% (52–85.8%)	25/31 80.7% (62.5–92.6%)	22/31 71% (52–85.8%)	25/31 80.7% (62.5–92.6%)
Sensitivity >15 days post symptoms or post + PCR	40/45 88.9% (76–96.3%)	39/45 87.7% (73.2–95%)	41/45 91.1% (78.8–97.5%)	42/45 93.3% (81.7–98.6%)	39/45 86.7% (73.2–95%)	33/45 73.3% (58.1–85.4%)
Overall Sensitivity From 0 to >15 days post symptoms or post + PCR	90/111 81.1% (72.6–87.9%)	78/111 70.3% (60.9–78.6%)	84/111 75.7% (66.6–83.3%)	92/111 82.9% (74.6–89.4%)	81/111 73% (63.7–81%)	72/111 64.9% (55.2–73.7%)
Specificity	96/96 100% (96.2–100%)	94/96 97.9% (92.7–99.8%)	92/96 95.8% (89.7–98.9%)	92/96 95.8% (89.7–98.9%)	96/96 100% (96.2–100%)	96/96 100% (96.2–100%)
Positive predictive value	100% (96–100%)	97.5% (91.3–99.7%)	95.5% (88.8–98.8%)	95.8% (89.7–98.9%)	100% (95.6–100%)	100% (95.1–100%)
Negative predictive value	82.1% (73.9–88.5%)	74.0% (65.5–81.4%)	77.3% (68.7–84.5%)	82.9% (74.6–89.4%)	76.2% (67.8–83.3%)	71.1% (62.7–78.6%)

respectively 62.5% for the Elecsys, 58.3% for the Liaison IgG, the Euroimmun IgG as well as the VIDAS IgG, 66.7% for Euroimmun IgA and 45.8% for the VIDAS IgM and were lower compared to those measured for mild to moderate COVID-19 patients. However, the difference was not statistically significant ($P > 0.05$). The detection rates were significantly higher for the 40 severe symptomatic patients compared with asymptomatic carriers (i.e. 87.5% for the Elecsys, the Euroimmun IgG, the Euroimmun IgA and the VIDAS IgG, 85% for the Liaison IgG, 77.5% for the VIDAS IgM, $P < 0.05$). When comparing the asymptomatic and the severe patient populations, the antibody titers were significantly lower for asymptomatic carriers when seroconversion was assessed with the Euroimmun IgG, the Euroimmun IgA, the VIDAS IgG as well as the VIDAS IgM ($P < 0.05$). However, the number of days post positive PCR (median: 3 days, range 0–15 days) for asymptomatic patients was significantly lower compared with the number of days post disease onset for mild to moderate (median: 16, range 0–54) as well as for severe COVID-19 patients (median: 14 days, range 1–39, $P < 0.0001$).

The kinetics of antibodies appearance was assessed in 6 COVID-19 patients (4 mild to moderate and 2 severe patients). Results are described in Figure 1. The Elecsys, the Euroimmun IgA, and the VIDAS IgM highlighted an earlier seroconversion for Patient 1 (Day 9) compared with the Euroimmun IgG or the VIDAS IgG (Day 11) and the Liaison IgG (Day 13). For Patient 2, an earlier seroconversion was observed for the Euroimmun IgA as well as the VIDAS IgM. All the immunoassays investigated underlined a seroconversion for Patient 3 at Day 3 except the VIDAS IgM for which the level of antibodies titer remains below the threshold value. The Elecsys and the Euroimmun IgA showed a seroconversion at Day 4 for Patient 4 whereas the Liaison IgG, the Euroimmun IgG, the VIDAS IgG and IgM underlined positivity at Day 11. Interestingly, the Patient 5, treated by chemotherapy for a lymphoma, had immunoglobulin levels remaining below the cut-off of positivity for three of the evaluated immunoassays (the Elecsys, the Euroimmun IgG and IgA, the VIDAS IgG and IgM) 21 days after the disease onset. The Liaison IgG underlined a borderline result for this patient at Day 21. The seroconversion of SARS-CoV-2 antibodies underlined with the different immunoassays was identical for Patient 6.

4. Discussion

Although at the time of writing (7 July 2020), nucleic acid amplification tests are still considered as the reference method by the WHO to screen for SARS-CoV-2 infection, the outcome is directly affected by the viral load obtained during the sampling process. The viral load depends on the time point of infection and pre-analytical factors (samples type, samples collection, storage conditions) (Yongchen et al., 2020). Monitoring antibody response could be complementary to nucleic acid testing given the potential false negative RNA results. Combining

RNA and antibody measurement significantly increases the positive detection rate in patients (Guo et al., 2020). Compared to molecular methods, most serological tests present a faster turn-around time, high-throughput and less workload (Zhao et al., 2020). Seroconversion assessment could allow the identification of individuals exposed to the virus as well as potentially protected people and, as a consequence, could guide the application of progressive relaxation of containment measures (Gilbert et al., 2020).

Many CE-labeled tests allowing the detection of SARS-CoV-2 antibodies are now available on the market. They differ in the type of immunoglobulin classes recognized (IgA, IgM, IgG or a combination IgM/IgG) as well as the nature of the antigen used for antibody recognition. Some of them are based on a recombinant nucleocapsid protein (i.e. the Elecsys Roche) while others use of a recombinant spike protein (i.e. Spike-S1/S2 protein for the Liaison, Spike-S1 protein for the Euroimmun IgG and IgA, sub domain of Spike protein for the VIDAS IgG and IgM), both proteins being highly immunogenic (Loeffelholz and Tang, 2020). A higher sensitivity was described for immunoassays based on recombinant spike protein attributed to the earlier response to the spike antigen compared to the nucleocapsid antigen in patients with COVID-19 (Liu et al., 2020; To et al., 2020). To our knowledge, this is the first evaluation of VIDAS Anti-SARS CoV-2 IgM and IgG recently CE marked (21 May 2020)

In this study, the diagnostic efficiency of different immunoassays as well as immunoglobulin classes were assessed for detecting seroconversion in 111 COVID-19 positive symptomatic or asymptomatic patients. To monitor the antibody response, the timeline started from the first symptoms for symptomatic patients whereas the timeline started from the qRT-PCR diagnosis for asymptomatic carriers. The overall sensitivities estimated for the Euroimmun IgA and the Elecsys were very close with respective positive rates of 82.9% and 81.1%. When comparing immunoassay measuring only IgG, both being based on a spike recombinant antigen, the Euroimmun IgG was slightly more sensitive than the VIDAS IgG followed by the Liaison IgG (respective positive rate of 75.7%, 73%, and 70.3%). The sensitivity of the VIDAS IgM was the lowest with a positive detection rate of 64.9%. The detection rate of the four tests increases with the number of days post disease onset or post positive PCR, these results being explained by the low antibody titers observed at early stages of the disease. The VIDAS IgM was the only immunoassay highlighting the highest sensitivity between 8 to 14 days post disease onset. These results are in agreement with Long et al. who underlined a rapid rise of IgM with a drop 3 weeks after the first symptoms (Long et al., 2020).

Conflicting results are described in the literature regarding the chronological order of IgM as well as IgG appearance following a SARS-CoV-2 infection and could be explained by the different sensitivity of immunoassays (To et al., 2020). Whereas some authors demonstrated that the dynamic pattern of SARS-CoV-2 antibody production is typical of acute

Table 3

The number of patients, the age, the ratio male to female, the number of days post + PCR (asymptomatic patients) or post disease onset (symptomatic patients) as well as the detection rates obtained for the different immunoassays are described according to the severity of COVID-19 disease presentation. Values are reported as the median (range).

|	Asymptomatic	Symptomatic (mild to moderate)	Symptomatic (severe)
Number of patients	24	47	40
Age	61	60	59
	(20–85)	(21–88)	(26–88)
Ratio male to female	11/13	24/23	28/12
Number of days post + PCR or post disease onset	3	16	14
	(0–15)	(0–54)	(1–39)
Detection rate Elecsys Anti-SARS CoV-2			
	62.5%	85.1%	87.5%
Detection rate			
Liaison SARS CoV-2 S1/S2 IgG	58.3%	63.8%	85%
Detection rate			
Euroimmun Anti-SARS CoV-2 IgG	58.3%	74.5%	87.5%
Detection rate Euroimmun Anti-SARS CoV-2 IgA	66.7%	87.2%	87.5%
Detection rate Anti-SARS CoV-2 VIDAS IgG	58.3%	68.1%	87.5%
Detection rate Anti-SARS CoV-2 VIDAS IgM	45.8%	63.3%	77.5%
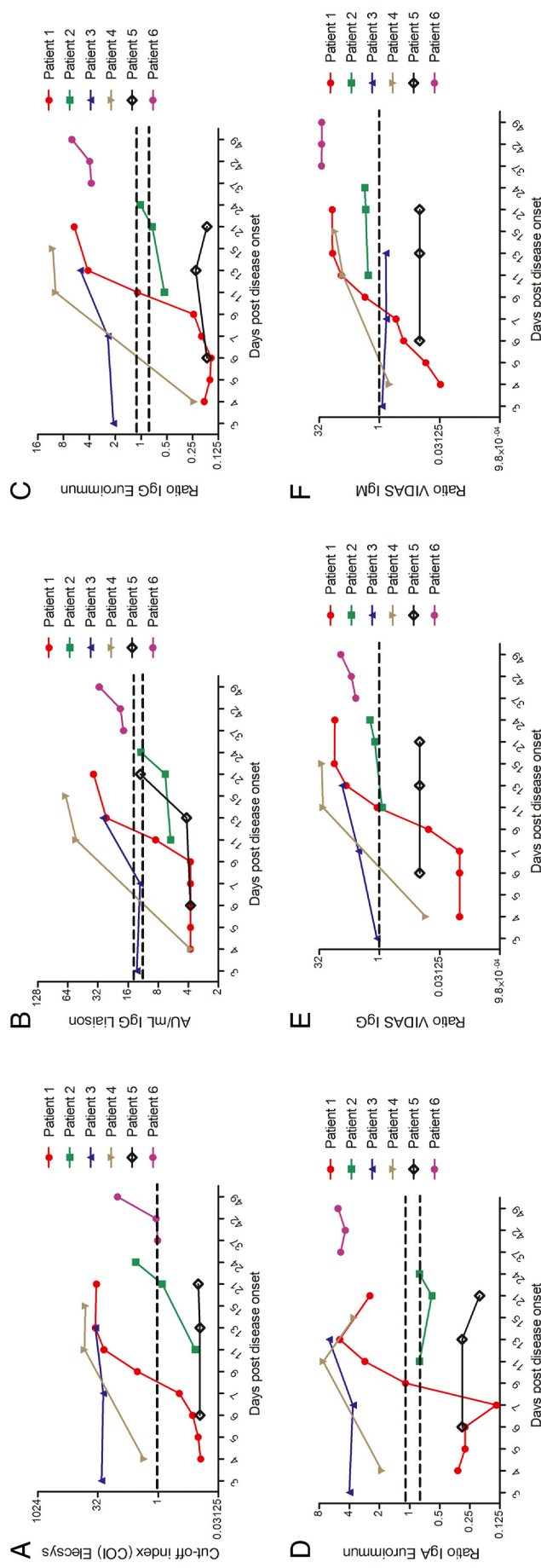
Figure 1. Detection rates for SARS CoV2 antibodies obtained using commercial immunoassays in 6 COVID-19 patients. The threshold value for positivity is reported for each test. Values between ≥12 and 15 for the Liaison SAsRS-CoV-2 S1/S2 IgG and between ≥0.8 and 1.1 for the Euroimmun IgG/IgA are considered as borderline data. The seroconversion was achieved earlier with the Elecsys (Patient 1, 4), the Euroimmun IgA (Patient 1, 2, and 4) and the VIDAS IgM (Patient 1, 2) tests for half of the studied patients.

viral infection with a rise of IgG that appears when IgM drop (Guo et al., 2020; Liu et al., 2020; Okba et al., 2020; Zhao et al., 2020), others showed an earlier conversion for IgG than IgM (Padoan et al., 2020; To et al., 2020; Zhang et al., 2020). This contradictory data support the detection on both antibodies simultaneously (Long et al., 2020).

Based on samples collected before the pandemic COVID-19, the Roche Elecsys, the VIDAS IgG and IgM assays showed a 100% specificity with the highest positive predictive value compared with other tests investigated. This maximal specificity obtained for the Roche Elecsys assay was recently confirmed by Favresse et al. (Favresse et al., 2020). The optimal threshold value calculated for the Elecsys was completely in agreement in those estimated by the manufacturer. A further refinement of the reactive cut-off value could be advised for the Euroimmun IgA/IgG and the VIDAS IgG/IgM to reach a better diagnostic efficiency. The results obtained for the Euroimmun are in line with those of Tré-Hardy et al. showing that a cut-off value adaptation could improve the clinical performance of the immunoassays (Tré-Hardy et al., 2020).

The results obtained for the Euroimmun IgA versus IgG are in line with those previously published highlighting a higher sensitivity for IgA versus IgG (Montesinos et al., 2020; Okba et al., 2020). Despite the use of a fully automated microtiter plate analyzer, the capacity of this non-random access assay is 90 tests per 3 hours only. Such a sample throughput is not compatible with massive testing. On the other hand, the Elecsys Roche kit assayed on a Cobas e801 is a fully automated random access test allowing to reach a capacity of 200 tests per hour with results delivered in 18 minutes.

Zhao et al. demonstrated a strong correlation between the disease severity and the antibody titer two weeks after disease onset (Zhao et al., 2020). On the other hand, Yongchen et al. underlined a different pattern of seroconversion between symptomatic and asymptomatic patients with only one (20%) out of 5 asymptomatic cases generating SARS-CoV-2 antibodies three weeks after the diagnosis. An earlier antibody response was also identified for severe COVID-19 patients. However, these data were obtained in a limited number of patients (i.e. 11 non-severe and 5 severe cases) (Yongchen et al., 2020). In this study, the detection rates of SARS-CoV-2 obtained using the different assays were compared according to the clinical presentation of the disease (i.e. none symptom, mild to moderate COVID-19 disease and severe COVID-19 disease). Although, no significant difference in sensitivity as well as serum antibody levels was underlined for mild/moderate versus severe patients, a lower detection rate was obtained for asymptomatic carriers regardless of the immunoassays used. This different pattern of seroconversion observed between asymptomatic versus severe symptomatic population is related to the lower median of days post positive qRT-PCR (asymptomatic patients) compared to the median of days post disease onset (symptomatic patients). The detection rates of immunoassays are indeed strongly impacted by the stage of the disease. These results were in agreement with those of To K et al. who did not observe a correlation between serum antibody levels and clinical severity (To et al., 2020).

The kinetics of SARS-CoV-2 antibodies appearance were highly variable among the patients studied. The results of the kinetics studies assessed in six patients were in agreement with the diagnostic efficiency previously reported for the Elecsys, the Liaison IgG, the Euroimmun IgG/IgA assays and the VIDAS IgG/IgM. The seroconversion was achieved earlier with the Elecsys, the Euroimmun IgA, and the VIDAS IgM tests for half of the studied patients.

This study presents some limitations. First, the median of sample time collection was 12 days, the results obtained for the diagnostic efficiency could have been different for a longer delay after the disease onset or the positive qRT-PCR. Second, all the patients included in this study have a positive SARS-CoV-2 qRT-PCR. Untypical antibody profiles might be missed for patients having a lower respiratory viral load with an overestimation of the sensitivity evaluated in this study.

To conclude, combining molecular diagnosis and serological tests could enhance the detection of infected individuals, an accurate diagnosis being essential to limit the spread of SARS-CoV-2 (Guo et al., 2020; Zhao et al., 2020). The choice of the most appropriate immunoassay for assessing SARS-CoV-2 seroconversion will be guided by its diagnostic efficiency but also by its capacity for samples throughput. The Roche Elecsys test showed a sensitivity of 81.1% with a specificity and PPV of 100%. Based on its important samples throughput, this immunoassay could be advised for assessing SARS-CoV-2 seroconversion in laboratories with a high demand for analysis. On the other hand, when combining the detection of IgG and IgM using the VIDAS diagnostic platform, a sensitivity of 78.7% was obtained by preserving the 100% specificity and PPV. This immunoassay uses unit tests with less problem of reagent stability and could be a good option for low demand laboratories.

Declarations of competing interest

None

Author statement

All authors contributed to the study conception, design, writing or reviewing. Material preparation was performed by Dahma Hafid and Cécile Duterme. Data collection and analysis, writing were performed by Fleur Wolff and Isabel Montesinos. Reviewing was performed by Sigi Van den Wijngaert, Olivier Vandenberg and Frédéric Cotton. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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