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Abstract

Objectives: Accurate SARS-CoV-2 serological assays are urgently needed to help diagnose infection, determine past exposure of populations and assess the response to future vaccines. The study aims at assessing the performance of the multiplex D-tek COVIDOT 5 IgG assay for the detection of SARS-CoV-2 IgG antibodies (N, S1+S2, S1, S2 and RBD).
Methods: Sensitivity and dynamic trend to seropositivity were evaluated in 218 samples obtained from 46 rRT-PCR confirmed COVID-19 patients. Non-SARS-CoV-2 sera (n=118) collected before the COVID-19 pandemic with a potential cross-reaction to the SARS-CoV-2 immunoassay were included in the specificity analysis.
Results: A gradual dynamic trend since symptom onset was observed for all IgG antibodies. Sensitivities before day 14 were suboptimal. At ≥21 days, sensitivities reached 100% (93.4–100%) for N, S1+S2, S2 and RBD-directed IgG and 96.3% (87.3–99.6%) for S1-directed IgG. In 42 out of 46 patients (91.3%), all five antibodies were detected at ≥14 days. The four remaining patients had between 2 and 4 positive antibodies at their respective maximal follow-up period. The specificity was 100 % for S1+S2, S2 and RBD, 98.3% for N and 92.4% (86.0–96.5%) for S1-directed IgG. The combined use of antigens increases the early sensitivity whilst enforcing high specificity.
Conclusions: Sensitivities at ≥21 days and specificities were excellent, especially for N, S1+S2, S2 and RBD-directed IgG. Caution is however required when interpreting single S1-directed reactivities. Using a multiplex assay complies with the orthogonal testing algorithm of the CDC and allows a better and critical interpretation of the serological status of a patient.

Keywords: COVID-19; kinetics; multiplex; SARS-CoV-2; serology.

Introduction

The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is responsible for the ongoing pandemic. As of the 7th of December, it has led to more than 65 million confirmed cases and to more than 1.5 millions deaths [1].

Currently, the gold standard method for the diagnosis of COVID-19 is detection of SARS-CoV-2 RNA in nasopharyngeal samples through real-time reverse transcription polymerase chain reaction (rRT-PCR), targets of which may include a combination of *N*, *E*, *RdRp*, *orf1a* and *orf1b* genes [2]. The detection of anti-SARS-CoV-2 antibodies represents an additional method for the diagnosis of COVID-19, especially in patients who present late, with a low viral load [3]. The combination of rRT-PCR and antibody detections significantly improved the sensitivity of pathogenic diagnosis for COVID-19 [4].

A wide range of serological immunoassays have been developed to complement the rRT-PCR, with different SARS-CoV-2 antigen targets and formats [2, 5–7]. The main SARS-CoV-2 antigens used are the nucleocapsid protein (N) and the spike protein (S) [6, 8–10]. The nucleocapsid participates in RNA package and virus particle release. The transmembrane spike glycoprotein comprises two functional subunits responsible for binding to the host cell receptor (*N*-terminal S1 subunit) and fusion of the viral and cellular membranes (*C*-terminal S2 subunit) [11, 12]. The receptor-binding domain (RBD) is located at the *C*-terminal region of the S1 subunit. Recombinant RBD has been shown to be sufficient to bind angiotensin-converting enzyme 2

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(ACE2) (cell entry receptor) [12]. Assays using the RBD-protein have also been developed [13, 14].

The performance of these assays varied because of the choice of the antigen for a particular target, the nature and structure of the target itself (purified vs. recombinant, full-length vs. truncated, eukaryotic vs. prokaryotic expression system), or the disparity of the patients cohorts [2, 3, 15, 16, 17, 18]. Furthermore, little is known about how antibody profiles across SARS-CoV-2 antigen specificities evolve early following infection and track differentially with disease trajectory. There is also a need for improvement of current serology immunoassays for detecting infection early after the symptom onset [19].

The aim of this study is to report the performance of the multiplex COVIDOT 5 IgG assay for the detection of SARS-CoV-2 total antibodies.

Materials and methods

Study design

This retrospective study has been conducted from June 4 to July 10, 2020, at the clinical biology laboratory of the University Hospital of Tivoli (CHU Tivoli, La Louvière, Belgium). Sera collected before the COVID-19 outbreak (between January and November 2019) were included in the specificity analysis (n=118). Case serum samples (n=218) with a confirmed rRT-PCR SARS-CoV-2 diagnosis were included in the sensitivity analysis and were obtained from 46 patients. Information on the days since the onset of symptoms was collected from the medical records. Only patients with at least two longitudinal sera samples and with a follow-up of at least 14 days since symptoms onset were included.

Sample collection

Blood samples were collected from patients into serum-gel tubes (BD SST II Advance®, Becton Dickinson, New Jersey, USA) or lithium-heparin plasma tubes (BD Vacutainer®) according to standardized operating procedure and manufacturer recommendations. Samples were centrifuged for 10 minutes at 1,740 × g (Sigma 3-16KL). Sera were stored in the laboratory serum biobank at −20 °C from collection date. Frozen samples were thawed during 1 hour at room temperature on the day of the analysis. Re-thawed samples were vortexed before the analysis.

Analytical procedures

The BlueDiver COVIDOT 5 IgG (D-tek sa, Mons, Belgium), a commercially available CE-marked enzyme immunoassay has been assessed for the *in vitro* semi-quantitative detection, in human sera or plasma, of IgG antibodies against SARS-COV-2 antigens. The assay is automated and performed on the BlueDiver Instrument. The BlueDiver COVIDOT 5 IgG is composed of 24 ready-to-use reagent cartridges and 24 multiplex strip tests that allow the simultaneous detection of antibodies targeting the five principal epitopes of the COVID-19: (1) the nucleocapsid (N) protein, (2) the spike (S) protein (ECD, ectodomain, S1+S2), (3) the S1 subunit of the S-protein, (4) the S2 subunit of the S-protein and (5) the Receptor Binding Domain (RBD) of the S1 subunit. The test strips are made of a plastic backing covered with nitrocellulose on which the antigens are coated. A schematic representation of a COVIDOT-5 IgG test strip is presented in Supplementary Material 1. During the automated test procedure, the BlueDiver Instrument sequentially incubates the strips in the wells of ready-to-use reagent cartridges. The strips are incubated with diluted patients' sera (10 µL of sample is required and diluted according to the instrument sequence using the diluent buffer provided in the kit). Enzyme activity, if present, leads to the development of purple dots on the membrane pads. The intensity of the coloration is directly proportional to the amount of antibody present in the sample. The Dr DOT software measures the color intensity by converting into Dr Dot Arbitrary Units (numeric values ranging from 0 [negative result] to 100 [high positive result]). Each strip contains the above-mentioned antigens plus 2 built-in controls (positive and negative). For each individual antigen, a result <5 arbitrary units (AU) is considered non-reactive (or negative), a result between 5 and 10 is considered doubtful, and a result >10 is considered reactive (or positive) [20]. A doubtful result was considered positive in our evaluation. The test is positive if at least one antibody is positive, whatever the antibody. The coefficient of variation obtained with low and high positive samples ranged from 1.8 to 9.9% [20].

The rRT-PCR for SARS-CoV-2 determination in respiratory samples (nasopharyngeal swab samples) was performed with the GeneFinder COVID-19 Plus RealAmpl kit (Osang Healthcare Co., Ltd) or with the Xpert Xpress SARS-CoV-2 kit (Cepheid). A dipstick immunochromatographic test (COVID-19 Ag Respi-Strip, Coris BioConcept, Gembloux, Belgium) designed to detect SARS-CoV-2 antigen in nasopharyngeal secretions was also used for the inclusion of two patients. This rapid test has been shown to be 100% specific compared to rRT-PCR [21].

Clinical specificity

Non-SARS-CoV-2 sera (n=118) with a potential cross-reaction to the SARS-CoV-2 immunoassay were analyzed. Samples included positive antinuclear antibodies (n=6), hepatitis B Ag (n=13), hepatitis C antibodies (n=6), IgM cytomegalovirus (n=6), IgM Epstein–Barr virus viral capsid (n=14), IgM *Mycoplasma pneumoniae* (n=19), IgM polyclonal activation (n=1), IgM *Toxoplasma gondii* (n=4), IgG monoclonal components (n=2), rheumatoid factor (n=4), pregnant women (n=6), random sera (n=11) and dialyzed patients (n=26) were also included for the specificity calculation.

Clinical sensitivity

Sera of 46 patients obtained at different time points since the onset of COVID-19 symptoms were used to calculate the clinical sensitivity. The demographic data of this population are presented in Table 1. From these 46 patients, a total of 218 serum samples were available. A minimal follow-up of at least 14 days was required [4, 22]. Samples were subdivided according to the following categories: 0–6 days: 43 sera; 7–13 days: 59 sera; 14–20 days: 62 sera; ≥21 days: 54 sera. The maximal follow-up time was 53 days since symptom onset.

Table 1: Demographic data of the COVID-19 population included.

Demography	
Age, mean (min–max)	63.7 (38.0–93.0)
Males, n (%)	28 (61%)
Females, n (%)	18 (39%)
Delay between symptoms and RT-PCR, median (min–max)	5 (0–14)
Number of blood sampling per patient, median (min–max)	4 (2–10)
Hospitalized (non ICU), n (%)	25 (54%)
Hospitalized (ICU), n (%)	21 (46%)

Dynamic trend to seropositivity

The average dynamic trend to seropositivity was evaluated using all serum samples since symptom onset. Samples were subdivided according to following categories: 0–6 days: 43 sera; 7–9 days: 24 sera; 10–12 days: 33 sera; 13–15 days: 42 sera; 16–21 days: 37 sera; >21 days: 39 sera.

Statistical analysis

Descriptive statistics were used to analyze the data. Sensitivity was defined as the proportion of correctly identified COVID-19 positive patients initially positive by rRT-PCR SARS-CoV-2 determination in respiratory samples and with COVID-19 symptoms. Specificity was defined as the proportion of naïve patients classified as negative. A ROC curve analysis was performed to determine the possibility of defining adapted cut-offs to improve clinical performance [23–25]. Samples included for ROC curves analyses were sera obtained from at least 14 days since symptom onset (n=116) and sera from the specificity study (n=118) (Supplementary Material 2). A heatmap was used to visualize the evolution of the number of positive antibodies (from 0 to 5 different antibodies) since the onset of symptoms for each patient. Inter-rater agreement (Cohen's kappa) was also evaluated. Data analysis was performed using GraphPad Prism® software (version 9.0.0, CA, USA) and MedCalc® software (version 14.8.1, Ostend, Belgium). The study protocol was in accordance with the Declaration of Helsinki and was approved by the Medical Ethical Committee of the CHU Tivoli (approval number 1351).

Results

Clinical specificity

Using the manufacturer's cut-off (i.e. AU >5), the specificity (95% confidence interval [CI]) for the five different IgG antibodies varied between 92.4% (86.0–96.5%) and 100% (96.9–100%). When antigens were considered separately, a specificity of 100% was observed for S2, S1+S2 and RBD-directed IgG and of 98.3% for N-directed IgG. The lowest specificity was observed with the S1-directed IgG (9 false positive results out of the 118 samples: 92.4% specificity). When all antigens were considered together, the cumulative specificity for the 5 antibodies was 90.7% (83.4–95.3%) or 98.3% (94.0–99.8%) if the contribution of S1-directed IgG was excluded. Using ROC curve adapted cut-offs increased the specificity for N (AU >7) and S1+S2-directed antibodies (AU >12) from 97.5 to 98.3% and from 98.3 to 100%, respectively (Table 2). ROC curve adapted cut-offs were equal to the manufacturer's lower cut-off for other antigens (i.e. AU >5). Noteworthy is the fact that all false positive samples were positive to only one antigen (Figure 1).

Clinical sensitivity

The sensitivity (95% CI) of each antibody during the first week since symptom onset (0–6 days) was low (<50%) and

Table 2: Overall diagnostic performance of the different IgG assays taken separately or combined (with or without S1 antigen).

	n samples	N	S1+S2	S1	S2	RBD	≥1 antibody	≥1 antibody*
Sensitivity (95% CI)	218	75.2% (69.0–80.8)	69.3% (62.7–75.3)	58.3% (51.4–64.9)	75.7% (69.4–81.2)	65.6% (58.9–71.9)	83.0% (77.4–87.8)	77.9% (71.9–83.3)
Day 0–6	43	26.1% (14.3–41.1)	16.3% (6.8–30.7)	16.3% (6.8–30.7)	27.9% (15.3–43.7)	11.6% (3.9–25.1)	46.5% (31.2–62.4)	37.2% (21.0–50.9)
Day 7–13	59	67.8% (54.4–79.4)	52.5% (39.1–67.7)	40.7% (28.1–54.3)	74.6% (61.6–85.0)	54.2% (40.8–67.3)	83.1% (71.0–91.6)	81.4% (61.9–90.3)
Day 14–20	62	91.9% (82.2–97.3)	85.5% (74.2–93.1)	71.0% (58.1–81.8)	88.7% (78.1–95.3)	83.9% (72.3–92.0)	93.6% (84.3–98.1)	96.3% (84.3–98.1)
Day ≥21	54	100% (93.4–100)	100% (93.4–100)	96.3% (87.3–99.6)	100% (93.4–100)	100% (93.4–100)	100% (93.4–100)	100% (93.4–100)
Specificity (95% CI)	118	98.3% (94.0–99.8)	100% (96.9–100)	92.4% (86.0–96.5)	100% (96.9–100)	100% (96.9–100)	90.7% (83.4–95.3)	98.3% (94.0–99.8)

ROC curve adapted cut-offs have been used for N and S1+S2-directed IgG (>7 and >12, respectively). The cut-off of 5 AU was used for S1, S2 and RDB-directed IgG.

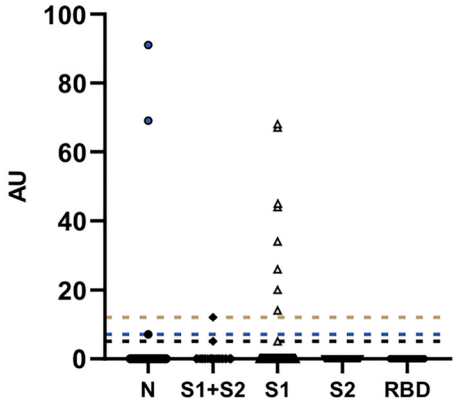


Figure 1: Cross-reactivity of the multiplex COVIDOT 5 IgG assay to non-SARS-CoV-2 sera (n=118).
Manufacturer's threshold is represented with a black dotted line (AU=5). The blue dotted line corresponded to the ROC curve adapted cut-off for N-directed IgG and blue points are false positive results (patients having hepatitis C antibodies or IgM *Toxoplasma gondii*). The brown dotted line corresponded to the ROC curve adapted cut-off for S1+S1-directed IgG. Beige points are false positive results for S1-directed antibodies (patients under dialysis (n=3), or positives for IgM *Toxoplasma gondii*, hepatitis B Ag (n=2), IgM *Mycoplasma pneumoniae* (n=2) or antinuclear antibodies). Black points are considered true negative results.

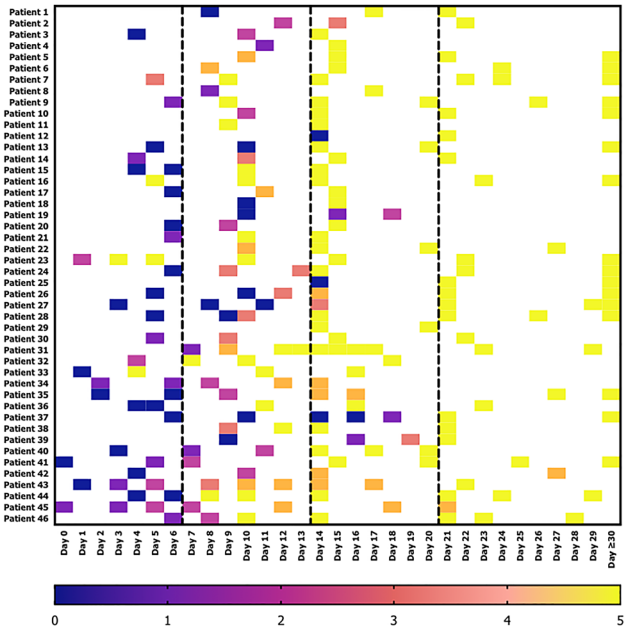


Figure 2: Heatmap of the dynamic trend to seropositivity for each patient.

ranged from 11.6% (3.9–25.1%) for RBD-directed IgG to 37.9% (15.3–43.7%) for N-directed IgG. The sensitivity increased during the second week (7–13 days) to achieve at least 50% except for S1-directed IgG (40.7% [28.1–54.3%]). During the third week (14–20 days), the sensitivity of S1-directed IgG was still the lowest (71.0% (58.1–81.8%)) while other antibodies had a sensitivity ranging from 83.9% (72.3–92.0%) for RBD to 91.9% (82.2–97.3%) for N-directed IgG. From 21 days since symptom onset, sensitivities reached 100% (93.4–100%) for all antibodies except for S1-directed IgG (96.3% [87.3–99.6%]) (Table 2). Interestingly, a substantial between-individual variation was observed in the antibody response generated following SARS-CoV-2 infection, and patients exhibited various antibody signatures over the first three weeks after symptoms onset (Figure 2). If considering that at least one antibody, whichever 1 out of 5, was sufficient to attest positivity, the sensitivity increased significantly, compared to any particular mono-plex interpretation, to 46.5% (31.2–62.4%), 83.1% (71.0–91.6%) and 93.6% (84.3–98.1%) at weeks 1, 2 and 3, respectively. Excluding the particular anti-S1 reactivity, if at least one antibody out of N, S1+S2, S2 or RBD-directed IgG was required for positivity, the sensitivity was 37.2% (23.0–53.4%), 81.4% (69.1–90.3%) and 93.6% (84.3–98.1%) at weeks 1, 2 and 3, respectively. However, the 100% sensitivity was not reached significantly earlier with the multiplex algorithm for interpretation (at least one antibody positive) as compared to any antibody considered separately, since all antibodies (except S1-directed IgG) were positive in all tested samples from three weeks after symptoms (Table 2). Using ROC curve adapted cut-offs did not have any impact on sensitivities from 14 days since symptom onset. The inter-rater agreement between the different antibodies varied from 0.48 (S1 vs. S2; moderate agreement) to 0.82 (S1+S2 vs. S2; almost perfect agreement) (Table 3).

Dynamic trend to seropositivity

A gradual increase in antibody titers (AU) and positivity rates (%) since symptom onset was observed for all IgG

Table 3: Inter-rater agreement (Cohen's kappa) between the different assays in COVID-19 patients (218 samples for sensitivity [95% confidence interval]).

	S1+S2	S1	S2	RBD
N	0.71 (0.60–0.81)	0.50 (0.39–0.60)	0.68 (0.57–0.79)	0.70 (0.59–0.80)
S1+S2		0.63 (0.51–0.73)	0.82 (0.73–0.90)	0.81 (0.73–0.90)
S1			0.48 (0.37–0.58)	0.65 (0.56–0.75)
S2				0.65 (0.54–0.76)

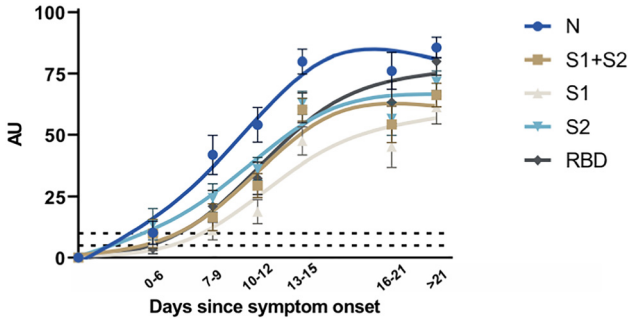


Figure 3: Dynamic trend of absolute signal for each IgG in 218 samples from 46 patients.
Manufacturer's thresholds are represented with dotted lines (5 and 10 AU).

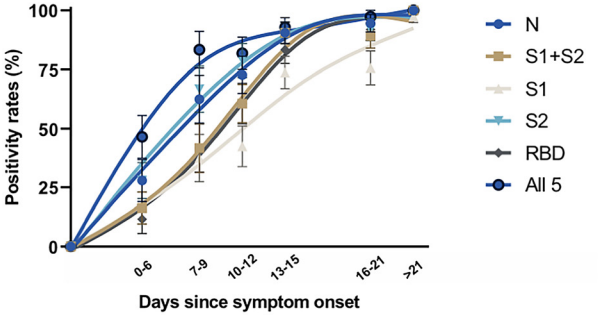
antibodies. In 42 out of 46 patients (91.3%), the full spectrum of all 5 antibodies was detected at ≥14 days. Higher titers were observed throughout the antibody kinetics for N-directed IgG while lower titers were observed for S1-directed IgG (Figure 3). A delayed increase in positivity rates was also observed in particular for S1-directed IgG (Figure 4).

Discussion

This study is the first to report the performance of the multiplex COVIDOT 5 IgG assay for the detection of five different SARS-CoV-2 IgG antibodies. Sensitivities at ≥21 days since symptom onset were excellent. However, sensitivities in the early phase of symptom onset were still too low to be used in clinical practice to confirm COVID-19 on the sole basis of serology testing, as confirmed in other studies [6, 14, 26].

There is accumulative data about the clinical performance of SARS-CoV-2 immunoassays [6, 14, 24, 27, 28]. These evaluations focused on the evaluation of mono-antigenic assays or, to a lesser extent, bi-antigenic assays. Currently, commercial serological assays are using the N antigen (Roche, Abbott, Euroimmun) [5, 6, 23, 24, 29], the S1 antigen (Euroimmun) [5, 30, 31], the S1+S2 antigen (DiaSorin) [5, 24, 30], the RBD antigen (Siemens, Wantai) [13, 14, 32] or a combination of both N and S antigens (iFlash, Maglumi, Mikrogen) [6, 24, 33]. Correlation between assays, using the same antigens or not, are often suboptimal and highlights a lack of harmonization for the detection of anti-SARS-CoV-2 antibodies [6, 14, 28, 34]. Overall, serological assays are mostly using N- or S-proteins [6, 8]. However, the RBD antigen represents a promising antigen because it is poorly conserved between SARS-CoVs and other pathogenic human coronaviruses [8], and is therefore less likely to cross-react in serological antibody tests. Furthermore, a strong correlation between levels of RBD binding antibodies and SARS-CoV-2 neutralizing antibodies in patients has been found [8].

So far, only very few studies have explored the possibility of using multiplex methods for SARS-CoV-2 antibodies [25, 35–38] and different technologies have been



	Days since symptom onset	0-6	7-9	10-12	13-15	16-21	≥21
	n	43	24	33	42	37	39
N	n positive (>7 AU)	12	15	24	38	35	39
	Positivity rate (%)	27.9	62.5	72.7	90.5	94.6	100
S1+S2	n positive (≥5 AU)	7	10	20	38	33	39
	Positivity rate (%)	16.3	41.7	60.6	90.5	89.2	100
S1	n positive (>12 AU)	7	9	14	31	28	38
	Positivity rate (%)	16.3	37.5	42.4	73.8	75.7	97.4
S2	n positive (≥5 AU)	12	16	26	38	34	39
	Positivity rate (%)	27.9	66.7	78.8	90.5	91.9	100
RBD	n positive (≥5 AU)	5	10	20	35	34	39
	Positivity rate (%)	11.6	41.7	60.6	83.3	91.9	100
≥ 1 antibody	n positive	20	20	27	39	36	39
	Positivity rate (%)	46.5	83.3	81.8	92.9	97.3	100

Figure 4: Dynamic trend to seropositivity for each IgG in 218 samples from 46 patients.

used, namely Luminex-based assay (N and S antigens) [35], protein micro-array assay (N and S antigens) [37], bead-based immune assay (S1, RBD and N antigens) [25], and solid-phase chemiluminescent assay (trimeric S, S1, RBD and N antigens) [36]. Noteworthy, the COVIDOT is the only multiplex assay that also target the S2 antigen.

There are several advantages and perspectives of using a multiplex method for the detection of anti-SARS-CoV-2 antibodies. We identified four different situations where it can be useful.

Advantage #1: improving clinical sensitivity

In our cohort, 42 patients out of 46 (91.3%) developed antibodies for the 5 antigens ≥14 days since symptom onset. Four patients developed ≤4 antibodies at their respective maximal follow-up periods. The first patient had only 2 different antibodies (N and RBD-directed IgG). The second and the third had N, S2, S1+S2, and RBD-directed IgG and the last had N, S1, S2, and S1+S2-directed IgG (Figure 4). If these patients had been analyzed with a mono-antigenic assay (against S1 or RBD), this would have classified these patients as negative. Patients that did not develop such antibodies have been described elsewhere [32, 34].

The patient with only two antibodies 18 days since symptom onset is not likely to be a false positive result because no false positive has been observed for RBD in our specificity evaluation and because an increase in N titers has been observed between day 15 (AU=51) and 18 (AU=86). It is possible that patients only presented N and RBD and not S1-directed IgG. Additionally, the cumulative detection of multiple antibodies significantly improved the sensitivity in the early phase since symptom onset (Table 2).

Advantage #2: improving clinical specificity

Multiplex methods increase the overall specificity in a testing workflow [25, 35], which is in line with the orthogonal testing algorithm proposed by the CDC [39]. The CDC algorithm has been used for large serological studies, and false positive results could have been identified [40–42]. A recent study also found that orthogonal test strategies improved the clinical specificity because false positive results across five different platforms were assay-specific [28]. Other reports also described assay-specific false positive results [7, 30, 43]. Having five different targets in the same run and on the same platform might therefore be ideal to identify real false positive patients [5, 6]. In our study, no pre-COVID-19 samples cross-reacted simultaneously with two different targets (Figure 1) while positive patients mostly had the five different antibodies 14 days since symptom onset (Figures 2, 4). Multiplex assays could therefore dispense the use of other analyses to confirm each positive patient and is relevant for seroprevalence studies. This approach might also improve the turn-around time and decrease overall costs in a routine testing workflow.

Advantage #3: vaccination

Multiplex methods could face the future widespread of vaccines by measuring the vaccine response. Because most vaccines will use the S protein or S-domains as immunogen [44], assays targeting the N-protein would therefore not be a good candidate to evaluate the vaccine response [45]. Having only assays targeting the S-protein might, however, be misleading in some situations. If a patient develops COVID-19-related symptoms following the vaccination, an assay with multiple targets might differentiate neo-COVID-19 infection from side effects due to the vaccination (i.e. flu-like syndrome). The rise of N-directed antibodies, in addition to S-directed antibodies, following natural SARS-CoV-2 infection, might be usefully tested is this situation.

Advantage #4: prediction of disease outcome

Recently, Atyeo et al. found that convalescent individuals developed a response mainly focused towards the spike protein, whereas deceased individuals developed a response mainly focused on the nucleocapsid protein [46]. RBD-specific responses were also more present in deceased individuals. The utilization of a multiplex method paves the way to define an antibody signature that differentiates disease trajectory and outcome. The clinical significance of variable antibody signatures, i.e. the presence or the absence of multiple subtypes of antibodies against the different antigens of the virus, remains to be determined.

Our study has some limitations. Because of the retrospective design of our study, the number of samples per patient was not harmonized and the follow-up of some patients was longer compared to others. Nevertheless, we decided to only include patients with a minimal follow-up period of 14 days. We were also not able to compare the COVIDOT 5 IgG multiplex assay to mono-specific assays and to correlate our result with a neutralization assay. We

also further highlight the need to evaluate the long-term kinetics of the different antibody responses. In this context, a multiplex method might also be of interest.

Conclusions

This study is the first to report the clinical performance of a multiplex assay for the simultaneous detection of five different SARS-CoV-2 antibodies. Sensitivities at ≥21 days or more since symptom onset were 100% for N, S1+S2, S2 and RBD-directed IgG with specificities ranging from 98.3 to 100%. The specificity of S1-directed IgG was however moderate (92.4%) and may require some caution when interpreting single S1-directed reactivities. Having multiple antigen targets in one assay complies with the orthogonal testing algorithm of the CDC and allows a better and critical interpretation of the serological status of a patient. Research perspectives are also promising especially in the field of vaccination or in predicting the disease trajectory and outcome.

Supplementary Material 1

Schematic representation of a COVIDOT-5 IgG test strip.

Supplementary Material 2

ROC curve analysis on the five different antigens.

Samples included for ROC curves analyses were sera obtained from ≥14 days since symptom onset (n=116) and sera from the specificity study (n=118).

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Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: The study protocol was in accordance with the Declaration of Helsinki and was approved by the Medical Ethical Committee of the CHU Tivoli (approval number 1351).

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