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Journal of Virological Methods 289 (2021) 114060

Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

Analytical and clinical evaluation of four commercial SARS-CoV-2 serological immunoassays in hospitalized patients and ambulatory individuals

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ARTICLE INFO

Keywords:
SARS-CoV-2
Antibody
CLIA
Validation
COVID-19
Serology

ABSTRACT

Background: This study aimed to compare four anti-SARS-CoV-2 immunoassays in populations presenting different clinical severity levels.
Methods: Three populations were included: "severe-to-critical" ICU-hospitalized patients (n = 18), "mild-to-moderate" hospitalized patients (n = 16) and non-hospitalized symptomatic patients (n = 24). Four commercial immunoassays were analyzed and validated: anti-IgG ARCHITECT® (Abbott), anti-Total antibodies (Ab) VITROS® (Ortho Clinical Diagnostics), anti-IgG NovaLisa® (NovaTec Immundiagnostica) and Healgen® IgM and IgG (Zhejiang Orient Gene Biotech). Sensitivities were evaluated according to days post-symptoms onset (pso). Specificities were evaluated on SARS-CoV-2-negative control sera collected before January 2020.
Results: A majority of severe-to-critically ill patients showed detectable Ab already at day 14 and sensitivities reached 100 % after 22 days pso. For patients with "mild-to-moderate" illness, sensitivities increased by at least 5-fold from day 0 to day 14 pso. Non-hospitalized symptomatic individuals already seroconverted at day 14 days pso with 100 % sensitivities for Total Ab VITROS®. Specificities were evaluated at 97 % for ARCHITECT® and NovaLisa®, 98 % for VITROS® and at 94 % for Healgen® combined IgM and IgG. Five "severe-to-critically" ill patients presented high positive Ab levels for at least 16 weeks pso.
Conclusion: The Ab levels and the evaluated sensitivities, representing the true positive rate, increased overtime and were related to the COVID-19 severity. Automated Total Ab immunoassay showed better sensitivities and specificity for immunological surveillance and vaccine evaluation.

1. Introduction

The emergence of a novel virus named SARS coronavirus 2 (SARS-CoV-2) late 2019 led to a pandemic coronavirus disease 2019 (COVID-19) with wide clinical presentations (WHO, 2020; Zhu et al., 2020; Sun et al., 2020; CDC, 2020a). The primary diagnostic tool for active infection is the detection of SARS-CoV-2 by RT-qPCR performed on respiratory specimens (Sethuraman et al., 2020). However, the sensitivity is largely impacted by the diagnostic testing window which may yield false negatives in approximately 20 % of cases, e.g., caused by variable viral shedding at different timepoints (Bohn et al., 2020; Gandhi et al., 2020; Sethuraman et al., 2020).

Unlike direct virological detection, serological tests can detect past COVID-19 infection even though the tested person did not develop

Abbreviations: Ab, antibody; AUC, area under the curve; CLIA, chemiluminescent immunoassay; CLSI, Clinical and Laboratory Standards Institute; COFRAC, Comité Français d'Accréditation; COVID-19, Coronavirus disease 2019; ELISA, enzyme-linked immunosorbent assay; ICU, intensive care unit; LFA, lateral flow assay; LOD, limit of detection; LOQ, limit of quantification; N, nucleocapsideprotein; NTU, NovaTec unit; Ppp, post-positive RT-qPCR; Pso, Post-symptoms' onset; ROC, receiver operator characteristic; RT-qPCR, reverse transcriptase-quantitative polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; S/C, signal on cut-off; WHO, World Health Organization.

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https://doi.org/10.1016/j.jviromet.2020.114060
Received 3 August 2020; Received in revised form 21 December 2020; Accepted 21 December 2020
Available online 24 December 2020
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symptoms or was not detected by molecular assays. The need for serological testing led to numerous rapidly developed and early commercialized tests, which were insufficiently characterized and evaluated (Bohn et al., 2020; Farnsworth and Anderson, 2020; Ismail, 2020).

Different types of assays can be used to detect binding antibodies (Ab): fully automated chemiluminescent immunoassay (CLIA), enzyme-linked immunosorbent assay (ELISA) and rapid immunochromatographic lateral flow assay (LFA). The two major antigenic targets for serological tests are the nucleocapsid phosphoprotein (N-protein) and the S (Spike) glycoprotein (S1 and S2 subunits) (Lu et al., 2020; Meyer et al., 2014; Qiu et al., 2005).

The use of well-characterized serological tests might provide key information for evaluating the transmission rate within a local community or a specific patient population and for evaluating host humoral immune response in previously infected individuals (Bermingham et al., 2020; CDC, 2020b). However it must be underlined that the presence and the persistence of immunity remain unknown and the possibility of re-infection or disease chronicity needs to be elucidated as well (Winter and Hegde, 2020). The patient population chosen for evaluating a serological test is an important parameter (Bohn et al., 2020). To date, serological studies on non-hospitalized symptomatic individuals are scare and mostly with limited sample numbers (Montesinos et al., 2020; Plebani et al., 2020; Theel et al., 2020; Yongchen et al., 2020).

This study aimed to analytically validate two automated CLIAs detecting anti-SARS-CoV-2 and commercialized by Abbott (ARCHITECT®, anti-IgG) and by Ortho Clinical Diagnostics (VITROS®, anti-Total Abs). The study compared the seroconversion profiles between both CLIAs, an ELISA (NovaLisa®, anti-IgG) and a rapid LFA (Healgen®, anti-IgM and IgG) since the day post-symptoms onset (pso) in hospitalized and ambulatory patients.

2. Material and methods

2.1. Sample collection

This retrospective study included samples collected from March 18th to May 1st, 2020 at CHU UCL Namur hospital during the COVID-19 pandemic. All individuals considered in this study were confirmed positive for COVID-19 infection by RT-qPCR targeting SARS-CoV-2 (AllplexTM 2019-nCoV Assay, Seegene) performed on nasopharyngeal swab samples and admitted in our hospital. Positive patients admitted to the intensive care unit (ICU) with endotracheal intubation were categorized as the group presenting a "severe-to-critical" form of the illness ($n = 22$). Secondly, positive hospitalized patients presenting a favorable evolution with a rapid withdrawal of oxygen therapy (< 10 days) were grouped into the "moderate-to-mild" category ($n = 30$) and finally, the third group included symptomatic healthcare professionals not requiring oxygen therapy nor hospitalization ($n = 37$). Only positive individuals with time of symptoms onset reported in the medical records were included in this study, representing 18 "severe-to-critically" ill patients, 16 "mild-to-moderately" ill patients and 24 healthcare professionals. This study was approved by the Ethics Committee of the CHU (agreement: CE Mont-Godinne 86/2020).

Blood samples were collected in serum tube (CAT serum Sep Clot Activator, VACUETTE®, Greiner Bio-One, Vilvoorde, Belgium), then centrifuged at 1500*g* for 10 minutes at room temperature. Residual serum samples from routine laboratory testing were used for the hospitalized patient groups and distributed according the days post-symptoms onset: week (W) 0 (0–6 days), W1 (7–13 days), W2 (14–20 days), W3 (21–27 days), W4 (28–34 days). Five "severe-to-critically" ill patients were followed for at least 16 weeks post-symptoms onset (> 112 days). Positive healthcare professionals were sampled at W2 (14–20 days) and at W4 (28–34 days). All aliquots were stored at −80 °C.

SARS-CoV-2-negative control sera were thawed from a collection stored at −20 °C before January 2020 (preceding COVID-19 outbreak in Belgium). A total of 90 sera from immune or infected patients with positive Ab for various viruses (human immunodeficiency virus [HIV], cytomegalovirus [CMV], hepatitis B virus [HB], hepatitis C virus [HCV], Epstein-Barr virus [EBV], parvovirus B19, herpes viruses), bacteria (*Mycoplasma pneumoniae*) or parasite (*Toxoplasma gondii)* were included to assess the cross-reactivity. Ten samples from patients with a pathological level of rheumatoid factor (RF) (> 12 IU/mL) were included.

2.2. Anti-SARS-CoV-2 assays

The four anti-SARS-CoV-2 assays are described in Table 1. In brief, the first assay was the fully automated measurement of anti-SARS-CoV-2 Total Ab using VITROS® 5600 integrated system (Ortho Clinical Diagnostics, USA). The reactive cut-off threshold was set at ≥ 1 index. The second immunoassay was the fully automated SARS-CoV-2 IgG assay on the ARCHITECT® *i*1000SR System (Abbott, USA). The positive cut-off threshold was set at ≥ 1.4 index. The third assay was the qualitative determination of anti-SARS-CoV-2 IgG based on an ELISA (NovaLisa®, SARS-CoV-2 IgG, NovaTec Immundiagnostica GmbH, Germany). The positive cut-off threshold was set at > 11 NovaTec Unit (NTU). The fourth assay was an immunochromatographic LFA using a COVID-19 IgG and IgM Rapid Test Cassette (Healgen®, Zhejiang Orient Gene Biotech Co Ltd, China). Results were interpreted visually by the appearance of colored lines on the IgM and/or IgG position (positive/negative) in addition to a color change (from blue to red) at the control line to validate the result. Each sample was analyzed by the four different methods, according the manufacturers' instructions, on the same day.

2.3. Analytical evaluation of the two fully automated immunoassays

The analytical validation procedure for the VITROS® and the ARCHITECT® was performed as semi-quantitative assay (based on the signal on cut-off ratio) in accordance with the Clinical and Laboratory Standards Institute (CLSI) ("EP15A3 User Verification of Precision & Bias Estimation, " n.d.) and Comité Français d'Accréditation (COFRAC) ("Guide de vérification/validation des méthodes en Biologie Médicale, " n.d.).

The trueness, referring to the closeness of agreement between the mean of test results and the accepted reference value, was established by comparing the mean value of 30 replicates of two levels quality controls (one positive/reactive and one negative/non-reactive, provided by the manufacturers) to the value indicated by the manufacturer, i.e. ≤0.78 for negative control and 1.65–8.40 for positive control for ARCHITECT® and < 1 for non-reactive control and ≥ 1 for "reactive" control for VITROS®.

The evaluation of precision included the within-run precision, corresponding to 30 replicates of the two levels quality controls, provided by the manufacturer, on the same day, and the between-run precision corresponding to 15 independent measurements of the two levels quality controls, tested in duplicate.

Water for injection was used as blank sample to determine the limit of detection (LoD) and the limit of quantification (LoQ). They were performed by running 30 replicates of blank sample and estimated by following equations ("Guide de vérification/validation des méthodes en Biologie Médicale, " n.d.): LoD = mean + 3*standard deviation (SD) and LoQ = mean + 10*SD.

The range of linearity of analytical response was determined in triplicate by a two-fold serial dilution (1:2 to 1:4098) from a positive patient sample with high Ab index.

The possibility of carry-over was verified by switching between positive sample and negative sample. A positive (Pos) sample was run in triplicate (Pos1, Pos2, Pos3) followed by a negative (Neg) sample run in triplicate (Neg1, Neg2, Neg3) for 5 days and carry-over was calculated as: (mean Neg1-mean Neg3)/(mean Pos-mean Neg3)*100.

Table 1
Description of the four anti-SARS-CoV-2 assays. CLIA (chemiluminescent immunoassay), ELISA (enzyme-linked immunosorbent assay), LFA (rapid immunochromatographic lateral flow assay), NTU (NovaTec Unit), N (nucleocapsid).

Manufacturer	Ortho Clinical Diagnostics	Abbott	NovaTec Immundiagnostica	Zhejiang Orient Gene Biotech
Assay name	anti-SARS-CoV-2 Total Ab	SARS-CoV-2 IgG assay	NovaLisa® SARS-CoV-2 IgG	COVID-19 IgG and IgM Rapid Test Cassette (Healgen®)
Assay type	CLIA	CLIA	ELISA	LFA
Automated systems	VITROS® 5600 integrated system	ARCHITECT® *i*1000SR System	Manual	Manual
Measurement	Semi-quantitative	Semi-quantitative	Qualitative	Qualitative
Antigenic target	S (S1 subunit)	N	N	Not applicable
Antibodies detected	IgA, IgM and IgG	IgG	IgG	IgM and IgG
Positive/reactive threshold	≥ 1 index	≥ 1.4 index	> 11 NTU	Colored line

2.4. Clinical evaluation and comparison between automated CLIAs, ELISA and rapid LFA

The sensitivity was assessed at different timepoints since the day post-symptoms onset for a total of 32 hospitalized patients and sampled as follows: W0 (n = 16), W1 (n = 30), W2 (n = 32), W3 (n = 25), W4 (n = 10), W5 (n = 10) and a total of 24 non-hospitalized individuals with n = 10 at W2 and n = 24 at W4. Among these collections, 4 serial serum samples for hospitalized patients or paired samples for non-hospitalized groups were used for investigating the kinetics and the longitudinal Ab changes.The specificity was evaluated on non-SARS-CoV-2 control sera (n = 100), as described in the **2.1. Sample collection**. We were not able to test specificity or the cross-reactivity with sera from patients infected by the most common human coronaviruses other than SARS-CoV-2.

2.5. Statistical analyses

Statistical analyses were performed using Medcalc (MedCalc Software Ltd, Ostend, Belgium) and Prism (GraphPad Software, CA, USA). Sensitivities and specificities were calculated for all anti-SARS-CoV-2 assays. The clinical evaluation was examined by constructing a receiver operator characteristic (ROC) curve and by comparing the area under the curves (AUC).A p-value < 0.05 was considered as statistically significant.

3. Results

3.1. Analytical performance of two automated CLIAs

All analytical results for the validation are reported in Table 2. The trueness was within the range reported by the manufacturers, as shown by a mean index of 3.64 ± 0.07 for the positive quality control and a mean index of 0.07 ± 0.00 for negative quality control for ARCHITECT®. For the VITROS®, reactive quality control reported an index of 3.91 ± 0.09 and non-reactive quality control a mean index of 0.04 ± 0.01. Non-reactive quality control for VITROS® (0.04 ± 0.00) was below the LoD (0.05) and LoQ (0.07). The evaluation of with-in and between-runs CVs for non-reactive quality control was performed on non-reactive quality control supplemented with 10 % (v/v) of positive quality control. Both CLIAs presented a polynomial analytical response ($R^2 = 0.99$) and an acceptable carry-over rate according the CLSI and COFRAC recommendations (Table 2).

Table 2
Analytical evaluation of two automated immunoassays performed on ARCHITECT® and on VITROS®. Data are expressed as mean index (signal/cut-off) ± SEM (standard error of mean). Precision profile and carry-over are expressed in percentage. CT (control).

Analytical parameters	ARCHITECT® anti-SARS-CoV-2 IgG		VITROS® anti-SARS-CoV-2 Total	
	Negative CT	Positive CT	Non-reactive CT	Reactive CT
Limits of				
Detection (index)	0.00		0.05	
Quantification (index)	0.00		0.07	
Trueness (index)	0.07 ± 0.00	3.64 ± 0.07	0.04 ± 0.01	3.91 ± 0.09
Precision				
With-in run (CV %)	6.7	1.9	5.7	2.3
Between-run (CV %)	9.2	2.8	10.2	3.3
Linearity				
Index range	From 0.06 to 6		From 0.06 to 196	
Equation	y = -0,3014x + 1,2213		y = -0,3277x + 3,7286	
R^2	0.99		0.99	
Carry-over (%)	0.04		0.00	

3.2. Seroconversion profiles and comparison between automated CLIAs, ELISA and LFA

Test sensitivities are summarized in Table 3 with the symptoms onset as the reference timepoint. True positive rates (sensitivities) and positive Ab levels in hospitalized groups increased overtime in all immunoassays. Overall, immunoassays detecting more than one type of Ab showed higher sensitivities in hospitalized groups from 7 days pso as compared to anti-IgG immunoassays. Sensitivities reached 100 % after 21 days of symptoms onset with all immunoassays. In non-hospitalized symptomatic group, immunoassays with more than one type of Ab detected showed a 100 % (95 % CI 69.2–100) sensitivity from 14 days post-symptoms onset.

VITROS® presented a specificity of 98 % (95 % CI 93–99.7), ARCHITECT® and NovaLisa®, a specificity of 97 % each (95 % CI 91.5–99.4). Healgen® combined IgM and IgG showed the lowest specificity of 94 % (95 % CI 87.4–97.8). Samples giving false-positive results by anti-IgM were different from those by anti-IgG (see Table 4). Nine samples were misclassified as positive/reactive (false-positive) by at least one of the tested methods: HIV positive serum (n = 1 with ARCHITECT®), serum containing CMV IgM (n = 1 with NovaLisa®), HBs antigen (n = 1 with ARCHITECT®), HCV Ab (n = 1 for Healgen® IgM and IgG), parvovirus B19 IgM and IgG (n = 2 with Healgen® IgM and IgG), *M. pneumonia* IgM and IgG (n = 1 for VITROS® and Healgen® IgM and IgG) and RF (n = 1 with NovaLisa® and Healgen® IgM and IgG) (Table 4). Interestingly, one negative serum from a patient infected by *M. pneumonia* was positive/reactive in all serological immunoassays, suggesting a possible true positive from January 2020 (no molecular diagnosis at the time). Sera from patients infected by other common human coronaviruses were not included in the evaluation of specificity.

The construction and the comparison of receiver operator characteristic (ROC) curves for ARCHITECT®, VITROS® and NovaLisa® showed that overall VITROS® yielded the largest area under the curve (AUC) of 0.95 as compared to ARCHITECT® and NovaLisa®, suggesting a better discrimination power (p < 0.005) (Fig. 1).

Fig. 2 shows the evolution of Ab (IgA/IgM/IgG or IgG) levels. Overall, disease severity and phases impacted the Ab kinetic profile with all immunoassays. The “severe-to-critical” group had a markedly longitudinal Ab change between W1 and W4 with ARCHITECT® and NovaLisa®. This observation was not observed with VITROS® where Ab

Table 3
Sensitivities and specificities for the four immunoassays in relation to the study groups and the different timepoints post-symptoms onset. Data are expressed as percentage (95 % confidence interval).

Sensitivities % (95 % CI)		Time from the onset of symptoms				
		0–6 days	7−13 days	14−20 days	21−27 days	28−34 days
Severe-to-critical patients		(n = 3)	(n = 15)	(n = 18)	(n = 16)	(n = 9)
	ARCHITECT®	33.3 (0.84–90.6)	66.7 (38.4–88.2)	88.9 (65.3–98.6)	100 (79.4–100)	100 (66.4–100)
	VITROS®	33.3 (0.84–90.6)	86.7 (59.5–98.3)	94.4 (77.7–99.9)	100 (79.4–100)	100 (66.4–100)
	NovaLisa®	66.7 (9.4–99.2)	66.7 (38.4–88.2)	88.9 (65.3–98.6)	100 (79.4–100)	100 (66.4–100)
	Healgen®	100 (29.2–100)	86.7 (59.5–98.3)	94.4 (77.7–99.9)	100 (79.4–100)	100 (66.4–100)
Mild-to-moderate patients		(n = 13)	(n = 15)	(n = 16)	(n = 9)	
	ARCHITECT®	15.4 (1.9–45.4)	33.3 (11.8–61.6)	81.3 (54.3–95.9)	100 (66.4–100)	
	VITROS®	7.7 (0.2–36)	33.3 (11.8–61.6)	93.8 (69.7–99.8)	100 (66.4–100)	
	NovaLisa®	15.4 (1.9–45.4)	33.3 (11.8–61.6)	87.5 (61.6–98.4)	100 (66.4–100)	
	Healgen®	15.4 (1.9–45.4)	46.7 (21.3–71.4)	100 (79.4–100)	100 (66.4–100)	
Non hospitalized symptomatic individuals				(n = 10)		(n = 24)
	ARCHITECT®			80 (44.4–97.5)		87.5 (67.6–97.3)
	VITROS®			100 (69.2–100)		95.8 (78.9–99.9)
	NovaLisa®			40 (12.2–73.8)		66.7 (44.7–84.4)
	Healgen®			100 (69.2–100)		100 (85.7–100)

Table 4
False-positive samples. Number indicates the false-positive samples number found by each method. HIV (human immunodeficiency virus), CMV (cytomegalovirus), HBs (hepatitis B virus surface antigen), HCV (hepatitis C virus), *M. pneumonia* (*Mycoplasma pneumoniae*), RF (rheumatoid factor).

	ARCHITECT®	VITROS®	NovaLisa®	Healgen® IgM	Healgen® IgG
HIV positive	1				
CMV IgM			1		
HBs antigen	1				
HCV Ab				1	
Parvovirus B19				2	
M. pneumoniae	1	2	1	2	1
RF			1		1

level plateaued. Regarding the "mild-to-moderate" group, the difference in Ab level between W0 and W3 was significantly different with ARCHITECT® and NovaLisa®, while it was not significant with VITROS® where Ab level rapidly plateaued after one week. Paired non-hospitalized symptomatic individuals showed a positive Ab level at 14 days post-symptoms onset (week 2) with all immunoassays, remaining stable two weeks later. However, the Ab level measured by NovaLisa® was just above the positive cut-off threshold (11.6 and 11.9 for a cut-off threshold set at 11).

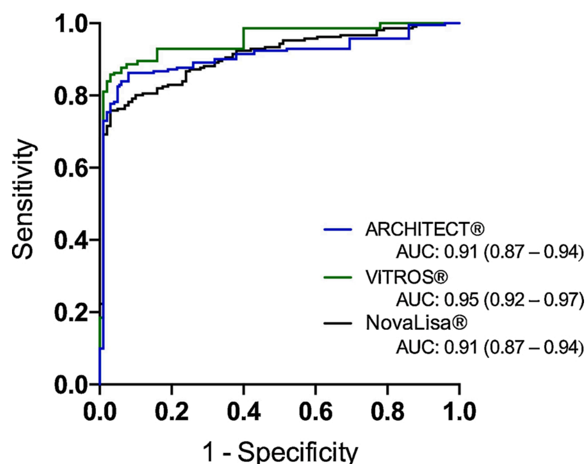

Fig. 1. ROC curves for ARCHITECT® (black), VITROS® (green) and NovaLisa® (blue). Data used for the ROC curves construction were not subdivided into the different groups. Area under the curves were calculated and compared (AUC with 95 % CI) using MedCalc.

In addition, we followed five "severe-to-critically" ill patients from one week post-symptoms onset for at least 16 weeks post-symptoms onset, this analysis was only performed by VITROS® Total Ab based on its better discrimination power as reported with the ROC curves. During this period, their clinical status was improved (e.g., withdrawal of oxygen therapy) but they remained hospitalized in revalidation care unit. All 5 patients presented high Ab levels throughout the 16 weeks. However, we observed that patients 2 and 3 presented a Ab drop of 40 % between W7 and W16, without difference in clinical evolution compared to other patients (Fig. 3).

4. Discussion and conclusion

Serological tests are of importance for the estimation of COVID-19 pandemic's extent, by evaluating different demographic and geographic patterns (Bohn et al., 2020; CDC, 2020b).

In our study, three patient groups were defined according to the severity degree of COVID-19 illness: "severe-to-critical", "mild-to-moderate" and non-hospitalized symptomatic forms.The time between the symptoms onset and the positive RT-qPCR was on average 7 ± 5 days for "severe-to-critically" ill patients, 3 ± 3 days for "mild-to-moderately" ill patients and 3 ± 3 days for non-hospitalized symptomatic individuals.

The longitudinal serological follow-up and Ab levels evolved differently in the three patients groups. Severe forms of the illness presented overall higher Ab levels after the symptoms onset as compared to "mild-to-moderate" forms of COVID-19. This difference was not detectable when IgG levels were measured by NovaLisa®. Total Ab (IgA, IgM and IgG) level achieved plateau more rapidly after the symptoms onset in "severe-to-critically" ill patients compared to anti-IgG assays. In addition, long-term follow-up of five"severe-to-critically" ill patients showed persistence of positive Ab level for at least 16 weeks post-symptoms onset with insignificant intra-patient variations. This persistence of Ab for a longer period of time would be further assessed with different clinical populations.

Patients presenting "mild-to-moderate" form of COVID-19 were not seropositive (IgG or IgM/IgG) at day 0, this timepoint was associated with poor sensitivities in all immunoassays. Non-hospitalizedsymptomatic individuals seroconverted already after 14 days post-symptoms onset with lower Ab level than observed in more severe illness' form.

Considering the diversity of the evaluated methods using different detection antigens and analyzers, we showed that overall total antibodies detection tended to be the most sensitive and discriminative method regardless of different timepoints or groups evaluated. Among the high-throughput immunoassays, Total Ab VITROS® presented the

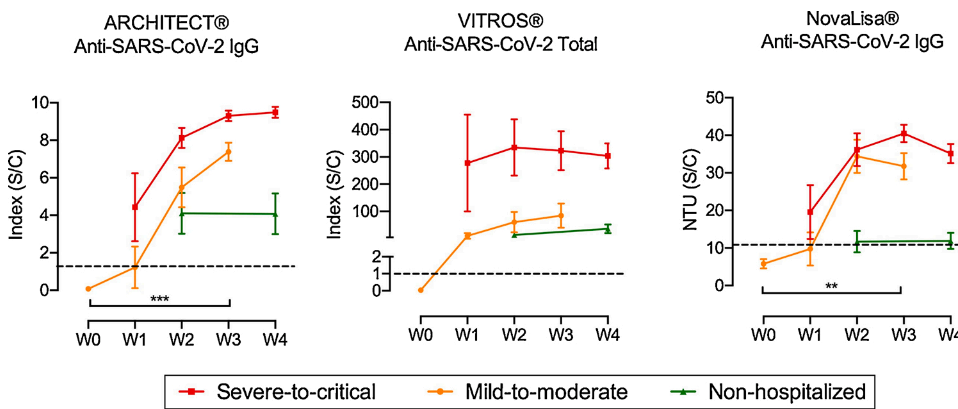

Fig. 2. Evolution of antibodies against SARS-CoV-2 in sequential samples. (A) Indexes (signal/cut-off) from ARCHITECT®, from VITROS® and NovaTec Units (NTU) (signal/cut-off) from ELISA with symptoms onset as the reference timepoint. Dotted line represents the positive/reactive cut-off threshold for each method (1.4 for ARCHITECT®, 1 for VITROS® and 11 for NovaLisa®). Data are expressed as mean index or unit ± SEM (standard error of mean). Evolution in severe-to-critical (red line, n = 5) and in mild-to-moderate patients (orange line, n = 6) are analyzed by one-way analysis of variance followed by Bonferroni's posttest (*** $p < 0.001$, ** $p < 0.01$ between D0 and D21). Difference in non-hospitalized symptomatic individuals (green line, n = 8) is analyzed by paired *t*-test (* $p < 0.05$).

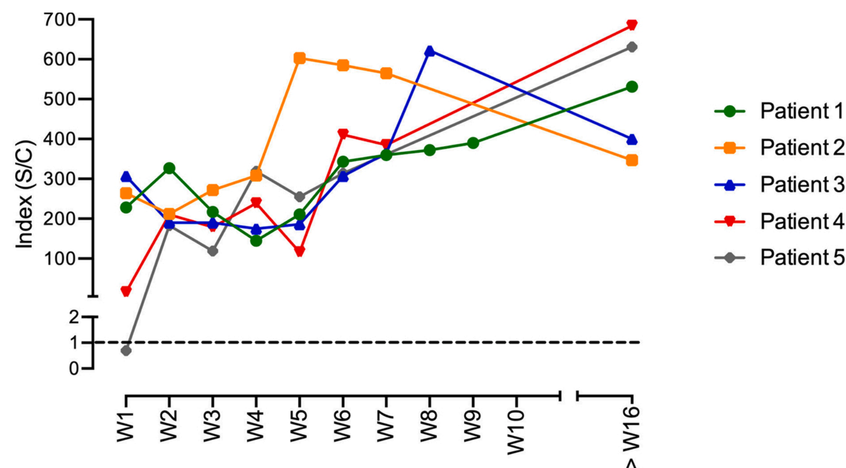

Fig. 3. Follow-up of five "severe-to-critically" ill patients. Week (W) 1 = 7-13 days, W2 = 14-20 days, W3 = 21-27 days, W4 = 28-34 days, W5 = 35-41 days, W6 = 42-48 days, W7 = 49-55 days, W8 = 56-62, W9 = 63-69, W10 = 70-76 days and > W16 ≥112 days post-symptoms onset.

highest sensitivities for non-hospitalized symptomatic individuals after14 days (100 % with 95 % CI 69.2–100) and after 28 days (95.8 % with 95 % CI 78.9–99.9). This observation could be of interest for the collective serological surveillance, contact tracing or for vaccine evaluation.

The two fully automated immunoassays and the ELISA detect different Abs against N protein (ARCHITECT®, NovaLisa®) and against the S (spike) glycoprotein (S1 subunit) (VITROS®). Similar to previous findings (Liu et al., 2020; Vashist, 2020), the detection of anti-S appeared to be more sensitive in early detection of Ab than anti-N protein. Secondly, the combination of IgA, IgM and IgG showed early better sensitivities; this effect could result from the early appearance of IgA (only for VITROS®) and IgM as observed in some patients (Long et al., 2020).

Several CLIAs have been analytically and clinically evaluated (Favresse et al., 2020; Montesinos et al., 2020; Padoan et al., 2020; Plebani et al., 2020; Tré-Hardy et al., 2020). They presented heterogeneity in the sensitivity assessment explained by the different tested populations and adapted cut-off limits. Three recent studies evaluated the performance of the ARCHITECT® SARS-Cov-2 IgG with similar sensitivities to ours with sensitivities greater than 90 % at ≥ 14 days after the symptoms onset (Nicol et al., 2020; Tang et al., 2020; Theel et al., 2020). A recent publication reported the clinical performance of anti-SARS-CoV-2 IgG from Ortho Clinical Diagnostics with 100 % sensitivity at > 15 days after the symptoms onset (Theel et al., 2020).

Despite high specificities of the immunoassays obtained in the present study, no firm conclusion can be drawn for their clinical performance in a routine setting. Given the low pre-test probability for SARS-CoV-2 seropositivity in the general population, an analytical specificity of 97–98 % could lead to a low positive predictive value (PPV) of serological testing in a low prevalence setting. A positive result should be interpreted with caution depending on the clinical and epidemiological context. In this regard, an orthogonal algorithm with two different immunoassays (e.g., anti-S and anti-N or IgG and Total Ab) could be advised to reinforce the PPV (CDC, 2020b).

Since both fully automated CLIAs are registered as qualitative immunoassays, little information about the analytical validation is provided by the manufacturers and the comparison between both CLIAs is also limited by the absence of a gold-standard method. Using values from signal on cut-off ratio for semi-quantitative validation, we estimated the analytical parameters acceptable according to the CLSI and the COFRAC ("EP15A3 User Verification of Precision & Bias Estimation, " n.d.; "Guide de vérification/validation des méthodes en Biologie Médicale, " n.d.).

This study has several limitations, such as the evaluation of specificity without sera from patients infected by the four endemic human coronaviruses that may generate false-positive results and without healthy donor's samples. This study and its conclusions are limited by the small sample numbers. Indeed, we collected only a few sera from hospitalized patients during the first week of symptoms due to the time of their hospitalization, resulting in overestimation of sensitivities at < 7 days. Finally, it would have been interesting to assess the Ab persistence beyond our tested period on a larger number of different clinical populations.

In conclusion, this study evaluated analytically and clinically four commercial anti-SARS-CoV-2 immunoassays in different clinical populations. The automated immunoassay (using S-protein as the antigen for detection) targeting IgA, IgM and IgG appeared to be the most sensitive method in our study of small hospitalized population and healthcare professionals. Studying the virus epidemiology in the general population needs to be evaluated on large-scale population including asymptomatic cases, uninfected individuals, and non-hospitalized cases.

Authorship statement

Conception and design: CE, JH, DM, CM, DO, GL, MF, HTD.
Acquisition of data: CE, JH, DM, SAI, LB, dfB, DB.
Analysis and Interpretation of data: CE, JH, DM, DO, GL, MF, HTD.
Writing – Original draft: CE, JH, DM, DO, GL, MF, HTD.
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Source of funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

The Authors confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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