Performance assessment of 11 commercial serological tests for SARS-CoV-2 on hospitalised COVID-19 patients

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ABSTRACT

Background: Commercial availability of serological tests to evaluate immunoglobulins (Ig) targeting severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has grown exponentially since the start of the coronavirus disease 2019 (COVID-19) outbreak. Thorough validation of these tests is important before use as epidemiological tools to infer seroprevalence in specific populations and as diagnostic tools to complement molecular approaches (e.g., quantitative reverse transcription-polymerase chain reaction).
Methods: Commercial serological tests from 11 suppliers were assayed side-by-side using 126 samples from SARS-CoV-2-infected inpatients and 36 from healthy and HIV-infected individuals.
Results: The majority of the tests assayed have >95% specificity. For the sensitivity calculation, samples were stratified by days since symptoms onset; sensitivity peaks at 16–21 days for IgM and IgA (maximum 91.2%, Euroimmun) and, dependant on the test, at 16–21 or >21 days for IgG (maximum 94.1%, Snibe). Data from semiquantitative tests show that patients with a severe clinical presentation have lower levels of Ig targeting SARS-CoV-2 at <10 days since symptoms onset and higher levels at >21 days, compared to patients with a non-severe presentation.
Conclusions: This study highlights the heterogeneity of sensitivity and generally high specificity of the serological tests and establishes a basis for their usefulness to complement diagnostic techniques and population seroprevalence studies.

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a large RNA virus from the Coronaviridae virus family that is currently causing global spread of coronavirus disease 2019 (COVID-19) (Lu et al., 2020). Considering the absence of an effective treatment for the SARS-CoV-2 infection, early diagnosis of infection and isolation of infected individuals is critical to control the ongoing pandemic (Gudbjartsson et al., 2020b). Case detection usually involves collection of swab samples from the upper respiratory tract and the amplification of viral nucleic acids sequences by quantitative reverse transcription-polymerase chain reaction (RT-qPCR) (Corman et al., 2020). Recently tests detecting SARS-CoV-2 proteins from swab samples were developed. While viral detection is essential to identify active infection, detection of antibodies against SARS-CoV-2 is a useful complementary surveillance tool. Following SARS-CoV-2 infection, most patients produce detectable immunoglobulins (Ig) targeting a set of viral antigens, particularly the immunodominant nucleocapsid (N) and spike (S) proteins (Guo et al., 2020; Tang et al.,

2020; Walls et al., 2020; Zhao et al., 2020). Current evidence suggests that Ig produced against these antigens may confer protection to SARS-CoV-2 infection (Cao et al., 2020; Ju et al., 2020; Poh et al., 2020). However, there is insufficient data on the timing of Ig production following infection. The literature suggests that IgA and IgM can be detected 6–8 days from symptoms onset (Guo et al., 2020; Montesinos et al., 2020; Okba et al., 2020; Padoan et al., 2020; Yongchen et al., 2020), while IgG seroconversion seems to occur slightly later at 9–10 days. However, many patients appear to seroconvert for both IgM and IgG simultaneously, peaking at around 21 days (Liu et al., 2020b; Sun et al., 2020; Yongchen et al., 2020; Zhang et al., 2020). It is not yet confirmed whether Ig levels vary with disease severity.

The performance (sensitivity and specificity) of serological assays can be affected by many variables including: timing of assessment relative to symptoms onset/infection, course of COVID-19 (from asymptomatic to lethal) and, potentially, population and virus genetics (Osório and Correia-Neves, 2020; Peeling et al., 2020). It is vital to evaluate the performance of commercially available serological tests, comparing their performance side-by-side using samples from well-characterised patients with well-defined clinical presentation and distinct time-points from symptoms onset, to be able to select the most appropriate tests for defined populations.

We evaluated the performance of serological tests (3 semiquantitative and 8 qualitative) from 11 suppliers using plasma samples of hospitalised patients with COVID-19 from the Minho region, in the North of Portugal. The particular tests were chosen due to previous reports on their sensitivity, specificity and availability.

Methods

Study population

Patients living in the Minho region of Portugal who were inpatients at Senhora da Oliveira Hospital (Guimarães) or Braga Hospital, admitted with COVID-19 (diagnosed by RT-qPCR at a reference laboratory; at least 2 positive RT-qPCR results were obtained from each patient), were invited to participate in the study. This study was approved by the ethics committees of both participating hospitals (Senhora da Oliveira Hospital: 25/2020; Braga Hospital: 37/2020). An explanation of the project was provided to those individuals and participants signed an informed consent form. The informed consent was prepared according to the Declaration of Helsinki principles, the Oviedo Convention and the General Data Protection Regulation–Regulation (EU) 2016/679. Patients' blood samples were collected throughout hospitalisation at different time-points following symptoms onset. The number of samples available from each participant varied depending on the duration of their hospitalisation.

For sensitivity calculation, COVID-19 patients were stratified based on the number of days since symptoms onset as follows: <10 days; 10–15 days; 16–21 days; >21 days. Days since symptoms onset were calculated based on each patient's self-report of symptoms manifestation. COVID-19 patients were further categorised according to the severity of their clinical presentation. Patients given oxygen therapy above 10 L/min and/or needing mechanical ventilation (non-invasive or invasive) were considered as having a severe clinical presentation. All other patients (i.e., needing supplementary oxygen therapy below 10 L/min and not requiring mechanical ventilator support) were considered as having a non-severe clinical presentation.

SARS-COV-2 non-infected controls were selected from banked human plasma samples from 2 pre-COVID-19 pandemic studies (the first COVID-19 case in Portugal was reported on 2 March 2020): (i) a study with healthy individuals >55 years old (samples collected between April 2019 and January 2020); (ii) a study with HIV-infected patients on antiretroviral therapy (54–60 months; samples collected between January 2016 and August 2018) (Rb-Silva et al., 2019). Matched samples were selected based on COVID-19 patients' sex and age. Control samples were collected, processed and preserved at −80 °C using a similar protocol as the one used for samples from COVID-19 inpatients (see below).

Data were handled anonymously. Individuals' sex, age and comorbidities are summarised in Table 1.

Sample processing

From each patient, venous blood was collected into K_2EDTA collecting tubes and processed on the same day: blood collecting tubes were centrifuged at 2000×g for 15 min, at 20 °C. Plasma was aliquoted into screw-cap tubes and frozen at −80 °C until tested.

Immunoassays

Semiquantitative (enzyme-linked immune-absorbent assays [ELISA] and chemiluminescence immunoassays [CLIA]) and

Table 1
Clinical and demographic characterisation of the cohort.

	COVID-19 patients			Pre-COVID-19 controls	
	All	Clinical presentation[a]		Healthy controls	HIV and other viral infections
		Severe	Non-severe		
n	89	32	57	25	11
Age (years), Median [min;max]	71 [30;96]	75 [45;96]	67 [30;94]	71 [59;80]	57 [33;72]
Female, n (%)	51 (57.3)	16 (50.0)	35 (61.4)	13 (52.0)	3 (27.3)
Hypertension, n (%)	59 (66.3)	25 (78.1)	34 (59.6)	n/a	n/a
Diabetes, n (%)	27 (30.3)	11 (34.4)	16 (28.1)	n/a	n/a
Obesity, n (%)[b]	13 (14.6)	4 (12.5)	9 (15.8)	n/a	n/a
Neoplasia, n (%)	8 (9.0)	4 (12.5)	4 (7.0)	n/a	n/a
Autoimmune disease, n (%)	5 (5.6)	2 (6.3)	3 (5.3)	n/a	n/a
Active smokers, n (%)	2 (2.2)	0 (0.0)	2 (3.5)	n/a	n/a
Corticosteroids (during hospitalization), n (%)	6 (6.7)	3 (9.4)	3 (5.3)	n/a	n/a
Other immunosuppressive drugs, n (%)					
Prior to hospitalization	5 (5.6)	3 (9.4)	2 (3.5)	n/a	n/a
During hospitalization	1 (1.1)	1 (3.1)	0 (0.0)	n/a	n/a

n/a, not applicable.
[a] Please see "Study Population" section on Methods. No differences between groups were observed in the percentages of the clinical conditions, as assessed by the Fisher Exact Probability test.
[b] Obesity defined as body mass index over 30 kg/m².

qualitative assays (lateral flow immunoassays [LFIA]) from 11 different suppliers were tested according to the manufacturer's instructions (Table 2). At least 2 different tests were performed for each sample (Supplementary Tables S1 and S2).

Data analysis

For each test, specificity was calculated as the percentage of negative tests among the pre-COVID-19 controls, and sensitivity as the percentage of positive tests among the SARS-CoV-2 confirmed cases. Sensitivity was calculated upon stratification in days since symptoms onset (<10, 10–15; 16–21 and >21 days). Whenever the same patient was tested multiple times within the same time range during the course of the disease the results of only 1 plasma sample were considered to calculate sensitivity (Supplementary Table S1). The 95% confidence intervals for sensitivity and specificity were calculated using the Wilson score with continuity correction method. Positive predictive values (PPV) and negative predictive values (NPV) were calculated for 3 prevalence scenarios, as described elsewhere (Tenny and Hoffman, 2020): 3% seroprevalence in a general population, which is the estimated prevalence for the Portuguese population (Rodrigues, 2020); 50% seroprevalence in a high-risk sub-population; and 95% seroprevalence in hospitalised patients suspected for COVID-19. Tests' agreement was evaluated using Cohen's Kappa. For the semiquantitative tests, receiver-operator characteristic (ROC) curves were constructed and used to calculate the area under the curve (AUC) of the different serologic tests. All variables analysed had a non-normal distribution, as verified by Shapiro–Wilk tests. Comparisons of the relative amounts of Ig at the various time ranges were performed using the Kruskal–Wallis test, followed by Dunn's post-hoc tests. Comparisons of the relative amount of Ig in the groups of patients with severe versus non-severe clinical presentation were performed using the Mann–Whitney U test. Comparison of demographic and clinical conditions percentages between groups of patients, with severe and non-severe clinical presentation, were performed using Fisher's Exact Probability Test.

Differences were considered significant when $P < 0.05$. Statistical analyses were performed on GraphPad Prism version 8.4.3 (La Jolla, San Diego, CA, USA).

Results

Study population

This study includes 89 inpatients infected with SARS-CoV-2 (diagnosed and re-confirmed during hospitalisation by RT-qPCR). Plasma samples from 40 individuals were tested at different time-points during hospitalisation (Supplementary Table S1). Most participants were women (57%) with a median age of 71 years (Table 1). None of the COVID-19 patients were HIV-positive or had a history of organ transplantation.

Performance of tests

Sensitivity and specificity

Serologic tests from 11 suppliers were assayed for their performance. In the IgM and IgG tests combined, specificity ranges from 95.8% to 100.0%, except for Cellex and Snibe (76.0% and 88.6%, respectively). In 4/10 tests assessing IgG and 5/9 tests assessing IgA or IgM specificity is 100.0% (Table 3 and Supplementary Figure S1). From the 36 samples used as negative controls (collected pre-COVID-19 pandemic), 10 are positive for at least 1 test, 5 of which are positive in more than 1 test (Supplementary Table S2). In the semiquantitative tests (Abbott, Euroimmun and Snibe) 2 of the negative control samples are positive in the Snibe IgG test but have a value close to the negative-to-positive cut-off value (1.124 and 1.652 AU/mL; cut-off 1.000 AU/mL; Supplementary Figure S2 and Supplementary Table S2).

To analyse sensitivity, samples were stratified according to time since symptoms onset (<10, 10–15, 16–21 and >21 days). For each Ig, the lowest sensitivities are observed at <10 days since symptoms onset: the Leccurate IgG test presents the highest sensitivity (71.4%) and Snibe IgM the lowest (33.3%; Table 3 and

Table 2
Assayed commercial tests to detect immunoglobulins specific for SARS-CoV-2 infection.

Assay	Supplier	Product	Catalog no.	Technology	Format	Assay Target	Obs
Semiquantitative assays	**Abbott** Diagnostics	SARS-coV-2 IgG assay	06R86	CLIA	IgG	N protein	Requires an Abbott Architect i2000
	Euroimmun	anti SARS-CoV-2 **IgG** ELISA kit	EI 2606-9601 G	ELISA	IgG	S1 protein	Requires a regular absorbance microplate reader
		anti SARS-CoV-2 **IgA** ELISA kit	EI 2606-9601 A	ELISA	IgA		
	Snibe Diagnostic	MAGLUMI® 2019-nCoV (SARS-CoV-2) **IgM** kit	130219016M	CLIA	IgM	S antigen and N protein	Requires a MAGLUMI chemiluminescence immunoassay system
		MAGLUMI® 2019-nCoV (SARS-CoV-2) **IgG** kit	130219015M	CLIA	IgG		
Qualitative assays	**Cellex**	qSARS-Cov-2 IgG/IgM Cassette Rapid Test	WI5513C	LFIA	IgM/IgG	N and S Proteins	–
	Getein Biotech, inc	One step test for novel coronavirus (2019-nCoV) IgM/IgG antibody (colloidal gold)	CG2057	LFIA	Total Ig	N and S Proteins	–
	Innovita	2019-nCoV Ab test (colloidal gold), IgM/IgG whole blood/Serum/Plasma Combo	n/a	LFIA	IgM/IgG	N and S Proteins	–
	Liming Bio	StrongStep® SARS-CoV-2 IgM/IgG Test	502090	LFIA	IgM/IgG	Not specified	Extremely faint bands.
	Leccurate	SARS-CoV-2 antibody test (colloidal gold immunochromatography)	K-20-RC-CoV-2	LFIA	IgM/IgG	Not specified	–
	Medomics	Rapid IgM-IgG Combined Antibody Test kit for SARS-CoV-2 (ICA)	n/a	LFIA	IgM/IgG	Not specified	–
	Render	COVID-19 IgM & IgG Test (immunocromatography)	3.2.01.0.1701	LFIA	IgM/IgG	Not specified	–
	SD Biosensor	Standard Q COVID-19 IgM/IgG Duo Test	Q-NCOV-01D	LFIA	IgM/IgG	N Protein	–

CLIA; Chemiluminescence Immunoassay; ELISA: Enzyme Linked Immuno-Sorbent Assay; Ig: Immunoglobulin; LFIA: Lateral Flow Immunoassay; n/a: not available; N: Nucleocapsid; S: Spike.

Table 3

Sensitivity and specificity of the assayed tests to detect immunoglobulins specific for SARS-CoV-2 infection. Sensitivity was evaluated in COVID-19 patients upon stratification by days since symptoms onset. Each colour gradient refers to higher (darker) towards lower (lighter) values of specificity (yellow) or sensitivity (green).

Commercial kit	Ig	SPECIFICITY					SENSITIVITY — Days since symptoms onset																			
		Tot Neg		Spec	95% CI		<10			95% CI		10-15			95% CI		16-21			95% CI		>21			95% CI	
		Tot n	Neg n	%	Lower	Upper	Tot n	Pos n	Sens %	Lower	Upper	Tot n	Pos n	Sens %	Lower	Upper	Tot n	Pos n	Sens %	Lower	Upper	Tot n	Pos n	Sens %	Lower	Upper
Abbott	IgG	39	38	97.4	84.9	99.9	22	12	54.5	32.7	74.9	32	24	75.0	56.2	87.9	33	27	81.8	63.9	92.4	33	29	87.9	70.9	96.0
Euroimmun	IgG	38	38	100.0	88.6	100.0	24	12	50.0	29.6	70.4	33	26	78.8	60.6	90.4	34	28	82.4	64.8	92.6	35	28	80.0	62.5	90.9
Snibe	IgG	35	33	94.3	79.5	99.0	21	9	42.9	22.6	65.6	30	18	60.0	40.7	76.8	34	32	94.1	78.9	99.0	32	29	90.6	73.8	97.5
Cellex	IgG	25	22	88.0	67.7	96.8	14	8	57.1	29.6	81.2	20	13	65.0	40.9	83.7	20	17	85.0	61.1	96.0	16	14	87.5	60.4	97.8
Getein	---	---					---					---					---					---				
Innovita	IgG	35	34	97.1	83.4	99.9	18	7	38.9	18.3	63.9	29	20	69.0	49.0	84.0	27	21	77.8	57.3	90.6	24	17	70.8	48.8	86.6
Leccurate	IgG	25	25	100.0	83.4	100.0	14	10	71.4	42.0	90.4	20	13	65.0	40.9	83.7	20	14	70.0	45.7	87.2	16	12	75.0	47.4	91.7
Liming	IgG	38	38	100.0	88.6	100.0	24	13	54.2	33.2	73.8	33	21	63.6	45.1	79.0	34	27	79.4	61.6	90.7	35	29	82.9	65.7	92.8
Medomics	IgG	24	23	95.8	76.9	99.8	12	6	50.0	22.3	77.7	12	6	50.0	22.3	77.7	20	15	75.0	50.6	90.4	18	13	72.2	46.4	89.3
Render	IgG	26	26	100.0	84.0	100.0	16	6	37.5	16.3	64.1	28	18	64.3	44.1	80.7	24	17	70.8	48.8	86.6	20	15	75.0	50.6	90.4
SD	IgG	25	24	96.0	77.7	99.8	14	6	42.9	18.8	70.4	20	13	65.0	40.9	83.7	20	16	80.0	55.7	93.4	16	11	68.8	41.5	87.9
Abbott	---	---					---					---					---					---				
Euroimmun	IgA	38	37	97.4	84.6	99.9	24	15	62.5	40.8	80.5	33	30	90.9	74.5	97.6	34	31	91.2	75.2	97.7	35	31	88.6	72.3	96.3
Snibe	IgM	35	33	94.3	79.5	99.0	21	7	33.3	15.5	56.9	30	19	63.3	43.9	79.5	34	25	73.5	55.3	86.5	32	21	65.6	46.8	80.8
Cellex	IgM	25	19	76.0	54.5	89.8	14	9	64.3	35.6	86.0	20	15	75.0	50.6	90.4	20	17	85.0	61.1	96.0	16	14	87.5	60.4	97.8
Getein	---	---					---					---					---					---				
Innovita	IgM	35	35	100.0	87.7	100.0	18	8	44.4	22.4	68.7	29	21	72.4	52.5	86.6	27	20	74.1	53.4	88.1	24	19	79.2	57.3	92.1
Leccurate	IgM	25	24	96.0	77.7	99.8	14	9	64.3	35.6	86.0	20	13	65.0	40.9	83.7	20	15	75.0	50.6	90.4	16	10	62.5	35.9	83.7
Liming	IgM	38	38	100.0	88.6	100.0	24	13	54.2	33.2	73.8	33	21	63.6	45.1	79.0	34	26	76.5	58.4	88.6	35	24	68.6	50.6	82.6
Medomics	IgM	24	24	100.0	82.8	100.0	12	6	50.0	22.3	77.7	12	5	41.7	16.5	71.4	20	15	75.0	50.6	90.4	18	12	66.7	41.2	85.6
Render	IgM	26	26	100.0	84.0	100.0	16	6	37.5	16.3	64.1	28	13	46.4	28.0	65.8	24	15	62.5	40.8	80.5	20	13	65.0	40.9	83.7
SD	IgM	25	25	100.0	83.4	100.0	14	9	64.3	35.6	86.0	20	15	75.0	50.6	90.4	20	17	85.0	61.1	96.0	16	13	81.3	53.7	95.0
Abbott	---	---					---					---					---					---				
Euroimmun	(IgG:IgA)	38	37	97.4	84.6	99.9	24	16	66.7	44.7	83.6	33	30	90.9	74.5	97.6	34	31	91.2	75.2	97.7	35	32	91.4	75.8	97.8
Snibe	(IgG:IgM)	35	31	88.6	72.3	96.3	21	10	47.6	26.4	69.7	30	22	73.3	53.8	87.0	34	33	97.1	82.9	99.8	32	29	90.6	73.8	97.5
Cellex	(IgG:IgM)	25	19	76.0	54.5	89.8	14	9	64.3	35.6	86.0	20	15	75.0	50.6	90.4	20	17	85.0	61.1	96.0	16	14	87.5	60.4	97.8
Getein	(Total Ig)	25	24	96.0	77.7	99.8	13	8	61.5	32.3	84.9	13	8	61.5	32.3	84.9	22	20	90.9	69.4	98.4	18	15	83.3	57.7	95.6
Innovita	(IgG:IgM)	35	34	97.1	83.4	99.9	18	9	50.0	26.8	73.2	29	21	72.4	52.5	86.6	27	21	77.8	57.3	90.6	24	20	83.3	61.8	94.5
Leccurate	(IgG:IgM)	25	24	96.0	77.7	99.8	14	11	78.6	48.8	94.3	20	13	65.0	40.9	83.7	20	15	75.0	50.6	90.4	16	12	75.0	47.4	91.7
Liming	(IgG:IgM)	38	38	100.0	88.6	100.0	24	15	62.5	40.8	80.5	33	22	66.7	48.1	81.4	34	28	82.4	64.8	92.6	35	29	82.9	65.7	92.8
Medomics	(IgG:IgM)	24	23	95.8	76.9	99.8	12	8	66.7	35.4	88.7	12	7	58.3	28.6	83.5	20	16	80.0	55.7	93.4	18	14	77.8	51.9	92.6
Render	(IgG:IgM)	27	27	100.0	84.5	100.0	16	8	50.0	25.5	74.5	28	18	64.3	44.1	80.7	24	17	70.8	48.8	86.6	20	16	80.0	55.7	93.4
SD	(IgG:IgM)	25	24	96.0	77.7	99.8	14	9	64.3	35.6	86.0	20	15	75.0	50.6	90.4	20	17	85.0	61.1	96.0	16	13	81.3	53.7	95.0

CI: confidence interval; Ig: immunoglobulin; Neg: negative; Pos: positive; Sens: sensitivity; Spec: specificity; Tot: total

Supplementary Figure S1). Detection of IgA or IgM reaches maximum sensitivity at 16–21 days in 7/9 assays. The maximum sensitivity for IgG peaks at 16–21 days in 50% of the tests, and >21 days in the other 50% (Abbott, Cellex, Innovita, Leccurate, and Render). The Getein test, which detects total Ig targeting SARS-CoV-2, has a peak detection at 16–21 days. For the combined Ig classes, at >21 days, the Euroimmun test (IgA and IgG) provides the highest sensitivity (91.4%), followed by the Snibe test (IgM and IgG, 90.6%). The combined IgM and IgG Leccurate test provides the lowest sensitivity value (75.0; Table 3 and Supplementary Figure S1).

Positive and negative predictive values

PPV and NPV vary according to the estimated disease seroprevalence. For the assayed tests, 3 distinct population seroprevalence scenarios were tested, from low (3% considered to be the prevalence in the general population and the estimate for the Portuguese population (Rodrigues, 2020)) to high (95%, for a hospitalised setting). To estimate the adjusted PPV and NPV, the overall sensitivity of each test was calculated (i.e., without stratification by days since symptoms onset); Abbot, Euroimmun, Snibe and Cellex present the highest overall sensitivity for the detection of IgG, ranging from 74.3% to 76.7% (Table 4). Detection of

Table 4

Negative and positive predictive values (PPV and NPV, respectively) of assayed commercial tests to detect immunoglobulins specific for SARS-CoV-2 infection. PPV and NPV are adjusted for different population prevalence scenarios (3%, 50% and 95%), as described in the "Data analysis" section of material and methods. Each colour gradient refers to higher (darker) towards lower (lighter) values of NPV (blue) or PPV (grey).

Commercial kit		OVERALL		NEGATIVE PREDICTIVE VALUE (%)			POSITIVE PREDICTIVE VALUE (%)		
		Sensitivity	Specificity	Population prevalence			Population prevalence		
		%	%	3%	50%	95%	3%	50%	95%
Abbott	IgG	76.7	97.4	99.3	80.7	18.0	48.0	96.8	99.8
Euroimmun	IgG	74.6	100.0	99.2	79.7	17.2	100.0	100.0	100.0
Snibe	IgG	75.2	94.3	99.2	79.2	16.7	28.9	92.9	99.6
Cellex	IgG	74.3	88.0	99.1	77.4	15.3	16.1	86.1	99.2
Getein	---	---	---	---	---	---	---	---	---
Innovita	IgG	66.3	97.1	98.9	74.3	13.2	41.8	95.9	99.8
Leccurate	IgG	70.0	100.0	99.1	76.9	14.9	100.0	100.0	100.0
Liming	IgG	71.4	100.0	99.1	77.8	15.6	100.0	100.0	100.0
Medomics	IgG	64.5	95.8	98.9	73.0	12.4	32.4	93.9	99.7
Renger	IgG	63.6	100.0	98.9	73.3	12.6	100.0	100.0	100.0
SD	IgG	65.7	96.0	98.9	73.7	12.8	33.7	94.3	99.7
Abbott	---	---	---	---	---	---	---	---	---
Euroimmun	IgA	84.9	97.4	99.5	86.6	25.4	50.0	97.0	99.8
Snibe	IgM	61.5	94.3	98.8	71.0	11.4	25.0	91.5	99.5
Cellex	IgM	78.6	76.0	99.1	78.0	15.7	9.2	76.6	98.4
Getein	---	---	---	---	---	---	---	---	---
Innovita	IgM	69.4	100.0	99.1	76.6	14.7	100.0	100.0	100.0
Leccurate	IgM	67.1	96.0	99.0	74.5	13.3	34.2	94.4	99.7
Liming	IgM	66.7	100.0	99.0	75.0	13.6	100.0	100.0	100.0
Medomics	IgM	61.3	100.0	98.8	72.1	12.0	100.0	100.0	100.0
Renger	IgM	53.4	100.0	98.6	68.2	10.1	100.0	100.0	100.0
SD	IgM	77.1	100.0	99.3	81.4	18.7	100.0	100.0	100.0
Abbott	---	---	---	---	---	---	---	---	---
Euroimmun	(IgG + IgA)	86.5	97.4	99.6	87.8	27.5	50.4	97.0	99.8
Snibe	(IgG + IgM)	80.3	88.6	99.3	81.8	19.2	17.9	87.5	99.3
Cellex	(IgG + IgM)	78.6	76.0	99.1	78.0	15.7	9.2	76.6	98.4
Getein	(Total Ig)	77.3	96.0	99.3	80.9	18.2	37.4	95.1	99.7
Innovita	(IgG + IgM)	72.4	97.1	99.1	77.9	15.7	44.0	96.2	99.8
Leccurate	(IgG + IgM)	72.9	96.0	99.1	78.0	15.7	36.0	94.8	99.7
Liming	(IgG + IgM)	74.6	100.0	99.2	79.7	17.2	100.0	100.0	100.0
Medomics	(IgG + IgM)	72.6	95.8	99.1	77.8	15.5	35.0	94.6	99.7
Renger	(IgG + IgM)	67.0	100.0	99.0	75.2	13.8	100.0	100.0	100.0
SD	(IgG + IgM)	77.1	96.0	99.3	80.8	18.1	37.4	95.1	99.7

Ig: immunoglobulin

IgA by the Euroimmun test has an overall sensitivity of 84.9, and Cellex and SD for IgM of 78.6% and 77.1%, respectively; all the other tests detecting IgM have overall sensitivities <69.4. For combined detection of IgG and IgA or IgM, semiquantitative tests present the highest overall sensitivity (Euroimmun 86.5%; Snibe 80.3%); all the qualitative tests have an overall sensitivity lower than 78.6% (Table 4). The estimated NPV in a low seroprevalence scenario (3%) varies from 98.6% to 99.6%, dropping drastically in a high seroprevalence scenario (95%) to 10.1%–27.5%. The estimated PPV reached 100% for some tests, irrespective of prevalence. The estimated PPV is >98.4% in high prevalence scenarios, >76.6% in intermediate prevalence scenarios and >9.2% in low prevalence scenarios. Euroimmun, Innovita, Leccurate, Liming, Medomics, Render and SD have PPV of 100%, independent of the Ig type detected; in the combined tests, only Liming and Render have 100% PPV (Table 4).

Table 5
Cohen's kappa coefficient for the strength of agreement between assayed tests to detect immunoglobulins specific for SARS-CoV-2 infection. Colour gradient refers to higher (darker) towards lower (lighter) values Cohen's kappa coefficient (purple).

Row	Metric	SD −	SD +	Render −	Render +	Medomics −	Medomics +	Liming −	Liming +	Leccurate −	Leccurate +	Innovita −	Innovita +	Getein −	Getein +	Cellex −	Cellex +	Snibe −	Snibe +	Euroimmun −	Euroimmun +
Abbott	Test result (n) −	40	6	52	3	39	5	29	7	39	5	61	3	40	7	35	11	57	11	56	17
	Test result (n) +	0	51	8	57	4	42	9	89	4	42	4	70	3	46	0	51	3	88	5	94
	κ	0.875		0.817		0.800		0.701		0.800		0.898		0.791		0.770		0.817		0.732	
	95% CI Lower	0.789		0.733		0.700		0.615		0.700		0.832		0.694		0.671		0.747		0.659	
	95% CI Upper	0.931		0.879		0.874		0.776		0.874		0.941		0.865		0.847		0.872		0.796	
Euroimmun	Test result (n) −	34	2	44	0	34	3	22	4	34	3	52	1	36	2	28	8	50	9		
	Test result (n) +	6	55	17	60	10	44	20	105	10	44	14	72	8	51	7	54	13	103		
	κ	0.827		0.720		0.713		0.552		0.713		0.782		0.790		0.667		0.723			
	95% CI Lower	0.734		0.629		0.607		0.469		0.607		0.702		0.693		0.563		0.650			
	95% CI Upper	0.894		0.796		0.800		0.632		0.800		0.845		0.863		0.757		0.787			
Snibe	Test result (n) −	33	4	44	1	35	3	28	4	35	3	54	1	38	0	31	6				
	Test result (n) +	7	52	16	58	9	44	11	97	9	44	12	71	6	53	4	55				
	κ	0.762		0.715		0.735		0.718		0.735		0.810		0.874		0.778					
	95% CI Lower	0.662		0.624		0.630		0.635		0.630		0.732		0.787		0.679					
	95% CI Upper	0.840		0.792		0.819		0.789		0.819		0.870		0.930		0.854					
Cellex	Test result (n) −	34	1	34	0	28	2	14	2	28	2	34	0	30	1						
	Test result (n) +	6	56	13	49	11	41	7	49	11	41	11	51	9	47						
	κ	0.848		0.728		0.679		0.675		0.679		0.767		0.763							
	95% CI Lower	0.758		0.625		0.565		0.553		0.565		0.667		0.658							
	95% CI Upper	0.910		0.811		0.775		0.778		0.775		0.844		0.845							
Getein	Test result (n) −	33	6	42	2	37	4	16	4	37	4	42	2								
	Test result (n) +	3	45	8	45	6	43	6	46	6	43	6	47								
	κ	0.789		0.794		0.777		0.664		0.777		0.835									
	95% CI Lower	0.686		0.698		0.675		0.542		0.675		0.743									
	95% CI Upper	0.867		0.867		0.855		0.769		0.855		0.900									
Innovita	Test result (n) −	38	7	55	1	42	3	25	7	42	3										
	Test result (n) +	1	50	5	59	2	44	5	67	2	44										
	κ	0.831		0.900		0.890		0.724		0.890											
	95% CI Lower	0.738		0.828		0.803		0.627		0.803											
	95% CI Upper	0.897		0.945		0.943		0.805		0.943											
Leccurate	Test result (n) −	37	7	42	1	37	2	15	5												
	Test result (n) +	3	50	5	48	2	41	6	46												
	κ	0.790		0.875		0.902		0.625													
	95% CI Lower	0.694		0.788		0.811		0.503													
	95% CI Upper	0.864		0.931		0.954		0.734													
Liming	Test result (n) −	15	1	26	8	17	4														
	Test result (n) +	6	50	4	56	5	41														
	κ	0.747		0.716		0.692															
	95% CI Lower	0.628		0.612		0.566															
	95% CI Upper	0.839		0.802		0.796															
Medomics	Test result (n) −	32	7	44	0																
	Test result (n) +	2	41	4	43																
	κ	0.779		0.912																	
	95% CI Lower	0.671		0.829																	
	95% CI Upper	0.860		0.959																	
Render	Test result (n) −	39	8																		
	Test result (n) +	0	49																		
	κ	0.833																			
	95% CI Lower	0.740																			
	95% CI Upper	0.898																			

CI: confidence interval. K - Cohen's Kappa

Tests result agreement

The 2 tests with the overall highest sensitivity (Euroimmun and Snibe; Table 4) show a moderate agreement index (Cohens' Kappa = 0.723; Table 5). Euroimmun has the best agreement with SD (Cohens' Kappa = 0.827), and Snibe correlates the best with Getein (Cohens' Kappa = 0.874). For semiquantitative tests, most of the samples from COVID-19 inpatients with contradicting results present values far from the negative-to-positive cut-off values and therefore do not represent borderline positive results (Supplementary Figure S2).

Recipient-operating characteristic (ROC) curves

Analysis of the ROC curves of the semiquantitative tests reveals that Euroimmun is the test that best distinguishes SARS-CoV-2 non-infected controls from the SARS-CoV-2 confirmed cases, both for IgA and IgG (AUC [IgG] = 0.911; AUC [IgA] = 0.935; Figure 1).

Comparison of immunoglobulins levels in COVID-19 inpatients with distinct clinical presentation

To further understand the immune response profile to SARS-CoV-2, Ig levels were associated with clinical presentation. COVID-19 inpatients were classified as having a severe or non-severe clinical presentation based on the need, or not, for oxygen therapy above or below 10 L/min, respectively, as specified in the Methods section. When comparing COVID-19 patients with a severe or non-severe clinical presentation, no differences were observed on their clinical and demographic characteristics (i.e., gender, hypertension, diabetes, obesity, neoplasia, autoimmune disease, smoking habits, and administration of corticosteroids or other immunosuppressive drugs) as assessed by the Fisher Exact Probability test (Table 1).

Independent of clinical presentation, all Ig measured in the semiquantitative tests are detected at <10 days since symptoms onset. IgM and IgG plateau from 16–21 days forwards, and a peak in the relative amount of IgA is observed at 16–21 days since symptoms onset (Supplementary Figure S3). When comparing COVID-19 inpatients with a severe versus a non-severe clinical presentation, IgG tends to be lower in the severe group at <10 days since symptoms onset, reaching statistical significance for Abbot and Euroimmun (Abbott: U = 7.5, P = 0.0006; Euroimmun IgG: U = 23.5, P = 0.0045; Figure 2). Euroimmun IgA and Snibe IgM also present lower relative amounts in the severe group at <10 days since symptoms onset when compared with the non-severe group

(Euroimmun IgA: U = 29.5, P = 0.0133; Snibe IgM: U = 33.5, P = 0.0446). For Snibe, both IgM and IgG levels at >21 days are higher in the severe group (Snibe IgM: U = 145.5, P = 0.0106; Snibe IgG: U = 110, P = 0.0064). No differences are observed in the other tests or time ranges analysed (Figure 2).

Discussion

Serological testing for SARS-CoV-2 infection is a rapid, inexpensive and easy-to-perform diagnostic approach complementary to RT-qPCR and an essential tool to access the presence and profile of Ig against the virus, both individually and at the population level. The present study compares the performance of serological tests for SARS-CoV-2 using well-characterised COVID-19 inpatients with a diverse presentation of clinical severity, at various time-points across the disease course. Many studies exploring the production of Ig against SARS-CoV-2 have been reported, however, few compare different tests applied to the same set of plasma samples. Comparison of test performance, as presented here, is relevant because it depends on inherent population genetic variations, on the time-point in the disease course and on disease severity.

Regardless of the test methodology applied (ELISA, CLIA, LFIA), we observe here, as reported previously, that test sensitivity is dependent on time since symptoms onset. In addition, the combined detection of IgG and IgA/IgM against SARS-CoV-2 leads, in the majority of cases, to a better performance than measurements of a single Ig class, irrespective of the time range evaluated. These observations were previously reported by others and are in agreement with the establishment of the immune response, where IgA, IgM and IgG have different dynamics throughout disease progression (Alter and Seder, 2020; Guo et al., 2020; Liu et al., 2020b; Montesinos et al., 2020; Okba et al., 2020; Padoan et al., 2020; Sun et al., 2020; Yongchen et al., 2020; Zhang et al., 2020). This dynamic nature of test sensitivity needs to be considered when interpreting performance. Inconsistent reporting by manufactures or lack of details about the time-points used to establish test performance may create confusion regarding the sensitivity levels achievable. This has contributed to the recent decision by the US Food and Drug Administration to remove some tests from the Emergency Use Authorization.

Previous reports concluded that N-protein/peptide-based tests achieve better sensitivities than those based on the S-protein/
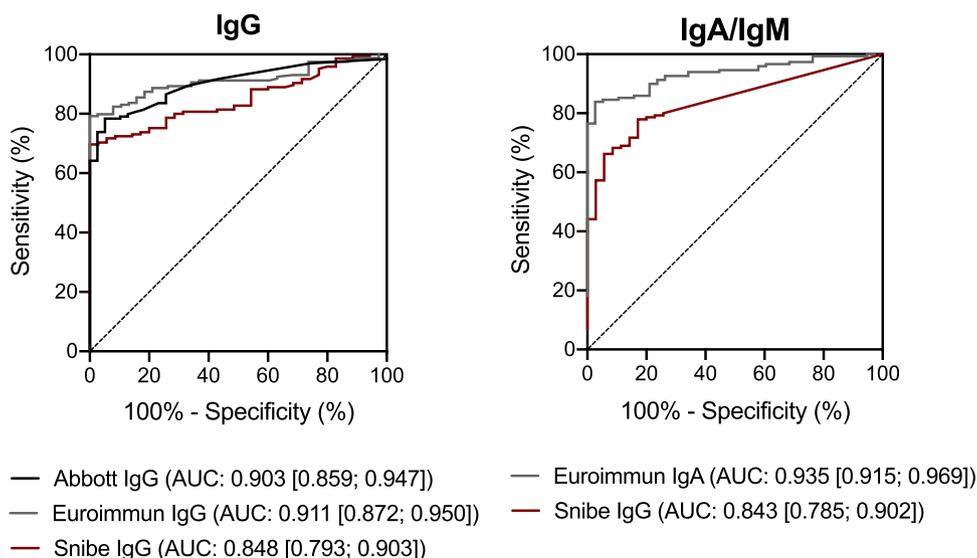
Figure 1. Receiver operator characteristic (ROC) curves of the assayed semiquantitative tests. AUC: Area Under the Curve. Ig: Immunoglobulin.
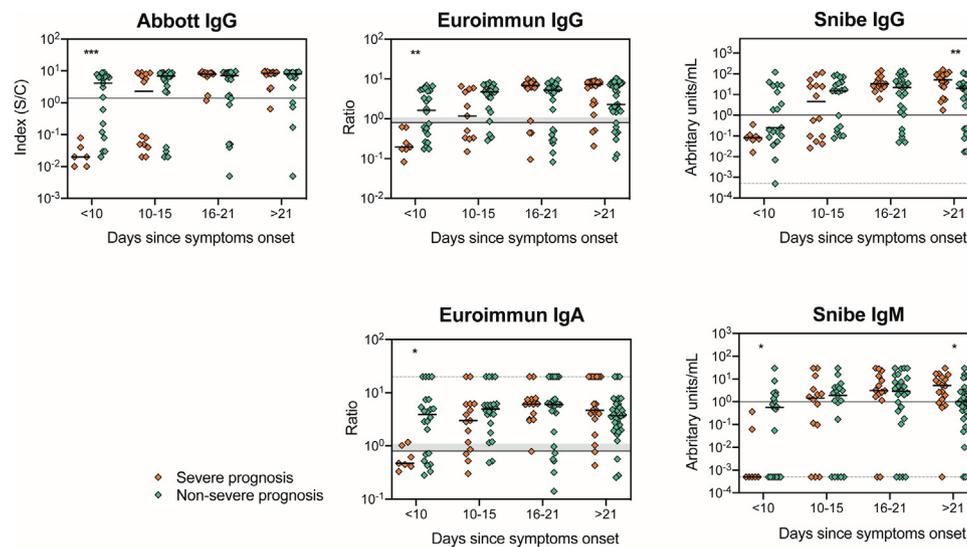
Figure 2. Comparison of the Ig levels in patients with a severe and non-severe clinical presentation using the semiquantitative tests Abbott, Euroimmun and Snibe. Each dot represents one sample and the solid thick lines correspond to the group's median. Solid thin lines represent each test's cut-off value. In the case of Euroimmun test, the shadowed grey banner refers to borderline values according to the manufacturer (between 0.8 and 1.1). Since the y-axis has a log scale that does not allow the representation of zero, in those situations (Snibe IgG and IgM) arbitrary values were attributed, and a dashed grey line was represented in this value. For values above the detection limit (Euroimmun IgA), a random value of 20 was attributed and represented as a dashed grey line. Groups median were compared using a Mann–Whitney U-tests; significant differences were represented as: * for $P < 0.05$; ** for $P < 0.01$; ***, $P < 0.001$.

peptide. This difference may result from an earlier immune response against the N-protein/peptide in comparison to the S-protein/peptide or it may be related to the higher specificity of Ig towards the N-protein/peptide (Caini et al., 2020; Kontou et al., 2020; Liu et al., 2020a). It is not possible to compare these results with the present study since 4 of the assayed tests displayed no information regarding the target antigen, 4 targeted both proteins, 2 targeted only the N-protein/peptide and 1 targeted only the S-protein.

This study reports that Ig targeting SARS-CoV-2 are detectable soon after symptoms onset for some patients (3–5 days for IgM, IgA or IgG; e.g., Patients 012, 033 and 070; Supplementary Table S1) while for others detection (with the same tests) occurred much later (>21 days; e.g., Patients 004, 037 and 064; Supplementary Table S1). Several factors might account for these differences; antibody production kinetics during SARS-CoV-2 infection is not yet fully elucidated nor are the factors responsible for the differences in patient response (Chen et al., 2020; Gudbjartsson et al., 2020a; Liu et al., 2020b; Wang et al., 2020; Zhao et al., 2020). Identifying the variable(s) responsible for distinct profiles of seroconversion is necessary to be able to fully understand false-negative results.

Time since symptoms onset is self-reported which might introduce variability taking into consideration the diversity of clinical manifestations of COVID-19 and of symptom perception by each individual. However, we consider that it introduces less variability than time since disease diagnosis by RT-qPCR, which is performed for some patients before symptoms onset and for others several days after.

Despite variability in the overall sensitivity among the assayed tests, the PPV in COVID-19 hospitalised patients when assuming ≥50% seroprevalence is >87.5%, except for Cellex. However, using serological tests as a seroprevalence and surveillance tool when the estimated prevalence is low e.g., 3%, which is the estimated seroprevalence for the Portuguese population (Rodrigues, 2020), is more challenging due to the observed variation. Still, some of the tests are promising in low prevalence scenarios. For the semiquantitative tests, that can only be used in a laboratory, Euroimmun presented the best PPV (mainly IgG) which was

expected due to its high specificity. For the rapid diagnostic tests, the combined IgG and IgM from Liming and Render has 100% PPV. The high NPV of these serological tests in low prevalence scenarios reinforces their suitability for use in the general population if the objective is to detect the presence of SARS CoV-2 antibodies in seroprevalence studies. However, for use in seroprevalence studies during a pandemic phase, a highly specific test is required to assure an acceptable PPV in a low seroprevalence population, as well as a reasonable sensitivity. Euroimmun IgG (ELISA) and the Liming IgG and IgM combination (LFIA) meet these criteria. Both tests have 100% PPV and an overall specificity close to 75% on a 3% seroprevalence scenario meaning that all the positive tests will be specific for SARS CoV-2 antibodies and 75% of subjects with Ig against SARS-CoV-2 will be detected (Table 4).

Our results highlight that Ig levels in a hospitalised COVID-19 population are associated with disease severity. Our data suggest a delay in Ig detection in patients with more severe disease presentation; at <10 days since symptoms onset they present relatively lower levels of Ig (IgA, IgM and IgG) compared to patients with non-severe clinical presentation. To our knowledge this has not previously been reported; some studies actually found no variability in Ig production between patients with different clinical presentation in the initial phase of the disease. As has been previously reported (Chen et al., 2020; Gudbjartsson et al., 2020a; Liu et al., 2020b; Wang et al., 2020; Zhao et al., 2020), we observed that patients with severe clinical presentation show higher relative amounts of Ig at later periods since symptoms onset (>21 days). A recent study showed that higher amounts of Ig in patients with a severe clinical presentation are associated with higher neutralising capability (Wang et al., 2020). The association of Ig production with disease severity is of utmost relevance in clinical settings and needs to be further explored in a larger cohort of patients and evaluated longitudinally from the earliest perceived onset of symptoms through the long-term course of the disease.

Our results are based on measurements performed in a specific population of hospitalised COVID-19 patients. Asymptomatic and non-hospitalised COVID-19 patients were not analysed, for this reason, extrapolation of these data to other COVID-19 settings or the general population should be made only cautiously. However,

the results provide the basis for an informed selection of a serological test to be assayed and applied in a larger population setting to evaluate the potential value of each test as a complement for COVID-19 diagnosis and to understand the dynamics of Ig production on infection with SARS-CoV-2 or on vaccination.

Data availability

Data will be available from the corresponding author upon justified request.

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Contribution

C.S-M., C.N., S.R., C.C., J.A.P., P.G.C. and M.C-N. conceptualized the study; C.S-M., C.N., S.R. and M.C-N. designed the experiments; J. C., A.R., M.F., H.S., O.P., A.C., C.C. and P.G.C. recruited the patients and collected the clinical data; C.S-M., C.N., S.R., JC-G., C.S.S., N.V., P.B-S. and P.A-P. performed the experiments; C.S-M., C.N. and S.R. prepared the figures and performed the statistical analysis; J.A.P., P. G.C. and M.C-N. supervised the study; and all authors discussed the results and contributed to the final manuscript.

Conflict of interest

Getein kits were provided free of charge by the manufacturer. No other conflicts of interest reported.

Acknowledgments

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2021.01.038.

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