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Comparison of six commercially available SARS-CoV-2 antibody assays—Choice of assay depends on intended use

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ABSTRACT

Objectives: Evaluate six commercial serological assays for detection of IgA, IgM or IgG SARS-CoV-2 antibodies in different disease severities.
Methods: Three lateral flow tests (LFTs) (Acro IgM/IgG, CTK IgM/IgG, Livzon IgM/IgG) and three ELISA assays (Euroimmun IgA and IgG, Wantai IgM) were included. Application was evaluated using samples from 57 patients with a positive SARS-CoV-2 reverse transcription polymerase chain reaction, stratified according to disease severity. Specificity was assessed using historical samples from 200 blood donors.
Results: While IgM LFTs failed to detect SARS-CoV-2 antibodies in 37–84% of non-hospitalised patients, the Wantai IgM ELISA detected antibodies in 79%. The Euroimmun IgG ELISA detected antibodies in 95% of non-hospitalised patients. IgA, IgM and IgG ELISA levels were initially low, increased over time, and correlated with disease severity. LFT sensitivity declined in samples taken >28 days after symptom onset/resolution. The Livzon IgG LFT had the highest specificity (98.5%), followed by the Euroimmun IgG ELISA (96.2%). The specificity for Euroimmun IgA ELISA improved (≥97.5%) using a custom cut-off value (4.0).
Conclusions: The sensitive and semi-quantitative ELISA assays are most appropriate for serologic detection of SARS-CoV-2 infection in mild cases. Livzon LFT and Euroimmun ELISA had the highest specificity among the IgG assays, making them most suitable for seroprevalence studies.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the cause of coronavirus disease 2019 (COVID-19), a novel infectious disease primarily manifesting as pneumonia. The clinical spectrum ranges from asymptomatic cases to severe or even fatal infections. However, in the majority of cases patients are not critically ill (Guan et al., 2020; Long et al., 2020b). Diagnosis of COVID-19 can be made by direct detection of SARS-CoV-2 RNA, typically by reverse transcription polymerase chain reaction (RT-PCR) on respiratory tract samples.

Several studies indicate that asymptomatic infections are frequent (Hoehl et al., 2020; Nishiura et al., 2020). Antibody responses during and after SARS-CoV-2 infection are poorly understood, especially in asymptomatic or mild cases, and the clinical usefulness of serological testing in early stages of COVID-19 remains uncertain. In general, it appears that SARS-CoV-2 antibodies can be detected within several days to weeks after first onset of clinical disease. IgM and IgA appear first, shortly after IgG antibodies to SARS-CoV-2 can be detected in most subjects (Guo et al., 2020; Long et al., 2020a; Zhang et al., 2020). However, solid data on the magnitude and duration of IgM, IgA and IgG responses in relation to disease severity are lacking, and accuracy and analytical sensitivity varies with the method and assay used (Lassaunière et al., 2020). Serological testing complements

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genomic methods and several testing modalities are available (Okba et al., 2020). Enzyme-linked immunosorbent assays (ELISA) offer several advantages compared to other serological assays including reduced workload, higher throughput, shorter turn-around time, and lower cost. Lateral flow tests (LFTs) have the advantage of being easy to implement and the potential for point-of-care testing (Li et al., 2020). However, concerns have been raised regarding both the sensitivity and specificity of LFTs (Van Elslande et al., 2020). Reports are emerging that serological testing using validated assays might add diagnostic information in patients suspected for COVID-19 with a negative SARS-CoV-2 RT-PCR (Guo et al., 2020; Zhao et al., 2020) and assist with identification of extant or recent asymptomatic infections (Long et al., 2020a; Zhao et al., 2020). Serological testing might also be used to assess the communal burden of disease and could potentially be an indicator of individual immunity.

The purpose of this study was to evaluate six commercially available CE-marked SARS-CoV-2 antibody assays and to evaluate the usefulness of these assays in different disease severities.

Material and methods

Patients and samples

A gold standard for the assessment of antibodies to SARS-CoV-2 is not available, hence a “true positive” antibody test was defined according to RT-PCR positivity.

The inclusion criteria were a positive SARS-CoV-2 RT-PCR test on a respiratory tract sample and the availability of a blood sample for SARS-CoV-2 antibody testing. There were no exclusion criteria. As part of a routine setting during late March to late April 2020, blood samples from all participants had been sent to the Department of Clinical Immunology and Department of Clinical Microbiology at Odense University Hospital for SARS-CoV-2 antibody testing. A total of 57 COVID-19 patients were retrospectively included, with 98 samples from this cohort tested for SARS-CoV-2 antibodies. Participants were stratified into three groups. The first group consisted of 19 non-hospitalised cases with mild symptoms, defined as combinations of mild flu-like symptoms, cough and fever. The participants were active blood donors diagnosed with COVID-19 and recruited for a convalescent plasma project. Samples from participants were collected at least four weeks after symptom resolution. The other two participant groups consisted of COVID-19 patients admitted to Odense University Hospital during the study period. One group of 27 patients with moderate COVID-19 disease requiring hospitalisation at a ward specialising in infectious diseases and one group of 11 patients with severe COVID-19 disease requiring admission to an intensive care unit (ICU).

Sensitivity was defined as the proportion of patients correctly identified as having SARS-CoV-2 infections, as initially diagnosed by RT-PCR detection of SARS-CoV-2 in respiratory samples.

200 randomly selected frozen plasma samples collected in April 2018 (one month after a severe influenza epidemic in Denmark) from anonymous voluntary Danish blood donors, were included as putative SARS-CoV-2 antibody negative controls. Specificity was defined as the proportion of SARS-CoV-2 immune naïve blood donors accurately identified as negative for COVID-19.

Receiver operating characteristics (ROC) analyses were performed on the semiquantitative ELISA assays, using all samples from both patients and healthy controls.

The detection limit of IgG and IgM assays was compared using a serum sample containing IgM and IgG SARS-CoV-2 antibodies, two-foldly diluted (1-1,024). The highest titer which yielded a positive reaction was recorded.

This study sought to adhere to the STARD criteria for reporting of studies of diagnostic accuracy (Bossuyt et al., 2015).

Enzyme-linked immunosorbent assay (ELISA) and lateral flow tests (LFTs)

Three SARS-CoV-2 antibody ELISAs and three LFTs were evaluated. Selection of assays and tests was according to availability on the Danish market at the time of the study (late March to late April 2020). All testing and evaluation was performed in tertiary care hospital laboratories at the Department of Clinical Immunology and the Department of Clinical Microbiology, Odense University Hospital. Both laboratories are accredited according to the EN:ISO 15189 standard. All samples were analysed using the same lot number for each of the six different assays.

Wantai IgM ELISA

WANTAI SARS-CoV-2 IgM ELISA is a CE/IVD-marked assay (Beijing Wantai Biological Pharmacy Enterprise Co., Ltd., China). The IgM ELISA is a solid-phase antibody capture ELISA assay in which polystyrene microwell strips are pre-coated with anti-human immunoglobulin M (anti-μ chain). Any IgM-class antibodies will be captured inside the wells and subsequently detected by the addition of recombinant SARS-CoV-2 antigen (spike protein including the receptor binding domain) conjugated with horseradish peroxidase, thus catalysing a colour reaction proportional with concentration of IgM antibody specific for SARS-CoV-2. The assay was performed according to the manufacturer’s instructions. Calibrations were performed in semiquantitative ratios, which are relative measures for the concentration of antibodies in the sample. Results were interpreted using the cut-offs recommended by the manufacturer; ratios <0.9 were interpreted as negative, ≥0.9 – <1.1 as borderline, and ≥1.1 as positive.

Euroimmun IgA and IgG ELISA

The EUROIMMUN anti-SARS-CoV-2 IgG and IgA ELISAs are CE/IVD-marked test systems (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany). Both the IgG and the IgA ELISA tests are classic sandwich ELISAs. Samples containing anti-SARS-CoV-2 IgG, IgA and IgM will bind to the antigen which is a recombinant structural protein (S1-domain including the receptor binding domain) of SARS-CoV-2. Bound antibodies are detected during a second incubation, using enzyme-labelled anti-human IgG or IgA, thus catalysing a colour reaction proportional with concentration of IgA of IgG antibody specific for SARS-CoV-2. Both assays were performed according to the manufacturer’s instructions. Calibrations were performed in semiquantitative ratios, which are relative measures for the concentration of antibodies in the sample. Results were interpreted using the cut-offs recommended by the manufacturer; ratios <0.8 were interpreted as negative, ≥0.8 – <1.1 as borderline, and ≥1.1 as positive.

Lateral flow tests (LFTs)

Three different serological LFTs were evaluated. These were the Acro IgM/IgG LFT (2019-nCoV IgG/IgM Rapid Test Cassette (Acro Biotech, Rancho Cucamonga, CA, USA)), Livzon IgM/IgG LFT (Diagnostic Kit for IgM/ IgG Antibody to Corona Virus (Zhuhai Livzon Diagnostics, Inc., Zhuhai, China)) and CTK IgM/IgG LFT (OnSiteTM COVID-19 IgG/IgM Rapid Test (CTK Biotech, Poway, CA, USA)). All tests are CE-IVD approved and they all apply a colloidal gold immuno-chromatography technology to detect either IgM or IgG SARS COV-2 antibodies. All three assays were performed according to the manufacturer’s instructions. The LFTs were read

by visual inspection 15 min after application of test material (one observer per test, but tests were performed by multiple laboratory technicians). Only tests in which the control line was visible were regarded as valid. If a line for IgM and/or IgG was observed, the test was considered positive for that isotype of antibody.

Statistical analysis

Data on analytical sensitivity and specificity were processed using Microsoft Excel (Microsoft, Redmond, WA, USA) and confidence intervals were calculated using the engine for calculation of exact confidence intervals for binomially distributed data on Statpages (statpages.info/confint.html).

Further statistical analysis and generation of boxplot figures was carried out using JMP for Mac ver. 14.2 (SAS Institute, Cary, NC, USA). Comparisons of antibody levels across patient groups were carried out using one-way ANOVA, with a statistically significant difference within a group followed by pairwise post-hoc Tukeys HSD test. Receiver operating characteristics (ROC) charts were created using Prism 8 for MacOS (GraphPad Software, LLC San Diego, CA, USA) and used to assess the performance of the ELISA tests. Statistical tests were performed as two-sided tests and $p < 0.05$ was considered statistically significant.

Results

Patient characteristics

One group consisted of non-hospitalised individuals with mild symptoms (n = 19, 24 samples), a second group comprised patients with moderate symptoms of COVID-19 requiring hospitalisation at a ward specialising in infectious diseases (n = 27, 51 samples), and the third group consisted of patients with severe disease requiring admission to an ICU (n = 11, 23 samples). For 25/57 (44%) patients a median of two (range 2–5) samples were available. For the remainder, 32/57 (56%), only one sample was available. For non-hospitalised individuals, samples were drawn at a median of 45 (range 34–51) days after symptom onset. For patients with moderate COVID-19, samples were drawn median 18 (range 6–31) days after symptom onset. For patients with severe COVID-19, samples were drawn at a median of 13 (range 5–26) days after symptom onset.

Diagnostic accuracy assessed by ROC

Diagnostic accuracy was highest for the Wantai IgM ELISA with an area under curve (AUC) of 0.993, 95% confidence interval (CI) 0.98–1.00 (Figure 1). In comparison, the Euroimmun assays, IgA ELISA (AUC 0.959, 95% CI: 0.93−0.98) and IgG ELISA (AUC 0.945, 95% CI: 0.92−0.97), had lower AUCs, though still above 0.94.

Specificity IgM, IgG and IgA

Assay specificities are shown in Table 1. For the IgM assays, the highest specificities were found using the Livzon IgM LFT and CTK IgM LFT (1/200, i.e., 97.6% one-sided 95%-upper confidence limit). The Wantai IgM ELISA had a specificity of 2/200 equal to 96.9% (one-sided 95%-upper confidence limit). The Livzon IgG LFT showed the highest specificity of all assays (0/200, i.e., 98.5% one-sided 95%-upper confidence limit).

The Euroimmun ELISA IgG had a specificity of 3/200, i.e., 96.2% (one-sided 95%-upper confidence limit). The Acro IgG LFT and CTK IgG LFT both had lower specificities (Table 1).

Using the manufacturer's recommended cut-off, the specificity of the Euroimmun IgA ELISA assay was 73.2% (one-sided 95%-upper confidence limit). A new cut-off was calculated and established at 4.0 in order to yield a specificity of ≥97.5%.

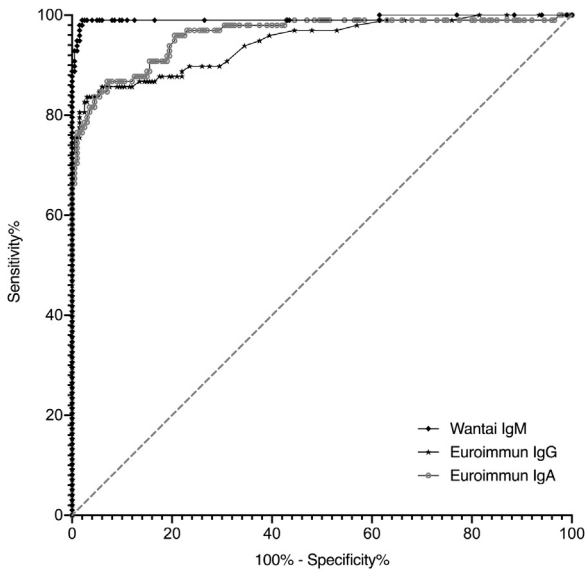

Figure 1. ELISA assay receiver operating characteristics (ROC) charts based on all tests in case subjects and in healthy controls (N = 298). Area under curve (AUC) for Wantai IgM ELISA 0.993 (95% CI: 0.98–1.00), Euroimmun IgA ELISA (95% CI: 0.93–0.98) and Euroimmun IgG ELISA 0.945 (95% CI: 0.92–0.97). ELISA: enzyme linked immunoassay.

Overall antibody response and assay sensitivity in relation to time

When grouping samples according to days since symptom debut, the Wantai IgM ELISA had the highest sensitivity in all time intervals ranging from 86 to 100% (Table 2a). All the IgM LTFs were inferior to the Wantai IgM ELISA, and showed peak detection rates in the time intervals ranging from day 15 to 28. In nine samples, SARS-CoV-2 IgM antibodies could not be detected by LFT. Using ELISA, four samples were negative in all assays (Table 2a). For the Euroimmun IgG ELISA assay the sensitivity increased in relation to days since symptom onset, reaching 96% in samples drawn >28 days (Table 2b). A similar pattern was also seen with the IgG LFTs, however, for samples drawn >28 days after symptom onset, there was a decline in sensitivity, especially so for the Livzon IgG LFT (Table 2b).

In Figure 2 a–c the antibody optical density (OD) ratios for the three semi-quantitative ELISA assays are shown in relation to time of symptom onset. For all isotypes, antibody levels were initially low and then increased steadily over the following weeks. For the Euroimmun IgA ELISA, a plateau was reached already after a few weeks, primarily due to many samples reaching the maximum OD ratio of the IgA ELISA assay.

Antibody response and assay sensitivity in relation to disease severity

Overall IgM and IgG antibody response in relation to disease severity was assessed on the last sample available from each patient. Overall the Wantai IgM ELISA appears to be the most sensitive assay for detection of SARS-CoV-2 IgM antibodies across all three patient groups, with detection rates ranging from 79 to 100% (Table 3a). In non-hospitalised subjects with samples taken a median of 45 days after symptom debut, the Wantai IgM ELISA detected antibodies in 79%. The LFTs all showed low sensitivity and failed to detect IgM antibodies in the majority of non-hospitalised cases. The Livzon IgM LFT and Acro IgM LFT both only detected IgM SARS-CoV-2 antibodies in 16% of this group. In samples from patients treated at the ICU (taken a median of 16 days after symptom onset) the Wantai IgM ELISA, Livzon IgM LFT and CTK IgM LFT detected SARS-CoV-2 antibodies in 100% of the samples. The

Table 1
ELISA assay and lateral flow test (LFT) specificity. Number of samples presenting antibodies against SARS-CoV-2 in a panel of 200 putative SARS-CoV-2 immune naïve blood donors (one-sided 95%-lower confidence limit on percentage true negative results i.e., assay specificity). ELISA: enzyme linked immunosorbent assay, LFT: lateral flow test.

SARS-Cov-2 ab	Euroimmun ELISA	Wantai ELISA	Livzon LFT	Acro LFT	CTK LFT
IgM	–	2 (96.9)	1 (97.6)	3 (96.2)	1 (97.6)
IgG	3 (96.2)	–	0 (98.5)	9 (92.8)	7 (93.5)
IgA	43 (73.2)	–	–	–	–

Table 2
(a and b) Number of samples (N = 98) presenting with antibodies against SARS-CoV-2 IgM (a) and IgG (b) (% positive) in relation to days from symptom debut. ELISA: enzyme linked immunosorbent assay, LFT: lateral flow test.

a

SARS CoV-2 IgM

Days since debut	No. of samples	Wantai ELISA	Livzon LFT	Acro LFT	CTK LFT	NEG in all LFT assays	NEG in all assays
1–7	3	3 (100)	1 (33)	3 (100)	2 (67)	–	–
8–14	23	20 (87)	20 (87)	18 (78)	19 (83)	1 (4)	1 (4)
15–21	32	32 (100)	30 (94)	26 (81)	29 (91)	1 (3)	–
22–28	12	12 (100)	11 (92)	11 (92)	12 (100)	–	–
>28	28	24 (86)	9 (32)	8 (29)	20 (71)	7 (25)	3 (11)

b

SARS CoV-2 IgG

Days since debut	No. of samples	Euroimmun ELISA	Livzon LFT	Acro LFT	CTK LFT	NEG in all LFT assays	NEG in all assays
1–7	3	0 (0)	1 (33)	2 (67)	1 (33)	1 (33)	1 (33)
8–14	23	13 (57)	17 (74)	21 (91)	18 (78)	2 (9)	2 (9)
15–21	32	28 (88)	30 (94)	32 (100)	30 (94)	–	–
22–28	12	11 (92)	12 (100)	12 (100)	11 (92)	–	–
>28	28	27 (96)	17 (61)	27 (96)	24 (86)	–	–

Acro IgM LFT had the weakest performance and only detected antibodies in 82% of these patients (Table 3a). Overall, SARS-CoV-2 IgM antibodies could not be detected by LFT in eight samples. Using ELISA, four samples were negative in all assays (Table 3a). Comparing the IgG assays, the Euroimmun IgG ELISA and the Acro IgG LFT showed equal sensitivity in non-hospitalised patients detecting antibodies in 95% (Table 3b). The Livzon IgG LFT failed to detect IgG antibodies in more than half of non-hospitalised patients, with a sensitivity at 42%. The CTK IgG LFT also had a low sensitivity in this group, detecting antibodies in 79% of non-hospitalised patients. Both the Livzon IgG LFT and the Acro IgG LFT detected SARS-CoV-2 antibodies in all ICU patients, while the Euroimmun IgG ELISA and CTK IgG ELISA detected antibodies in 91% of ICU patients (Table 3b).

Development of antibody response in patients with repeated sampling

When comparing detection rates using a subset of ten hospitalised patients (five from the Department of Infectious Diseases, five from the ICU) for whom two samples taken ≥7 days apart were available, the Wantai IgM ELISA showed excellent sensitivity, detecting antibodies against SARS-CoV-2 in all samples (Table 4). The earliest sample was taken five days after symptom onset and the last sample were taken after 31 days. The IgM LTFs, however, were not able to detect antibodies in all patients. The Livzon IgM LTF and the CTK IgM LTF were able to detect antibodies in all of the late samples, but the Acro IgM LTF failed to detect antibodies in a late sample from an ICU patient (Table 4). The Euroimmun IgG ELISA and IgG LFTs, except the CTK assay, were able to detect IgG antibody in all of the late samples (Table 4). However, detection ability with regard to the early samples varied (Euroimmun IgG ELISA 5/10, Livzon IgG LFT 6/10, Acro IgG LTF 9/10 and CTK IgG LFT 7/10). Using the manufacturer's recommended cut-off for the Euroimmun IgA ELISA, all samples tested positive.

Antibody level determined by OD ratios

When assessing anti-SARS-COV-2 IgM, IgG and IgA by ELISA OD ratios across the three patient categories (using all 98 samples), the general pattern of mean antibody level (OD ratio) for all three ELISA assays were in the order: non-hospitalised patients < hospitalised patients < ICU patients (Figure 3a–c). In detail, for IgM, mean antibody levels determined by OD ratios were 9.2 (non-hospitalised patients), 18.6 (hospitalised patients) and 27.2 (ICU patients), with all differences being statistically significant ($p < 0.0001$–$p = 0.021$) (Figure 3a). For IgG, mean antibody levels were 4.0 (non-hospitalised), 5.4 (hospitalised) and 6.3 (ICU), but differences were not statistically significant ($p = 0.14$) (Figure 3b). Finally, for IgA, mean antibody levels were 4.4 (non-hospitalised), 7.4 (hospitalised) and 9.5 (ICU), with all differences being statistically significant ($p < 0.0001$–$p = 0.016$) (Figure 3c).

Detection limit IgM and IgG

The Wantai IgM ELISA had the lowest detection limit, yielding a positive reaction at 64-fold sample dilution. The IgM LFTs all had higher (i.e., inferior) detection limits; the CTK IgM LFT showed a positive reaction at a maximum of 16-fold sample dilution, the Livzon IgM LFT at eight-fold sample dilution, and the Acro IgM LFT at four-fold sample dilution. Regarding the IgG assays, the Acro IgG LFT showed the lowest detection limit, detecting a positive reaction at 64-fold sample dilution. Euroimmun IgG ELISA and CTK IgG LFT both showed positive reactions at 32-fold sample dilutions. Livzon IgG LFT, yielded a positive reaction at eight-fold sample dilutions.

Discussion

Overall, the number of samples presenting antibodies against SARS-CoV-2 is related to disease severity and to days since

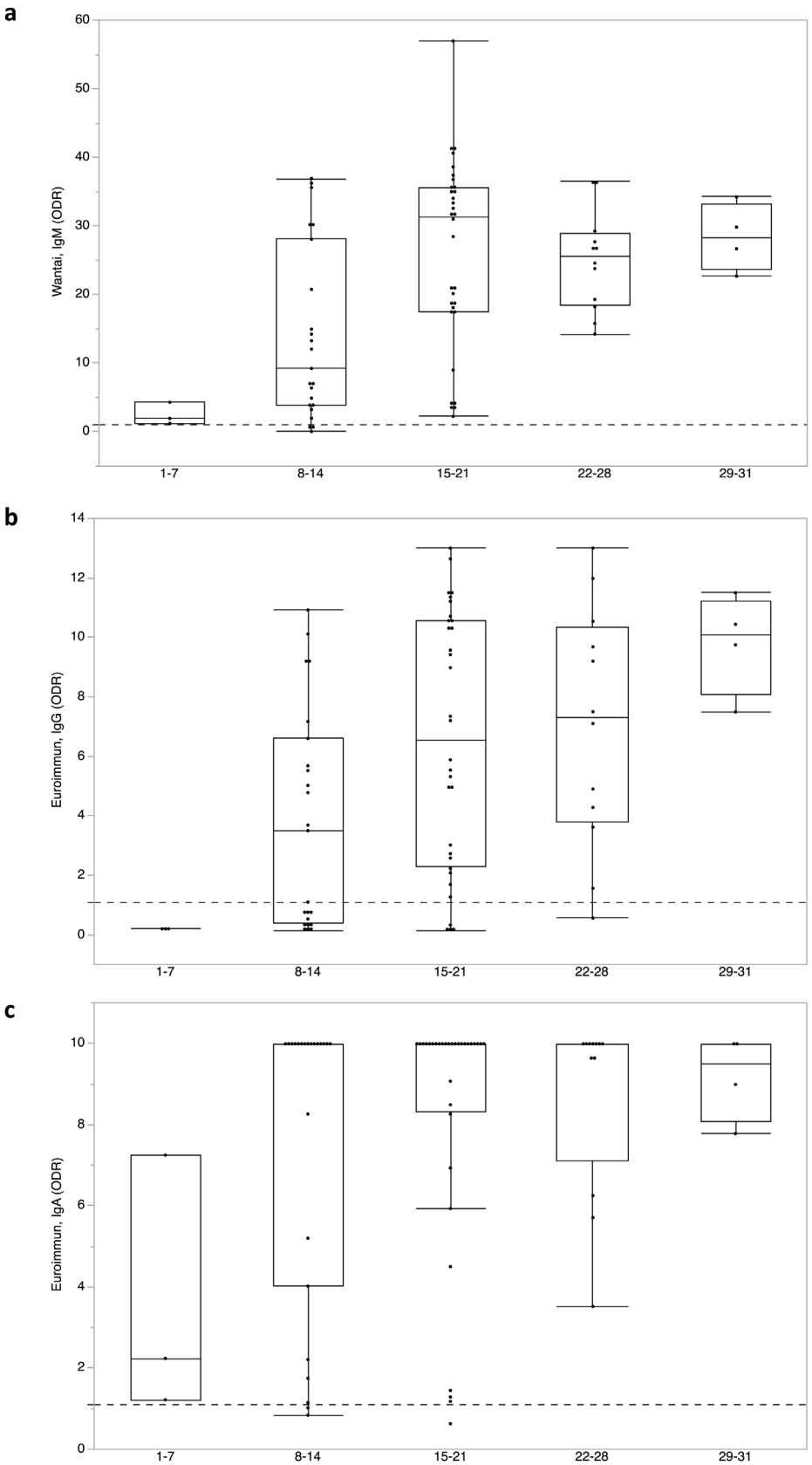

Figure 2. (a–c) Outlier box plot of level of IgM antibody (Wantai IgM ELISA) (a), IgG antibody (Euroimmun IgG ELISA) (b) and IgA antibody (Euroimmun IgA ELISA) (c) vs. days after symptom debut. Based on samples from hospitalised and intensive care unit patients only (n = 38 patients, 74 samples). Dotted line indicate assay cut-off (ratio 1.1). ELISA: enzyme linked immunoassay.

symptom onset, but differs with the type of assay. The Wantai IgM ELISA was the best assay for detection of SARS-CoV-2 antibodies in

Table 3
(a and b) Assay sensitivity according to disease severity (last patient sample, N = 57). Days since symptom debut are given as median of days (range). Number of samples presenting antibodies against SARS-CoV-2 IgM (a) and IgG (b) (% positive) in relation to disease severity. ELISA: enzyme linked immunosorbent assay, LFT: lateral flow test.

a

SARS CoV-2 IgM			
	Non-hospitalised	Hospitalised	Intensive care unit
No. patients:	19	27	11
Days since debut	45 (34–51)	18 (8–31)	16 (10–26)
Livzon LFT	3 (16)	24 (89)	11 (100)
Acro LFT	3 (16)	22 (81)	9 (82)
CTK LFT	12 (63)	25 (93)	11 (100)
Wantai ELISA	15 (79)	25 (93)	11 (100)
Negative in all LFT assays	6 (32)	2 (7)	–
Negative in all assays	3 (16)	1 (4)	–

b

SARS CoV-2 IgG			
	Non-hospitalised	Hospitalised	Intensive care unit
No. patients:	19	27	11
Days since debut	45 (34–51)	18 (8–31)	16 (10–26)
Livzon LFT	8 (42)	24 (89)	11 (100)
Acro LFT	18 (95)	25 (93)	11 (100)
CTK LFT	15 (79)	24 (89)	10 (91)
Euroimmun ELISA	18 (95)	22 (81)	10 (91)
Negative in all LFT assays	–	2 (7)	–
Negative in all assays	–	2 (7)	–

Table 4
Changes in reactivity of SARS-CoV-2 antibody assays in 10 hospitalised patients (#6–10 represent ICU patients). Samples were drawn at least 7 days apart. For the lateral flow tests (LFTs), the intensity of the test band was read and reported as "-", "(+)" or "+". #Euroimmun IgA assay cut-off set at 1.1 (manufacturer's instructions). ELISA: enzyme linked immunosorbent assay, LFT: lateral flow test.

Patient ID	Days since debut	Wantai ELISA	Euroimmun ELISA		Livzon LFT		Acro LFT		CTK LFT	
		IgM	IgG	IgA#	IgM	IgG	IgM	IgG	IgM	IgG
1	16	+	+	+	+	+	+	+	+	+
	23	+	+	+	+	+	+	+	+	+
2	6	+	–	+	–	–	(+)	–	(+)	–
	18	+	+	+	+	+	+	(+)	+	+
3	19	+	–	+	(+)	–	–	(+)	(+)	–
	26	+	+	+	(+)	(+)	(+)	+	+	+
4	19	+	+	+	+	+	(+)	(+)	–	(+)
	31	+	+	+	+	+	+	+	+	+
5	21	+	+	+	(+)	+	(+)	(+)	(+)	+
	30	+	+	+	(+)	+	+	(+)	+	+
6	8	+	–	+	–	–	(+)	(+)	(+)	(+)
	15	+	+	+	(+)	+	(+)	+	+	+
7	5	+	–	+	–	–	(+)	(+)	–	–
	12	+	+	+	(+)	(+)	(+)	(+)	(+)	–
8	19	+	+	+	+	+	(+)	+	+	+
	26	+	+	+	+	+	(+)	+	+	+
9	13	+	–	+	(+)	(+)	(+)	+	+	+
	20	+	+	+	+	+	(+)	+	(+)	+
10	11	+	+	+	(+)	(+)	–	(+)	+	+
	18	+	+	+	(+)	+	–	+	(+)	+

all three disease severities, with detection rates ranging from 79 to 100% and showed excellent diagnostic accuracy (AUC 0.993). While all IgM LFTs failed to detect SARS-CoV-2 antibodies in the majority of non-hospitalised subjects, the Wantai IgM ELISA detected antibodies in 79% of these cases. In particular, the Livzon IgM LFT and Acro IgM LFT both had extremely low detection rates at 16%. The Euroimmun IgG ELISA detected SARS-CoV-2 antibodies in 95% of non-hospitalised patients, as did the Acro IgG LTF. The Livzon IgG LFT had the lowest detection rate (42%) in non-hospitalised patients.

To our knowledge, this is one of the first accuracy studies including samples from both non-hospitalised patients with mild disease and patients with more severe disease, thus, providing the opportunity to assess test performance in different disease severities. Samples from non-hospitalised patients were taken 45 days (median, range 34–51) after symptom onset. In a real life setting, this is typically when there is a need for serologic testing. High sensitivity, as seen with the Wantai IgM ELISA and the Euroimmun IgA and IgG ELISA, is important in late presenting cases with milder symptoms and in patients suspected of COVID-19 despite negative SARS-CoV-2 RT-PCR (Sethuraman et al., 2020).

All ELISA assays tested have the advantage of being semi-quantitative, providing an estimate of antibody levels through OD ratios. All LFTs have the inherent limitation of being qualitative tests and being subject to inter-observer variation.

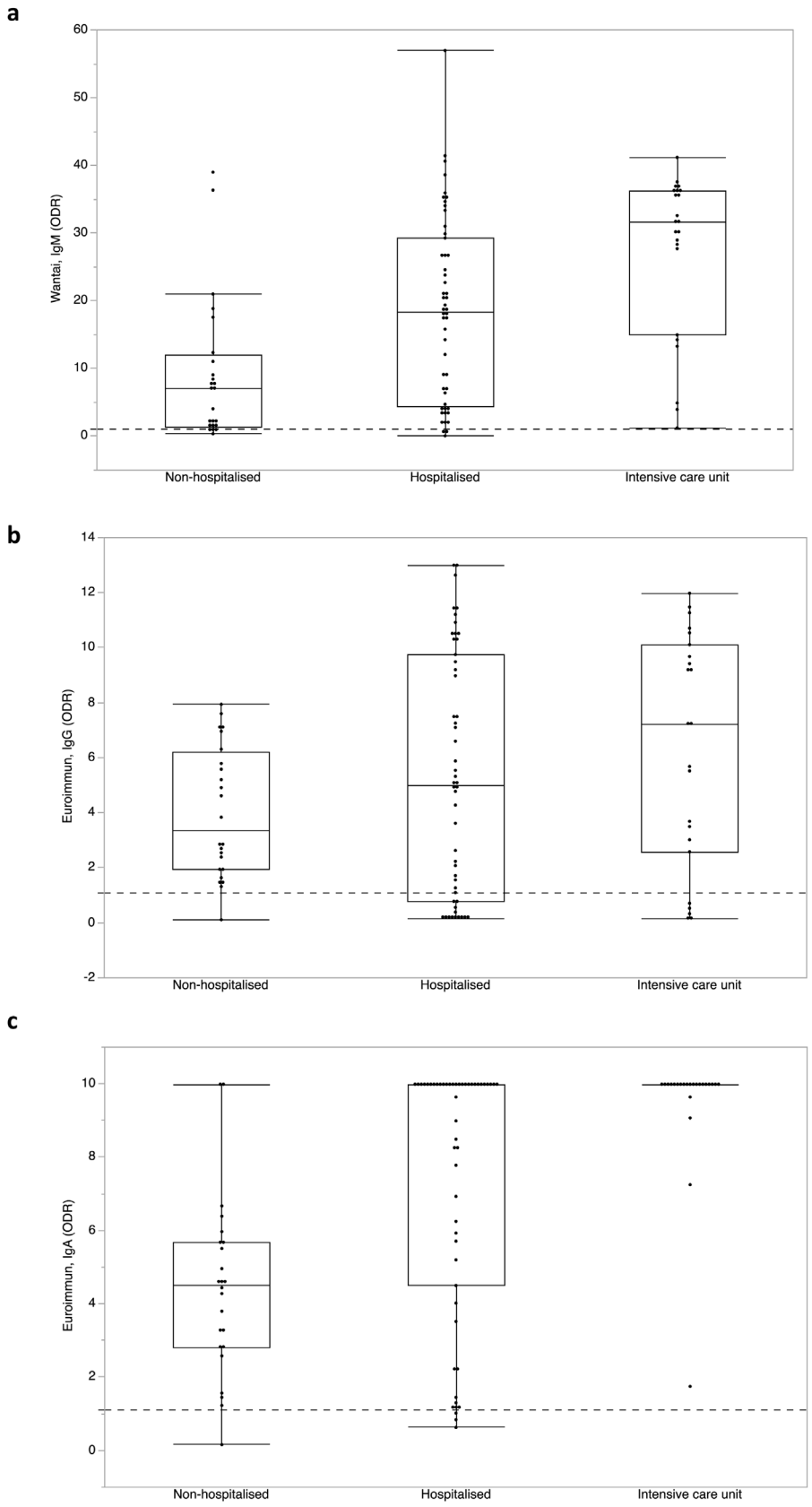

Figure 3. (a–b) Outlier box plot of level of IgM antibody (Wantai IgM ELISA) (a), IgG antibody (Euroimmun IgG ELISA) (b) and IgA antibody (Euroimmun IgA ELISA) (c) vs. patient category (n = 57 patients, 98 samples). Dotted line indicate assay cut-off (ratio 1.1). ELISA: enzyme linked immunoassay.

The performance and applicability of each test depends on the setting in which it is used. In a low prevalence screening situation, an assay with high specificity is required. Otherwise the positive predictive value will be unacceptably low. This can be achieved by

using a highly specific assay, or, when suitable, raising the cut-off limit for the assay used. The Livzon IgG LFT showed a high specificity (98.5%) and an adequate sensitivity in moderately and severely ill patients, but low sensitivity when applied to patients with only mild symptoms. Hence, none of the LFTs are suitable in a low-prevalence setting where each individual result is important, i.e., diagnostics, however if the aim is communal seroprevalence, the Livzon IgG test could be a suitable assay due to the high specificity (Erikstrup et al., 2020).

Antibody levels (OD ratios) were initially low and then increased steadily over time. The antibody levels of IgM, IgG and IgA also correlated well with clinical disease severity. Seow et al. has demonstrated that the magnitude of the neutralising antibody response is dependent on disease severity (Seow et al., 2020). In our study, samples from non-hospitalised patients were taken a median of 27 or 29 days later than samples from hospitalised patients, but this fact is not the sole explanation for the difference in antibody strength. The longevity of SARS-CoV-2 spike IgG antibodies, also in cases with mild disease, has been described (Wajnberg et al., 2020). The general pattern of mean antibody level for all three assays were in the order: non-hospitalised patients < hospitalised patients < ICU patients, significantly so for IgA and IgM, but not for IgG antibodies. In particular, IgA antibody levels are significantly higher for severely ill patients treated in the ICU. High anti-SARS-CoV-2 antibodies have previously been linked to disease severity (Hsueh et al., 2004; Tan et al., 2020; Zhao et al., 2020). We speculate that a high positive IgA antibody level could be an indicator of disease severity. Importantly, whether SARS-CoV-2 antibody levels correlate to protective immunity is yet to be determined.

IgA antibodies are detected in early and late stages of SARS-CoV-2 infection, and the detection of IgA antibodies can have an added value in diagnosing SARS-CoV-2 (Guo et al., 2020). Importantly, the Euroimmun IgA ELISA should not be used for screening purposes, due to a high rate of false positives when using the manufacturer's recommended cut-off. As we have shown, multiple thresholds for positivity can be considered for different settings, giving some flexibility in use of the assay. The specificity for Euroimmun IgA ELISA was improved ($\geq$97.5%) using a custom cut-off value (4.0). However, in high-prevalence settings, i.e., among hospitalised patients, test sensitivity should be prioritised and the manufacturer's cut-off (1.1) applied instead.

This study has several limitations. At the start of the pandemic, serologic assays were pushed onto the market in a haste. Not all assays performed adequately and some of these assays need be optimised further, independently validated, and used in algorithm formats to achieve the highest possible accuracy for decision making. The retrospective nature of the study means that samples were not collected at pre-determined time intervals and clinical data on patients are scarce. Moreover, the number of subjects and samples included are few.

Conclusion

Routine serologic testing using validated anti-SARS-CoV-2 assays could prove beneficial when used in the correct settings. The three LFTs evaluated in this study are not suitable for diagnosis or monitoring of SARS-CoV-2 infection, especially not in mild cases. Instead, sensitive ELISA assays will perform better in these settings. For evaluation of seroprevalence, IgG tests with high specificity, either ELISA or LFT, would be appropriate.

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Ethical approval

Not required.

Conflict of interest

The authors declare no conflict of interest.

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