






## RESEARCH ARTICLE

# Evaluation of commercially available immuno-magnetic agglutination in comparison to enzyme-linked immunosorbent assays for rapid point-of-care diagnostics of COVID-19

Maria E. Moeller<sup>1</sup>  | Jeppe Fock<sup>2</sup>  | Pearlyn Pah<sup>2</sup> | Antia De La C. Veras<sup>2</sup> |  
Melanie Bade<sup>2</sup> | Marco Donolato<sup>2</sup> | Simone B. Israelsen<sup>1</sup>  |  
Jesper Eugen-Olsen<sup>3</sup>  | Thomas Benfield<sup>1,4</sup>  | Frederik N. Engsig<sup>1</sup>

<sup>1</sup>Department of Infectious Diseases, Copenhagen University Hospital, Amager and Hvidovre Hospital, Hvidovre, Denmark

<sup>2</sup>BluSense Diagnostics ApS, Copenhagen, Denmark

<sup>3</sup>Department of Clinical Research, Copenhagen University Hospital, Amager and Hvidovre Hospital, Hvidovre, Denmark

<sup>4</sup>Institute of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark

## Correspondence

Maria E. Moeller, Department of Infectious Diseases, Copenhagen University Hospital, Amager and Hvidovre Hospital, Kettegaard Allé 30, 2650 Hvidovre, Denmark.  
Email: [Maria.elizabeth.engel.moeller@regionh.dk](mailto:Maria.elizabeth.engel.moeller@regionh.dk)

## Funding information

Blusense Diagnostics ApS

## Abstract

**Introduction:** Coronavirus disease 2019 (COVID-19) is caused by Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). Fast, accurate, and simple blood-based assays for quantification of anti-SARS-CoV-2 antibodies are urgently needed to identify infected individuals and keep track of the spread of disease.

**Methods:** The study included 33 plasma samples from 20 individuals with confirmed COVID-19 by real-time reverse-transcriptase polymerase chain reaction and 40 non-COVID-19 plasma samples. Anti-SARS-CoV-2 immunoglobulin M (IgM)/immunoglobulin A (IgA) or immunoglobulin G (IgG) antibodies were detected by a microfluidic quantitative immunomagnetic assay (IMA) (ViroTrack Sero COVID IgM + IgA/IgG Ab, Blusense Diagnostics) and compared to an enzyme-linked immunosorbent assay (ELISA) (EuroImmun Medizinische Labordiagnostika).

**Results:** Of the 33 plasma samples from the COVID-19 patients, 28 were positive for IgA/IgM or IgG by IMA and 29 samples were positive by ELISA. Sensitivity for only one sample per patient was 68% for IgA + IgM and 75% IgG by IMA and 80% by ELISA. For samples collected 14 days after symptom onset, the sensitivity of both IMA and ELISA was around 91%. The specificity of the IMA reached 100% compared to 95% for ELISA IgA and 97.5% for ELISA IgG.

**Conclusion:** IMA for COVID-19 is a rapid simple-to-use point-of-care test with sensitivity and specificity similar to a commercial ELISA.

## KEYWORDS

blood, immuno-magnetic agglutination assay, rapid IgG-IgM-IgA combined test, SARS-CoV-2, surveillance

## 1 | INTRODUCTION

Coronavirus disease 2019 (COVID-19) is caused by Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and has spread globally since its discovery in Wuhan, China in December 2019.<sup>1,2</sup>

In spite of advances in antiviral treatment, it remains a disease with considerable morbidity and mortality.<sup>3,4</sup>

Real-time reverse transcription-quantitative polymerase chain reaction (RT-qPCR) detection of SARS-CoV-2 RNA is the recommended test to diagnose active COVID-19, but several serological tests for COVID-19

have been developed.<sup>5-8</sup> Immunoassays detect different antibodies to SARS-CoV-2, namely antibodies to different parts of the spike or the nucleocapsid protein.<sup>9-12</sup> Although SARS-CoV-2 RNA can be demonstrated at the onset of COVID-19 symptoms, antibodies against SARS-CoV-2 can in most cases be demonstrated after 11 days (interquartile range [IQR] = 7.0–14.0).<sup>13</sup>

So serological testing, in general, cannot replace RT-PCR for diagnosing acute COVID-19 but may serve as a valuable supplement in persons with classical symptoms of COVID-19 and repeated negative RT-qPCR for clarification of diagnosis, although its main application is to assess immunity.

Enzyme-linked immunosorbent assay (ELISA) tests may take hours to perform, are usually batched, and require laboratory facilities and skilled personnel. Lateral flow assays for antibody detection are quick single sample tests but have lower sensitivity compared to ELISA, the read-out is operator dependent, and the result is qualitative.<sup>14-16</sup> An automated, real-time, and quantitative point-of-care (POC) test using capillary blood with high sensitivity would offer the ability of testing for SARS-CoV-2 antibodies both within and outside of a hospital setting.

In this study, we used a novel POC analysis for SARS-CoV based on automated immunomagnetic assay (IMA) technology. The analysis is performed on a portable POC testing device. Readout of results is automated, real-time, and quantitative using capillary blood.

We compared the performance of a well-tested commercial ELISA for COVID-19 with IMA for rapid testing for COVID-19 antibodies. The aim was to establish the sensitivity and specificity of the IMA, for future use in the clinic during the COVID-19 pandemic.

## 2 | MATERIALS AND METHODS

### 2.1 | Subjects and samples

We included individuals with confirmed COVID-19 by RT-qPCR for SARS-CoV-2 RNA on naso-/oropharyngeal swabs or lower respiratory tract specimens, from March 20 to May 1, 2020, with at least one available plasma samples.<sup>17</sup> Demographic and clinical data on the study population were transferred from electronic health records. Plasma samples collected before July 2019 from a biobank for Danish HIV-infected individuals (10 samples) and non-HIV-infected individuals (30 samples) served as COVID-19 negative controls.<sup>18</sup> Samples were stored at  $-80^{\circ}\text{C}$  until testing. A waiver of individual informed consent was granted by the Regional Ethics Committee of the Capital Region of Denmark (record no. H-20040649). The study was further approved by the Danish Patient Safety Authority (record no. 31-1521-309) and the Regional Data Protection Center (record no. P-2020-260). Data were entered into an electronic data capture tool hosted by the Capital Region of Denmark.<sup>19,20</sup> Variables included age, gender, comorbidity, radiographic findings, duration of symptoms, supplemental oxygen, do not resuscitate orders, intensive care, mechanical ventilation and 30-day mortality. In this paper, severe disease was defined as need of more than 15 L of supplementary oxygen per minute.

### 2.2 | Blinded samples were measured in singlicates using IMA and ELISA

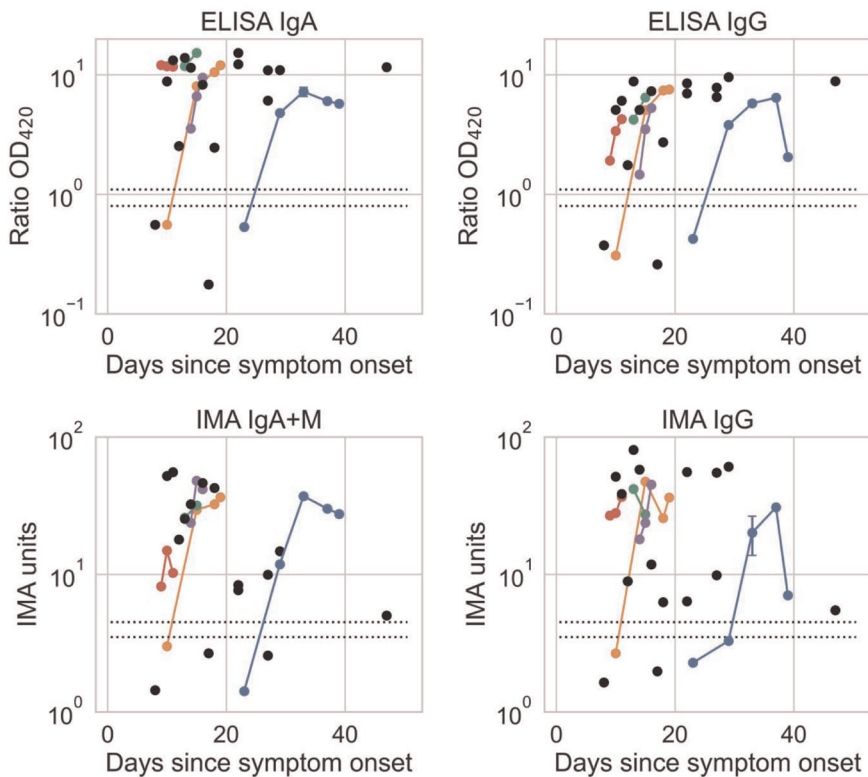
#### 2.2.1 | IMA

In the ViroTrack Sero Covid immunoglobulin A (IgA) + M/immunoglobulin G (IgG) (Blusense Diagnostics) (IMA), 10  $\mu\text{l}$  of plasma was mixed with 150  $\mu\text{l}$  sample dilution buffer, vortexed and 50  $\mu\text{l}$  of the diluted plasma was loaded on to the microfluidic cartridge. The IMA tests utilize a centrifugal microfluid platform together with optomagnetic readout based on the agglutination of magnetic nanoparticles. The samples are manipulated on cartridges with the help of the centrifugal force, Coriolis force, and Euler force to allow for separation, sedimentation, aliquoting, and reagent resuspension by the design of microfluidic chambers and channels and control over the angular velocity profile of the cartridge rotation.<sup>21</sup> The optomagnetic signal is obtained by measuring the modulated transmitted light through a suspension of magnetic nanoparticles in response to an alternating magnetic field.<sup>22</sup> The magnetic particles are covalently coupled to antigens or antibodies. Upon target induced magnetic particle agglutination, the change in optical and magnetic anisotropy results in a change in the optomagnetic signal which can be used to quantify the target concentration.<sup>23-25</sup> Incubating the particle in homogeneous magnetic fields speeds up the reaction kinetics.<sup>23,26</sup> IMA does not require secondary antibodies.

The magnetic particles were functionalized with commercial SARS-CoV-2 nucleocapsid recombinant protein in the IMA immunoglobulin M (IgM) + IgA/IgG Ab kit (BluSense Diagnostics APS). Negative results were reported with values below 3.5 IMA units and positive results with 4.5 IMA and above. The equivocal region (borderline results) is between 3.5 and 4.5 IMA. The cutoff value of 4.5 IMA was determined from multiple measurements on negative samples to ensure less than 0.1% false-positives with 95% confidence intervals. Values above 20 units were classified as high.

#### 2.2.2 | ELISA

The EI ELISA IgA/IgG (IgG test, REF EI2606-9601 G LOT E200420AW—IgA test REF EI2606-9601 A LOT: E200417AD, EuroImmun Medizinische Labordiagnostika) was performed according to the manufacturer's specifications. In both ELISAs, the antigen used is glycosylated Spike 1 protein. In the initial step, 1.2  $\mu\text{l}$  of plasma was diluted in 118.8  $\mu\text{l}$  sample buffer provided in the kit. After this dilution, 110  $\mu\text{l}$  of the sample was first transferred to an uncoated 96-well plate, and subsequently transferred to a coated 96-well ELISA plate using an eighth channel pipette and incubated at  $37^{\circ}\text{C}$  for 1 h. In the next step, the wells were emptied and subsequently washed three times with wash buffer provided in the kit. After that, 100  $\mu\text{l}$  of conjugate solution was added to each well, and the plate incubated at  $37^{\circ}\text{C}$  for 30 min and subsequently washed three times with wash buffer. Subsequently, 100  $\mu\text{l}$  of the substrate was added to each well and the plate incubated in the dark at room temperature for 30 min. After the addition of 100  $\mu\text{l}$  of stop-solution, the ELISA plate was measured in a Multiskan FC Microplate Photometer (Thermo Scientific,



**FIGURE 1** Severe acute respiratory syndrome coronavirus-2 antibody titer versus days after symptom onset. One sample, measured negative or borderline by microfluidic quantitative IMA (Virotrack) and ELISA, did not have information on the days since symptom onset and is not included in the plot. Black points are from patients with only one sample, and colored are from POS 01 (blue), POS 02 (yellow), POS 03 (green), POS 04 (red), and POS 05 (purple). The dotted lines indicate the cut-off values to determine positive and negative test results and the zone in between represents the borderline results. EI, euroimmun; ELISA, enzyme-linked immunosorbent assay; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; IMA, immunomagnetic assay; POS, positive

type 357) at 450 nm. The results were evaluated in terms of the absorbance ratio of the sample and a calibrator. Negative results were reported with ratio below 0.8 and positive results with ratios above 1.1 and above. The equivocal region (borderline results) is between 0.8 and 1.1.

### 2.3 | Statistics

Patient characteristics were presented as median with IQR or count with percentage. The data analysis included calculation of the following parameters: sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy. Confidence intervals for sensitivity and specificity were calculated using the Exact Binomial method.<sup>27</sup> As case samples were taken from individuals identified as having COVID-19 from a positive RT-PCR, these were considered “true positive.” The plots including receiver operating characteristics (ROC)-curves were constructed in python using the matplotlib, seaborn, and sklearn packages. Differences in titers were calculated by Fisher's exact test using SPSS statistical software, Version 25.0 (Norusis; SPSS Inc).

## 3 | RESULTS

We included a total of 20 individuals, who contributed 33 plasma samples. The individuals were mostly male (65%) with a median age of 71 (IQR = 53–76) years, where most had at least one comorbidity (75%) (Table S1). All had chest radiograph infiltration, and of these,

84.5% had multilobular infiltration. Ten plasma samples from Danish HIV-infected individuals and 30 non-HIV-infected patients were collected before July 2019 were included as controls.

All 20 patients received supplementary oxygen; the maximum support during admission was 0–14 L: two persons (10%), 15–29 L; five persons (25%) and more than 30 L; 13 persons (65%). Thirty-five percent of the patients had a “do-not-resuscitate” order and 20% were limited in treatment in terms of intensive care unit (ICU) admission. Forty-five percent of the patients were admitted to the ICU. Twenty percent of the patients died within 30 days (Table S1).

Five individuals were sampled over multiple days (positive [POS] = 1–5). Figure 1 shows the evolution of the obtained results from the ELISA and IMA. For patient POS 1 and POS 2 we observed a change from negative at day 23 and 10, respectively, to stable positive for all subsequent samples by both assays (ELISA IgA and IMA IgA + IgM). Similarly, the levels of IgG turned positive. POS 3, 4, and 5 were stable too, increasing over time. POS 1 (day 37) showed a decrease of IgG levels over time by ELISA and IMA. Of the 18 patients with severe disease, 13 (72%) had high IgM + A and/or IgG IMA titers (Table S1).

### 3.1 | Assay performance

#### 3.1.1 | Sensitivity analysis

Out of the 33 positive RT-qPCR SARS-CoV-2 samples (all samples, single and longitudinal, included), 28 were IgA + IgM positive and 28

**TABLE 1** Analytical sensitivity, specificity, and predictive values for the detection tests

		Sensitivity	Specificity	Agreement	PPV	NPV
EI ELISA (eqv. Neg)	IgA	29/33 (87.9%)	38/40 (95%)	67/73 (91.8%)	29/31 (93.5%)	38/42 (90.5%)
	IgG	29/33 (87.9%)	39/40 (97.5%)	68/73 (93.2%)	29/30 (96.7%)	39/43 (90.7%)
	IgA + IgG	29/33 (87.9%)	38/40 (95%)	67/73 (91.8%)	29/31 (93.5%)	38/42 (90.5%)
EI ELISA (eqv. Pos)	IgA	29/33 (87.9%)	35/40 (87.5%)	64/73 (87.7%)	29/34 (85.3%)	35/39 (89.7%)
	IgG	29/33 (87.9%)	39/40 (97.5%)	68/73 (93.2%)	29/30 (96.7%)	39/43 (90.7%)
	IgA + IgG	29/33 (87.9%)	35/40 (87.5%)	64/73 (87.7%)	29/34 (85.3%)	35/39 (89.7%)
ViroTrack	IgA + IgM	28/33 (84.8%)	40/40 (100%)	68/73 (93.2%)	28/28 (100%)	40/45 (88.9%)
	IgG	28/33 (84.8%)	40/40 (100%)	68/73 (93.2%)	28/28 (100%)	40/45 (88.9%)
	IgA + IgM + IgG	29/33 (87.9%)	40/40 (100%)	69/73 (94.5%)	29/29 (100%)	40/44 (90.9%)

Note: Considering all 33 samples from the 20 individual patients as positive samples. Equivocal/borderline results for ELISA were treated as negatives (eqv. Neg) and as positives (eqv. Pos). No equivocal/borderline results were found in the microfluidic quantitative IMA (Virotrack). The confidence intervals of the analytical sensitivities, specificities, and predictive values are given in Table S3.

Abbreviations: EI, euroimmun; ELISA, enzyme-linked immunosorbent assay; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; IMA, immunomagnetic assays; NPV, negative predictive value; PPV, positive predictive value.

were IgG positive by IMA and 29 positive for IgA and IgG by ELISAs (Table 1). This corresponds to a sensitivity of 84.8% for IgA + IgM and IgG by IMA and to 87.9% by ELISA.

None of the 33 positive samples showed borderline results for IMA and for ELISA assays.

By considering the first drawn samples from each individual, the sensitivity was 75% for IgA + IgM and 80% for IgG by IMA and 80% by ELISA. By combining the IMA IgA + IgM and IgG and the ELISA IgA and IgG results, the sensitivity was comparable for both assays (80%). Days from the first symptom to drawing of the first sample ranged from 8 to 47 days, with a median of 15 days. For samples collected 14 days or more after symptoms onset, the sensitivity of both IMA and ELISA test was around 91%.

### 3.2 | Specificity analysis

The specificity of IMA was 100.0% for the IgM + IgA and the IgG assays. Three of the 40 control samples (two IgA and one IgG) were positive by ELISA resulting in a specificity of 95.0% and 97.5%, respectively (Table 1). Additionally, three non-COVID-19 samples were borderline positive by ELISA for IgA corresponding to a lower specificity of 87.5% (Table S1). All borderline and false-positive ELISA results were from HIV-negative samples. No sample was indeterminate by IMA.

The PPV of ELISA is likely to be 85.3%–96.7% and of the IMA it is 100%. The NPV for ELISA is measured to be 89.7%–90.5% and of the IMA 88.9%–90.5% (Table 1).

### 3.3 | Semiquantitative results of IgA, IgM, and IgG detection

The distance of data points from the cut-off values and confidence in assigning a positive or negative result differed between the IMA and

ELISA assays (Figure 2). The distribution of positive and negative data points was distinct for the IMA cartridge, with a cut-off value above all the negative samples, which allowed for unequivocal interpretation of all measurements. In contrast, the ELISA data had less separation, especially for IgA, resulting in a “grey zone” of borderline data points to which a positive or negative result could not be assigned. Both positive and negative samples have borderline data points. ROC curves of the assays all showed an area under the curve above 0.93 (see Figure S1).

## 4 | DISCUSSION

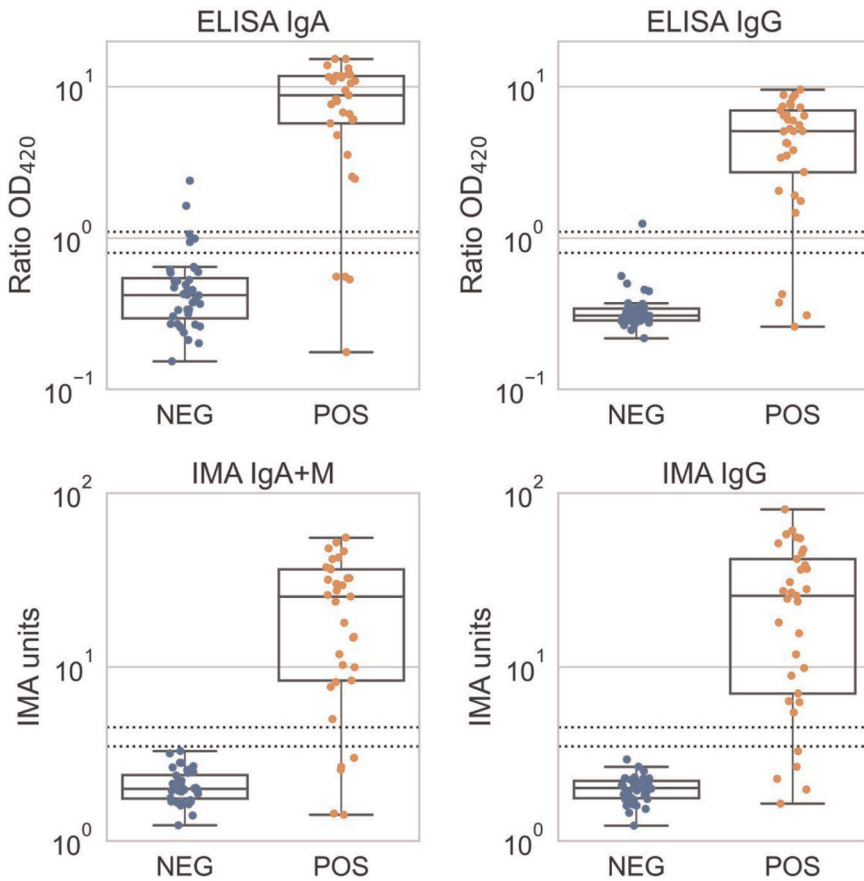
This study showed that IMA IgM + IgA/IgG performed similarly to a commercial ELISA with a sensitivity for each assay of 84.9% and a combined sensitivity of 87.9% to detect antibodies to SARS-CoV-2 in patients with moderate to severe disease. Sensitivity for only one sample per patient (first sample drawn) was 75% for IgA + IgM and 80% IgG by IMA and 80% by ELISA. For samples collected 14 days after symptom onset, the sensitivity of both IMA and ELISA was around 91%.

The specificity was 100% by IMA and 95%–97.5% by ELISA. The use of serial samples increased the sensitivity of both tests and emphasizes the importance of retesting individuals with a high suspicion of COVID-19.

The PPV of ELISA is likely to be 85.3%–96.7% and for the IMA it is 100%. The NPV of ELISA is measured to be 89.7%–90.5% and of the IMA 88.9%–90.5% (Table 1).

The IMA had an overall sensitivity of 87.9%—considering all samples at different days from symptoms onset—which is more or less comparable to that of other POCs commercially available, depending on days from the debut of symptoms to test<sup>14,28–31</sup> but 100% for serial samples.

The sensitivity parameter of the IgM, IgA, and IgG tests may vary according to the study design, a low NPV of a given test indicates that



**FIGURE 2** Severe acute respiratory syndrome coronavirus-2 antibody assay performance. Data distribution obtained for POS and NEG samples, confirmed by real-time reverse-transcriptase polymerase chain reaction using (A) ELISA IgA and IgG and (B) microfluidic quantitative IMA (Virotrack) IgM + IgA and IgG. Lines represent median values with minimum to maximum ranges. The dotted lines indicate the cut-off values to determine POS and NEG test results, the zone in between represents the borderline results. EI, Euroimmun; ELISA, enzyme-linked immunosorbent assay; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; IMA, immunomagnetic assay; NEG, negative; POS, positive

individuals testing negative but presenting clinical symptoms of COVID-19 need to be retested using another serological test or RT-qPCR.<sup>28,32,33</sup> Here, we found that the NPV of the IMA is likely to be comparable to that of a commercially available ELISA test (90%–91%). The limitation of a given sample producing a false-negative result is correlated to different factors, such as time of testing in relation to symptom onset, changes in antibody levels during illness, and severity of the disease. Several studies covering the use of ELISA, Chemiluminescence immunoassays (CLIA), and qualitative assays show that full diagnostic sensitivity for neither IgM nor IgG is reached before approximately 14–22 days from onset of symptoms.<sup>30,31,34–36</sup>

It has been reported that IgM detection was more variable than IgG, and detection was the highest when IgM and IgG results were combined for both ELISA and POCs.<sup>28</sup> The addition of IgA may improve sensitivity as it has been found to have higher titers than IgM.<sup>37</sup> Using IMA cartridges, we observed better performance of the IgA + IgM/IgG combination in terms of sensitivity while keeping the specificity at 100%.

In this study, we compared two different serological technologies against two different antigens. Preferably it should have been two different serological technologies against the same antigen but this was not available at the time. Two previous studies have shown that antibodies to the nucleocapsid antigen, which is smaller than the spike protein and lacks a glycosylation site, can be measured earlier than antibodies to the spike protein antigen.<sup>13,38,39</sup> This could increase the sensitivity of the IMA but in this study, we only found

little difference. An explanation for this may be that the samples were taken at different time points and only five study subjects had serial sampling performed.

Four individuals had samples that were negative on both IMA and ELISA. Two (POS 2 and POS 18) of the four negative samples were taken less than 10 days after symptom onset, which is still within the normal window period for production of antibodies for SARS-CoV-2.<sup>12</sup> POS 2 later tested positive at day 15 while POS 18 had no follow-up sample. Two persons (POS 1 and POS 9) had not developed antibodies after 23 and 17 days after symptom onset, respectively. POS 1 was positive after 29 days and POS 9 was not tested again. The median time for detection of antibodies for COVID-19 is generally 11 days (IQR = 7.0 – 14.0) but several studies have shown that some individuals take longer to develop antibodies against COVID-19.<sup>12,40,41</sup> In general, the immune response decrease with age which may be the reason why POS 1, who was in his seventies, took longer time to produce antibodies.<sup>42,43</sup> Antibody production correlates with disease severity and could explain low or prolonged antibody production but all persons with negative tests had severe infections. These results underline the importance of timely testing and retesting.

The detailed clinical data including symptom onset and disease severity improved the interpretation of the results as antibody titers were found to be affected by both as previously reported.<sup>33,44</sup> Comparison of a well-tested commercial ELISA strengthens the evaluation of the novel IMA.



The current study lacks sera of individuals infected with other coronaviruses to test for cross-reactivity. A prior infection with other human coronaviruses may theoretically cause false-positive results due to cross-reactivity.<sup>33,45</sup> However, the amino acid sequences coding for nucleocapsid and spike proteins have low homology between alpha coronaviruses and beta coronaviruses, and cross-reactivity is mainly detected for other beta coronaviruses, namely Middle East Respiratory Syndrome (MERS) and SARS-CoV-1.<sup>45-48</sup> MERS and SARS-CoV-1 has never been diagnosed in Denmark. The IMA did not cross-react with other common viral pathogens (Table S2). The HIV-positive negative control group may have secondary hypogammaglobulinemia that potentially could lead to a falsely higher specificity. The analyses were performed on plasma and analysis on whole blood may decrease sensitivity of the POC. Patients tested were all hospitalized, symptomatic and presented with moderate to severe disease. Studies have shown that the titers are higher in those with more severe symptoms.<sup>49</sup> Finally, the small number of individuals made it difficult to estimate the association between antibody response and disease severity.

The IMA tests are sold at similar prices as the existing rapid test based on lateral flow technology but require a reader, which is sold for a few hundred USD. This makes it a bit more expensive than other POC tests for COVID-19 antibodies but considerably more affordable than most commercial ELISA platforms.

The advantage of POCs with high sensitivity and an easily read result is that they can reduce the amount of confirmatory testing and are portable.

Our aim was to test the efficacy of the IMA and compare it to a commercial ELISA. In conclusion, our results show that the IMA IgM + IgA/IgG system is an effective supplemental diagnostic tool for COVID-19 with high sensitivity and specificity in hospitalized patients with moderate to severe disease. The test is rapid and can be performed at POC as a supplement to RT-qPCR in testing for active COVID-19 as well as a potential screening and testing tool for epidemiological studies in community settings enabling a rapid result without the need for phlebotomy and handling of test tubes. Several lateral flow assays are already in use for COVID-19 IgM and IgG detection, but to our knowledge, this is the first semiquantitative POC assay with automatized readout, which measures IgA, IgM, and IgG.

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## AUTHOR CONTRIBUTIONS

*Study planning:* Marco Donolato, Jesper Eugen-Olsen, Thomas Benfield and Jeppe Fock. *Sample collection:* Maria Engel Moeller, Jesper Eugen-Olsen and Thomas Benfield. *Laboratory analysis:* Pearlyn Pah, Melanie Bade, Antia De La Campa Veras. *Manuscript drafting:* Maria

Engel Moeller, Simone Bastrup Israelsen, Thomas Benfield, Frederik N. Engsig and Jeppe Fock. *Critical revision of manuscript:* All authors approved the final manuscript.

## CONFLICT OF INTERESTS

Pearlyn Pah, Antia De La C. Veras, Melanie Bade, Jeppe Fock, Simone B. Israelsen, and Marco Donolato are employed at Blusense Diagnostics APS and have been part of the developing of ViroTrack Sero COVID IgM + IgA/IgG.

## PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1002/jmv.26854>

## DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

## ORCID

Maria E. Moeller  <http://orcid.org/0000-0003-4276-495X>

Jeppe Fock  <https://orcid.org/0000-0002-7515-4026>

Simone B. Israelsen  <https://orcid.org/0000-0002-0653-1289>

Jesper Eugen-Olsen  <https://orcid.org/0000-0002-4630-4275>

Thomas Benfield  <https://orcid.org/0000-0003-0698-9385>

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#### SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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