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Diagnostic and analytical performance evaluation of ten commercial assays for detecting SARS-CoV-2 humoral immune response

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1. Introduction

In December 2019 several cases of pneumonia of unknown cause occurred in Wuhan, Hubei Province, China. On January 72,020, a novel betacoronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was isolated from the patients in Wuhan (Wang et al.,...
2020a). This virus is responsible for a viral pneumonia called coronavirus disease 2019 (COVID-19) (Zhu et al., 2020; Lu et al., 2020). Although most people with COVID-19 disease have mild to moderate symptoms, the disease can cause severe medical complications such as acute respiratory distress syndrome, septic shock, bleeding and coagulation disorders, and can lead to death in pre-disposed people (Chen et al., 2020). Due to a combination of high human-to-human transmissibility, absence of natural immunity in the population and a lot of international traffic, the virus has quickly spread around the world and evolved to a global pandemic (World Health Organization, 2020a; Wroblewski et al., 2020). At the time of writing, the virus is globally disrupting society. Therefore, a rapid and correct identification of the virus is crucial, not only for the diagnosis of COVID-19 disease and subsequent correct treatment, but also to take necessary isolation precautions and thereby avoid further spreading. Also, vaccination will probably soon be a possible solution for reducing the spread of the virus by evoking humoral immunity in the vaccinated people.

The current gold standard for the diagnosis of COVID-19 is the detection of viral RNA in respiratory tract samples with real-time reverse transcriptase-polymerase chain reaction (rRT-PCR) targeting SARS-CoV-2 specific sequences coding for spike (S), envelope (E), or nucleocapsid proteins (Corman et al., 2020; Wang et al., 2020b; Chu et al., 2020; World Health Organization, 2020b; Bohn et al., 2020). rRT-PCR is highly sensitive and specific, especially in the acute phase of the infection (Infantino et al., 2020; Tang et al., 2020). The sensitivity of the PCR test depends on the time of sample collection in relation to the diagnostic testing window. Negative PCR results may be caused by extremely low viral load when tested shortly after exposure or at late stages of infection. Maximum viral load in throat swabs was observed 2 days before until 5 days after symptom onset (Wölfl et al., 2020; Kampf et al., 2020). The median [interquartile range] period between symptom onset and a negative rRT-PCR result has been reported to be 20 [17–24] days (Xiao et al., 2020). Furthermore, higher viral load and a longer mean duration of viral detection in respiratory samples correlate with disease severity (Clementi et al., n.d.). rRT-PCR can be false negative due to pre-analytical issues such as the sample collection technique. As to be expected, bronchoalveolar lavage (BAL) and sputum samples have shown to contain a higher viral load, and thus to remain positive for a longer time, compared to nasopharyngeal, nose or throat samples (Wang et al., 2020b). However, the Centers for Disease Control and Prevention (CDC) recommends using upper respiratory specimens for initial diagnostic testing, for logistical purposes and to limit invasive sampling procedures (Centers for Disease Control and Prevention, 2020). The nasopharyngeal swab (NP) is currently proposed as the gold standard sample for detection of SARS-CoV-2 due to the higher sensitivity for the detection of SARS-CoV-2 compared to oropharyngeal swabs and saliva samples (Wang et al., 2020b), restricting the latter sample types to specific screening strategies (Williams et al., 2020).

Serological assays have the potential to play a complementary role in the diagnosis of rRT-PCR-negative COVID-19 cases (Xiang et al., 2020). Seroconversion for SARS-CoV-2 is typically detected between 7 and 14 days post symptom onset (Guo et al., 2020; Burbeio et al., 2020; Long et al., 2020a). Among the four SARS-CoV-2 structural proteins, the spike (S) and nucleocapsid (N) proteins are the most immunogenic (Meyer et al., 2014; Qiu et al., 2005). Different types of tests are available to detect anti-SARS-CoV-2 antibodies: rapid lateral flow assays (LFA) as point of care tests, enzyme-linked immunosorbent assays (ELISA) and automated immunochromatographic assays. The contribution of serological assays to serorelevance studies and evaluation of the results of vaccine trials is currently under debate (Okba et al., 2020a).

The aim of this study is to compare the diagnostic performance of ten commercial SARS-CoV-2 antibody test assays: five ELISA’s, one fluoroenzyme-immunoassay (FEIA), two rapid LFA’s, and two chemiluminescence immunoaassays (CLIA) (Supplementary Material 1). The study was performed in co-operation with the Belgian Federal Agency for Medicines and Health Products (FAMHP) that had set up a validation scheme for serological SARS-CoV-2 assays, whereby positively evaluated laboratory assays are reimbursed by the national health insurance.

2. Materials and methods

2.1. Patient selection

This retrospective study was performed using 166 patient samples collected at the OLV Hospital Aalst, Belgium.

Specificity was assessed on a selection of 85 serum samples from unique patients, collected before the COVID-19 pandemic, from March 2017 to March 2020. This cross-reactivity panel consisted of a) 35 samples of patients with a rRT-PCR confirmed non-coronavirus respiratory pathogen infection b) 19 samples of patients with a rRT-PCR confirmed non-SARS-CoV-2 coronavirus infection c) 10 samples of patients with a confirmed systemic auto-immune rheumatic disease and d) 21 samples of patients with antibodies against other viral/bacterial/parasitic pathogens. A detailed description of this specificity cohort is listed together with the results description in Table 3.

Sensitivity was assessed on a selection of 81 serum samples from 77 patients with a rRT-PCR confirmed SARS-CoV-2 infection on nasopharyngeal swab. rRT-PCR was performed using an in-house method complying with the WHO guidelines (Corman et al., 2020). The time between symptom onset and sampling date was a) less than ten days (n = 30) b) between 10 and 20 days (n = 25) and c) more than 20 days (n = 14). Also 12 samples of asymptomatic patients were included. The median time between symptom onset and serum sampling was 11 days (range 1–51). The group consisted of 53 male and 28 female patients with a median age of 66 years (range 17–97). Of note, in case of multiple samples per patient, only the first sample per time-category was used to assess sensitivity.

All samples were stored at –20 °C until analysis.

2.2. Data collection

The protocol was approved by the local Ethics Committee OLV Hospital Aalst with Belgian registration number B126202000015. For all COVID-19 patients, disease severity status was collected. Patients were classified as a) “mild” if no hospital admission was required b) “moderate” in case of admission to a non-ICU ward, c) “critical” in case of admission to the ICU-ward or death and d) “asymptomatic”. Serum samples of immunosuppressed patients (hematological malignancies, solid organ transplant) and patients younger than one year were excluded from the data set (specificity and sensitivity).

2.3. Assays

Four new ELISA’s, one FEIA and two new rapid LFA’s were evaluated and compared to one established ELISA and two established CLIA’s. The new ELISA’s and FEIA included were respectively Anti-SARS-CoV-2-NCP (Euroimmun, Germany) targeting IgG anti-N antibodies (used abbreviation throughout the manuscript: EI-N), ID Screen® SARS-CoV-2-N IgG Indirect ELISA (id-vef, France) targeting IgG anti-N antibodies (abbreviation: Id-N), Covid-19 ELISA THERA02 IgG (Theradiag, France) targeting IgG anti-S antibodies (abbreviation: TD-S), COVID19-LISA IgG (Theradiag, France) targeting IgG anti-S + anti-N antibodies (abbreviation: TD-SN) and EliA SARS-CoV-2 Sp1 IgG (Thermo Fisher Scientific, Sweden) targeting IgG anti-S1 antibodies (abbreviation: TF-S). The new rapid LFA’s included TDR Covid-19 IgG + IgM Thera (Theradiag, France) targeting IgG + IgM anti-S antibodies (abbreviation: LFA TD-S) and COVID-PRESTO® (AAZ-LMB, France) targeting IgG + IgM anti-S1 and anti-N antibodies (abbreviation: LFA PR-SN).

The established ELISA and CLIA’s included concerned respectively Anti-SARS-CoV-2 (Euroimmun, Germany) targeting IgG anti-S antibodies (abbreviation: EL-S), LIAISON SARS-CoV-2 S1/S2 IgG (DiaSorin S.P.A., Italy) targeting IgG anti-S antibodies (abbreviation: DS-S) and...
Elecsys Anti-SARS-CoV-2 (Roche, Germany) targeting total Ig anti-N antibodies (abbreviation: R-N). A detailed description of the different assays, including type of analyzer used, is provided in Supplementary Material 1. The samples were analyzed within controlled pre-analytical sample conditions in batch by the laboratory of OLV Hospital Aalst according to the instructions of the different collaborating companies.

2.4. Performance measures and statistical analysis

Analytical performance of each assay was assessed by calculating imprecision (coefficient of variation (CV), %) using the manufacturer’s internal quality control materials (iQC) and three patient serum samples with a low, medium and high SARS-CoV-2 Ab concentration. All iQC samples were measured before and after every run during 10 runs (CLSI EP5-A2) (Clinical and Laboratory Standard Institute (CLSI), 2004). Linearity was assessed by diluting a high level serum SARS-CoV-2 Ab sample with increasing amounts of a serum sample with very low levels of SARS-CoV-2 Ab (CLSI EP06-A) (Clinical and Laboratory Standard Institute (CLSI), 2003).

Diagnostic performance characteristics (sensitivity, specificity) were calculated for every SARS-CoV-2 antibody assay based on the manufacturer’s cut-off and compared with the McNemar test. For calculation of performance characteristics, borderline results were considered as positive. For (semi-)quantitative assays, receiver operating characteristic (ROC) curve analyses were performed to verify company cut-off values. Cut-off values at the 95% specificity level were determined and corresponding sensitivity for diagnosing COVID-19 was compared between the different assays. Finally, a Box and Whisker analysis was performed between the different disease severity patient cohorts. Quantitative variables are presented as median and range and categorical variables with number and percentage or frequency. Data analysis was performed in MEDCALC® Statistical Software version 17.1 (MedCalc Software Ltd., Ostend, Belgium), except for ROC curves, which are performed in Microsoft Excel + Analyse-it® Software version 5.65.3 (Leeds, UK). A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Patient demographics

An overview of the demographic features of the different patient cohorts is shown in Supplementary Material 2a-d. In general, we retained no significant difference in gender distribution between the sensitivity and specificity patient cohorts (p = 0.1174), but regarding age, the sensitivity patient group was significantly older (p < 0.0001).

3.2. Analytical performance

3.2.1. Imprecision

Results of the imprecision study are presented in Supplementary Material 3. For the ELISA’s of Euromimmun (EI-S & EI-N), the imprecision obtained for the patient sample iQC was higher than for the kit iQC which can be explained by the fact that the kit iQC’s are prediluted and their imprecision results didn’t include a predilution step. The latter is not true for the ELISA’s of Theradiag (TD-S & TD-SN) and Id-vet (Id-N), with comparable imprecision results for the kit and patient sample iQC. Assays based on ELISA format obtained the highest CV% results.

3.2.2. Linearity

No deviation from linearity was revealed for any of the assays, which is illustrated in Supplementary Material 4a-h. The lower results for TD-S are related to imprecision rather than to non-linearity.

3.3. Diagnostic performance

3.3.1. Specificity cohort

Based on the 85 pre-pandemic serum samples of patients with non-coronavirus respiratory infections (n = 35), other human coronavirus infections (n = 19), systemic rheumatic diseases (n = 10) and other pathogens (n = 21), a specificity of 100% [95.8–100] was obtained for the R-N and TF-S assay (Tables 1, 2). The lowest specificity was obtained for the LFA TD-S IgM assay (82% [72.6–89.8]), with the highest cross-reactivity in the cohort of anti-nuclear antibody associated disease (AARD) and non-SARS-CoV-2 coronaviruses (15/85 false positive results). All other specificities ranged from 93% to 99% (EI-N 92.9% [83.3–97.4], LFA TD-S IgG 94.1% [86.8–98.1], EI-S 95.3% [88.4–98.7], DS-S 95.3% [88.4–98.7], TD-SN 96.5% [90.0–99.3], LFA PR-SN IgG 96.5% [90.0–99.3], LFA PR-SN IgM 98.8% [93.6–100], Id-N 98.8% [93.6–100], TD-S 98.8% [93.6–100]). An overview of the specific reactivities is given for every SARS-CoV-2 antibody assay in Table 3.

3.3.2. Sensitivity cohort

The evaluated SARS-CoV-2 antibody assays showed a significant difference in diagnostic performance with the 81 serum samples selected from patients with a rRT-PCR confirmed SARS-CoV-2 infection. Data of all (semi-)quantitative assays are shown in Table 1 and corresponding receiver operating characteristic (ROC) curves in Fig. 1. The observed differences are mainly related to the diagnostic performance in the early phase of antibody detection. The diagnostic performance of the antibody tests was directly proportional to the time period after onset of symptoms: the longer this time period, the higher the diagnostic performance of all antibody tests and consequently, the lower the difference in diagnostic performance between tests. In addition, assays using a recombination N-antigen revealed generally higher sensitivities compared to those targeting the S-antigen, although specificity results were generally comparable. Based on these results, the R-N assay showed the best diagnostic performance characteristics.

Equally, all data on diagnostic performance characteristics of the rapid LFA are shown in Table 2. Overall, LFA PR-SN showed the most favorable results, with the highest sensitivity in all cohorts (<10 days after symptom onset: 66.7% [41.2–82.7] to ≥20 days after symptom onset: 100.0% [76.8–100]) and overall specificity of 96.5% [90.0–99.3]). For all assays, Box and Whisker analysis revealed significantly higher antibody tests in the ‘critically ill’ patient cohort (n = 33) compared to the ‘moderately ill’ cohort (n = 33) (Supplementary Material 5; for all assays p < 0.05). However, the proportion of samples collected ≥20 days after symptom onset was significantly higher in the ‘critically ill’ patient cohort (36% versus 3%; p = 0.0008), which could attribute to the higher antibody levels. No significant difference was observed when comparing antibody results between patient cohort ‘asymptomatic’ (n = 12) and cohort ‘mildly ill’ (n = 3) or ‘mildly ill’ and cohort ‘moderately ill’ patients for all assays (p > 0.05).

4. Discussion

Since the start of the COVID-19 pandemic, an increasing number of serological SARS-CoV-2 assays have been introduced to the diagnostic market (Deeks et al., 2020). The expertise of laboratory professionals is critical in the validation of these diagnostic assays to ensure sufficient analytical performance in respect to the intended use (Vermeersch et al., 2020).

This study evaluated the diagnostic performance of eight (semi-) quantitative (IgG/total Ig) and two rapid LFA (IgM and IgG) serological assays for the detection of SARS-CoV-2 (N/S protein). Taking into account the manufacturer’s threshold, the overall sensitivity in our cohort (n = 81) ranged from 56.8% [45.3–67.8] (TD-S) to 84.0% [74.1–91.2] (R-N). The lower overall sensitivity is not surprising given the median time of 11 days between symptom onset and sample collection in our...
Table 1
Diagnostic performance characteristics of all (semi-)quantitative assays according to different sensitivity cohorts.

| Assay | ID-N (n = 85) | R-N (n = 85) | Total | E-I (n = 81) | D-S (n = 73) | T-F (n = 100) | D-SN (n = 85) |
|-------|---------------|--------------|-------|--------------|--------------|---------------|---------------|
|        | EI-N IgG      | EI-N IgG     |       | ESI IgG      | ESI IgG      | ESI IgG       | ESI IgG       |
|        |              |              |       |              |              |               |               |
|        | CU units      |              |       |              |              |               |               |
| Anti-N | 0.8          | 0.3          | 1.0   | 0.8          | 1.0          | 1.0           | 1.0           |
| Anti-S | NS           | NS           | NS    | NS           | U/mL         | AU/mL         | AU            |
| Anti-N/S | NS          | NS           | NS    | NS           | 0.8          | 1.0           | 1.0           |

**Legend**
- <50%
- 50-60%
- 60-70%
- 70-80%
- 80-95%
- 95-100%

Sensitivity is calculated at both the manufacturer’s cut-off (SN) and at the cut-off corresponding to a specificity level of 95% (CU 95% SP SN).

Abbreviations: anti-N, anti-nucleocapsid protein; anti-S, anti-spike protein; AU, arbitrary units; AUC, area under the ROC curve; CI, confidence interval; CU: cut-off; DS-S, Liaison SARS-CoV-2 S1/S2 IgG (DiaSorin S.P.A., Italy); EI-N, Anti-SARS-CoV-2 IgG (Euroimmun, Germany); EI-S, Anti-SARS-CoV-2 IgG (Euroimmun, Germany); Id-N, ID screen SARS-CoV-2-N (Id-vet, France); NS, not stated; ROC, receiver operating curve analysis; R-N, Elecsys Anti-SARS-CoV-2 (Roche, Germany); SN, sensitivity; SP, specificity; TD-S, COVID-19 THERA02 (Theradiag, France); TD-SN, COVID19-LISA IgG (Theradiag, France); TF-S, Eia SARS-CoV-2 Sp1 IgG (Thermo Fisher Scientific, Sweden). * in concordance to reference (World Health Organization, 2020c).

cohort. Our sensitivity findings (range 23.3–50.0%) in the subgroup of patients < 10 days of symptoms’ don’t support the use of serological SARS-CoV-2 assays in patients presenting at the emergency ward, as confirmed by previous studies (Zhao et al., 2020). Furthermore, the addition of IgM analysis for the LFA didn’t significantly improve sensitivity. Not taking into account the quicker seroconversion of total antibodies (R-N) possibly attributed to the double-antigen sandwich, previous antibody kinetic studies have shown a similar median time of 50.0% in the subgroup of asymptomatic patients.
The sensitivity in the asymptomatic cohort was significantly lower than the overall sensitivity. Importantly, 9 of the 12 samples were taken earlier in this patient category (Jiang et al., 2020). In the study of Jiang and colleagues, IgG/IgM titers and plasma neutralisation capacity were, at the time of virus clearance, significantly lower in recovered asymptomatic patients. Reinforced by the fact that the major part of asymptomatic and pauci-symptomatic patients is not even tested for viral RNA, serology ultimately offers the greatest potential to understand the true scale of SARS-CoV-2 infections. The persistence of the SARS-CoV-2 specific antibody response is still under review. It is observed that IgG levels and neutralizing antibodies in a high proportion of individuals who recovered from SARS-CoV-2 infection start to decrease within 2–3 months after infection, especially for the asymptomatic patients. 40.0% (120) of asymptomatic individuals, but only 12.9% (4/31) of symptomatic individuals, became seronegative for IgG eight weeks post hospital discharge (Long et al., 2020b).

The added value of SARS-CoV-2 antibody detection for the diagnosis of COVID-19 in rRT-PCR negative patients presenting in the late stage of the disease is well known (Long et al., 2020a). As we have shown in Table 1, antibody detection shows a high sensitivity from 10 days post symptom onset onwards. In this regard, serology can potentially offer added value in patients with a single respiratory sample with low viral load (cycle threshold ≥32), to distinguish acute from past infection. At this stage of the pandemic, quite some people have already gone through a COVID-19 infection some without knowing. As SARS-CoV-2 RNA remains detectable for several weeks to months after an infection, this can result in unexpected positive rRT-PCR results (with low viral load) at routine (pre-)admission screening of patients. In the absence of respiratory symptoms, the presence of antibodies in combination with a low viral load on rRT-PCR is highly suggestive for late stage of the disease or past infection. The patient can be considered noncontagious and there is no need for specific isolation precautions. When the antibody test is negative, the high cycle threshold most likely indicates a very recent infection, and thus an infectious patient. A new respiratory sample for detection of SARS-CoV-2 RNA is warranted to confirm a recent onset of infection. The patient can be considered noncontagious and there is no need for specific isolation precautions. When the antibody test is negative, the high cycle threshold most likely indicates a very recent infection, and thus an infectious patient. A new respiratory sample for detection of SARS-CoV-2 RNA is warranted to confirm a recent onset of infection. The potential role of serology in monitoring the immune response after vaccination is a next topic of research.

Regarding specificity, N protein-based serological assays were more often associated with cross-reactivity than the S-based assays (Infantino et al., 2020). For the latter, recombinant and more standardized S-
based assays have shown to be more specific compared to assays to full viral antigens (Okba et al., 2020b; Amanat et al., 2020) (Supplementary Material 1). Overall specificity in the samples collected prior to the pandemic (n = 85) ranged from 82.4% (LFA TD-S) to 100% (R-N & TF-S) (Table 1). Cross-reactivity is mainly attributed to antigens well-conserved among different coronaviruses and to cross-reaction with antibodies of autoimmune diseases (Wang et al., 2004). When using antibody assays on a population level, a high specificity is of utmost importance, as every small drop in specificity will seriously reduce the positive predictive value (Galli and Plebani, 2020). In the future, if antibodies prove to be protective, false positive results can potentially also have an important impact on the individual patient level if these results are used to decide whether or not to administer (re)vaccination or to use personal protective equipment.

In accordance with preceding studies (Long et al., 2020a; Okba et al., 2020a), we found that all (semi-)quantitative assays result in significantly (p < 0.05) higher antibody levels in the ‘critically ill’ patient cohort compared to the ‘moderately ill’ cohort. However, the proportion of samples collected ≥20 days after symptom onset was also significantly higher in the ‘critically ill’ patient cohort (36% versus 3%; p = 0.0008), which could attribute to the higher antibody levels. Nevertheless, our observations are completely in line with earlier findings that antibody levels are associated with disease severity (Gudbjartsson et al., 2020).

A strength of our study is the parallel evaluation of the diagnostic performance of several new serologic SARS-CoV-2 assays and assays with established diagnostic performance. Furthermore, we’ve performed a separate diagnostic performance analysis in asymptomatic

### Table 3

Overview of the cross reactivity of every SARS-CoV-2 antibody assay for the total specificity cohort.

| Sample group | micro-organism or diagnosis | number of samples tested | El-S | El-N | Id-S | Id-N | TD-S | TD-N | DS-S | DS-N | TD-SN | TF-S | LFA PR-SN | LFA TD-S |
|--------------|-----------------------------|--------------------------|------|------|------|------|------|------|------|------|-------|------|---------|---------|
|              |                             |                          | IgG  | IgM  | IgG+ IgM | IgG  | IgM  | IgG+ IgM |
| Non-coronavirus |                             |                          |      |      |        |      |      |        |
| Respiratory infection |                              |                          |      |      |        |      |      |        |
| (n = 35)                   | adenovirus                  | 1                        |      |      |        |      |      |        |
|                           | bocavirus                   | 2                        |      |      |        |      |      |        |
|                           | Chlamydophila pneumonia     | 3                        |      |      |        |      |      |        |
|                           | enterovirus                 | 3                        |      |      |        |      |      |        |
|                           | human                      | 2                        |      |      |        |      |      |        |
|                           | metapneumovirus             | 2                        |      |      |        |      |      |        |
|                           | influenza A virus           | 7                        |      |      |        |      |      |        |
|                           | influenza B virus           | 1                        |      |      |        |      |      |        |
|                           | Legionella                  | 1                        |      |      |        |      |      |        |
|                           | pneumophila                 | 3                        |      |      |        |      |      |        |
|                           | Mycoplasma                  | 1                        |      |      |        |      |      |        |
|                           | parainfluenza virus 1       | 1                        |      |      |        |      |      |        |
|                           | parainfluenza virus 2       | 1                        |      |      |        |      |      |        |
|                           | parainfluenza virus 3       | 1                        |      |      |        |      |      |        |
|                           | parainfluenza virus 4       | 1                        |      |      |        |      |      |        |
|                           | rhinovirus                  | 4                        |      |      |        |      |      |        |
|                           | respiratory syncitial virus | 3                        |      |      |        |      |      |        |
| Other human coronavirus infections (n = 19) |                              |                          |      |      |        |      |      |        |
|                              | human coronavirus 229E      | 4                        |      |      |        |      |      |        |
|                              | human coronavirus HKU      | 6                        |      |      |        |      |      |        |
|                              | human coronavirus NL63      | 5                        |      |      |        |      |      |        |
|                              | human coronavirus OC43      | 4                        |      |      |        |      |      |        |
| Systemic rheumatic disease (n = 10) |                              |                          |      |      |        |      |      |        |
|                                          | mixed connective tissue disease | 2                  |      |      |        |      |      |        |
|                                          | rheumatoid arthritis        | 3                        |      |      |        |      |      |        |
|                                          | systemic lupus             | 3                        |      |      |        |      |      |        |
| Other pathogens (n = 21) |                              |                          |      |      |        |      |      |        |
|                              | Borrelia burgdorferi (IgG) | 1                        |      |      |        |      |      |        |
|                              | cytomegalovirus            | 3                        |      |      |        |      |      |        |
|                              | epstein-barr virus         | 3                        |      |      |        |      |      |        |
|                              | hepatitis A virus          | 1                        |      |      |        |      |      |        |
|                              | hepatitis B virus          | 2                        |      |      |        |      |      |        |
|                              | (HBsAg)                    |                          |      |      |        |      |      |        |
|                              | hepatitis C virus          | 2                        |      |      |        |      |      |        |
|                              | hepatitis E virus          | 2                        |      |      |        |      |      |        |
|                              | parovirus                  | 2                        |      |      |        |      |      |        |
|                              | Toxoplasma gondii           | 2                        |      |      |        |      |      |        |
|                              | Treponema pallidum          | 2                        |      |      |        |      |      |        |
| Total                     |                             | 85                       | 4    | 6    | 1     | 1    | 0    | 4    | 3    | 0    | 3     | 1    | 4      | 5      | 15    | 15 |

Abbreviations: DS-S, Liaison SARS-CoV-2 S1/S2 IgG (DiaSorin S.P.A., Italy); El-N (Anti-SARS-CoV-2-2-NCP (Euroimmun, Germany); El-S, Anti-SARS-CoV-2 (Euroimmun, Germany); Id-N, Id screen SARS-CoV-2-N (Id-vet, France); LFA PR-SN, COVID-PRESTO® (AAZ-LMB, France); LFA TD-S = TDR Covid-19 IgG + IgM Thera (Theradiag, France); R-N, Elecsys Anti-SARS-CoV-2 (Roche, Germany); TD-S, COVID-19 THERA02 (Theradiag, France); TD-SN, COVID19-LISA IgG (Theradiag, France); TF-S, ELISA SARS-CoV-2 Sp1 IgG (Thermo Fisher Scientific, Sweden).
people. In this subgroup, overall sensitivity revealed to be lower than the overall sensitivity obtained for the several assays, as mentioned above. This is not surprising taking into account the earlier mentioned difference in Ab kinetics in the asymptomatic population.

A limitation of our study is that the samples used to evaluate specificity were all challenging. We thus expect a higher specificity in a routine laboratory setting. Another limitation is the limited sample size, which results in a small number of cases in the subgroup analyses concerning timing post symptom onset and severity of symptoms. Finally, the categorization of the patient cohorts “mild”, “moderate”, “critical” was only based on whether or not the patient was admitted to the hospital/intensive care unit. Information on duration and severity of symptoms of individual cases is lacking, due to the retrospective design of this study.

We can conclude that, in this study, the R-N serological assay revealed the best overall performance. However, for the intended use of antibody detection (>20 days after symptom onset), the R-N, Id-N and TF-S assays all met the sensitivity (95–98%) and specificity (97–99%) targets determined by the WHO (World Health Organization, 2020c).

**Data availability**

Data will be available from the author upon request.

**Ethics approval**

The protocol was approved by the local Ethics Committee OLV Hospital Aalst with Belgian registration number B1262020000015. The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

**Authors’ contributions**

MM, EVH, LC, AB and LVH contributed to the study conceptualization. Data curation and project administration were performed by MM, EVH, LN, SVDB and LH; Formal data analysis was performed by LVH. MM, EVH, LN and LVH wrote-original draft. Writing-review & editing was performed by MM, EVH, LN, LC, AB and LVH. The final manuscript was read and approved by all authors.

**Declaration of Competing Interest**

AB and LVH have been consultants for Thermo Fisher Scientific.

**Acknowledgments**

We thank Euroimmun, Id-vet, AAZ-LMB, Theradiag and Thermo Fisher Scientific for the donation of the assays. We are very grateful to the laboratory technicians for their most appreciated efforts.

**Appendix A. Supplementary data**

Supplementary data to this article can be found online at https://doi.org/...
