

Letter to the Editor

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Assessment of immune response to SARS-CoV-2 with fully automated MAGLUMI 2019-nCoV IgG and IgM chemiluminescence immunoassays

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To the Editor,

The recent emergence of 2019 coronavirus disease (COVID-19) in December 2019 in China, and its ensuing widespread propagation all around the world, have finally persuaded the World Health Organization (WHO) to upgrade COVID-19 from an epidemic to a pandemic disease [1]. According to the WHO and US Centers for Disease Control and Prevention (CDC), the etiological diagnosis of COVID-19 still requires the identification of the responsible microorganism (i.e. the severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2]) in upper or lower respiratory tract specimens by means of molecular biology techniques, namely with (real-time) reverse transcriptase-polymerase chain reaction (rRT-PCR) [2]. Besides molecular biology, serological testing is emerging as an additional option in COVID-19 diagnostics. Although the role of serological tests will remain almost confined to surveillance or epidemiological purposes, as also clearly endorsed by the CDC [3], accurate assessment of immunological response provides clinical

and societal benefits, especially for establishing whether a person has been infected by the virus and has then developed antibodies which, if neutralizing, may be effective to prevent re-infections [4]. Notably, as for any other laboratory test, novel serological assays shall be evaluated in clinical laboratories before broad introduction into clinical practice and diagnostic protocols. Therefore, the aim of this study was to analyze the immunological response to SARS-CoV-2 using novel fully automated chemiluminescence immunoassays (CLIAs).

The MAGLUMI 2019-nCoV IgG and IgM are two indirect CLIAs for assessment of IgG and IgM antibodies against SARS-CoV-2 in human serum or plasma samples, on the fully automated MAGLUMI analyzers (SNIBE – Shenzhen New Industries Biomedical Engineering Co., Ltd, Shenzhen, China). According to the manufacturer's declarations, the antibodies used in these assays are directed against both CoV-S (spike) and e CoV-N (nucleocapsid). A test result ≥ 1.10 AU/mL is considered reactive, whilst the overall reproducibility declared by the manufacturer comprises between 6.8% and 8.7%. Major details of these techniques have been summarized in the recent article by Padoan et al. [5], which also thoughtfully describes the analytical performance of these methods. The results of MAGLUMI 2019-nCoV were compared with those obtained with automated Anti-SARS-CoV-2 IgA and IgG enzyme-linked immunosorbent assays (ELISAs; Euroimmun AG, Luebeck, Germany), which are CE mark tests available for comparison studies. The technical and analytical characteristics of these ELISAs have been reported elsewhere [6]. A test result ≥ 1.1 (absorbance of patient sample/absorbance of calibrator) is considered reactive, whilst the overall reproducibility declared by the manufacturer comprises between 2% and 16%. The results were also compared with those of a reference RT-PCR assay in upper respiratory specimens (nasopharyngeal and oropharyngeal swabs) [7]. Respiratory specimens were tested for SARS-CoV-2 infection with a commercial RT-PCR method, Seegene Allplex™2019-nCoV Assay (Seegene,

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Seoul, South Korea), according to the manufacturer's protocols. Automated RNA extraction and PCR setup were carried out using Seegene NIMBUS, a liquid handling workstation. Real-time PCR was run on a CFX96TMDx platform (Bio-Rad Laboratories, Inc., CA, USA) and subsequently interpreted by Seegene's Viewer software. The Seegene Allplex™2019-nCoV Assay identifies the virus by multiplex real-time PCR targeting three viral genes (*E*, *RdRP* and *N*), thus complying with international validated testing protocols.

The final study population consisted of 131 consecutive patients (56 ± 21 years; 71 women and 60 men), hospitalized in the University Hospital of Verona for suspected COVID-19, in whom nasopharyngeal and oropharyngeal swabs were collected along with blood samples during hospital stay, for purposes of COVID-19 diagnosis and/or monitoring. Upper respiratory specimens were obtained in accordance with the WHO indications [7], whilst venous blood sampling was drawn in agreement with the current guidelines [8]. Date of symptom onset was recorded when available. The statistical analysis was carried out with Analyse-it (Analyse-it Software Ltd, Leeds, UK). The study has been cleared by the local Ethical Committee (University Hospital of Verona; SOPAV-2; protocol no. 35747).

The direct comparison by receiver operating characteristic (ROC) curve analysis of MAGLUMI 2019-nCoV IgG positive/negative vs. Euroimmun Anti-SARS-CoV-2 IgG positive/negative results yielded an overall concordance of 88% (kappa statistics, 0.47; 95% CI, 0.26–0.68; $p < 0.001$), whilst the area under the curve (AUC) was as high as 0.85 (95% CI, 0.72–0.97; $p < 0.001$). The Spearman correlation of absorbance values was 0.47 (95% CI, 0.32–0.59; $p < 0.001$). The ROC curve, recalculated using MAGLUMI 2019-nCoV IgG absorbance data vs. AUC vs. Euroimmun Anti-SARS-CoV-2 positive/negative IgG results, yielded substantially increased AUC (0.93; 95% CI, 0.84–1.00; $p < 0.001$) and slightly better agreement (89%; kappa statistics, 0.49; 0.27–0.70; $p < 0.001$). The newly identified ROC curve cut-off was ≥ 1.30 AU/mL.

Despite being different immunoglobulin classes, an analogous analysis between MAGLUMI 2019-nCoV IgM positive/negative vs. Euroimmun Anti-SARS-CoV-2 IgA positive/negative results yielded an overall concordance of 90% (kappa statistics, 0.39; 95% CI, 0.14–0.65; $p = 0.001$), AUC of 0.64 (95% CI, 0.53–0.75; $p = 0.001$) and Spearman correlation (absorbance) of 0.26 (95% CI, 0.09–0.41; $p = 0.003$). The ROC curve, recalculated using MAGLUMI 2019-nCoV IgM absorbance data vs. Euroimmun Anti-SARS-CoV-2 positive/negative IgA results, yielded a substantial increase in the AUC (0.87; 95% CI, 0.77–0.97; $p < 0.001$).

Either test positive of MAGLUMI 2019-nCoV IgM and IgG yielded an overall concordance of 90% (kappa statistics, 0.64; 95% CI, 0.46–0.82) and AUC as high as 0.85 (95% CI, 0.76–0.95; $p < 0.001$) vs. Euroimmun Anti-SARS-CoV-2 IgA and IgG (Figure 1). In the 95 patients in whom upper respiratory specimens yielded a clearly reactive ($n = 48$) or non-reactive ($n = 47$) RT-PCT test result (in the remaining 36 samples results were classified as inconclusive), the AUCs of either test positive of MAGLUMI 2019-nCoV IgM and IgG vs. Euroimmun Anti-SARS-CoV-2 IgA and IgG were 0.56 (95% CI, 0.50–0.63; $p = 0.033$) and 0.51 (95% CI, 0.45–0.57; $p = 0.377$), respectively. When inconclusive samples were included in statistical analysis and classified as reactive, the AUC performance of MAGLUMI vs. Euroimmun assays slightly increased to 0.59 (95% CI, 0.53–0.65; $p = 0.001$) and 0.55 (95% CI, 0.49–0.60; $p = 0.056$), respectively.

The rate of positivity of the different methods in the 48 patients in whom the date of symptom onset was available is shown in Table 1. In patients with symptom onset ≤ 5 days the rate of positive antibodies was very low, always $< 5\%$, whilst in those with symptom onset between 5 and 10 days the rate of positive antibodies ranged between 15.4% and 53.8%. Notably, in patients with symptom onset between > 10 and 21 days, the rate of positive antibodies was always $> 100\%$ except for MAGLUMI IgM, which were only positive in 60% of patients. These results are substantially aligned with those previously published using the same immunoassays by Padoan et al. [5], and especially by Jin et al. [9],

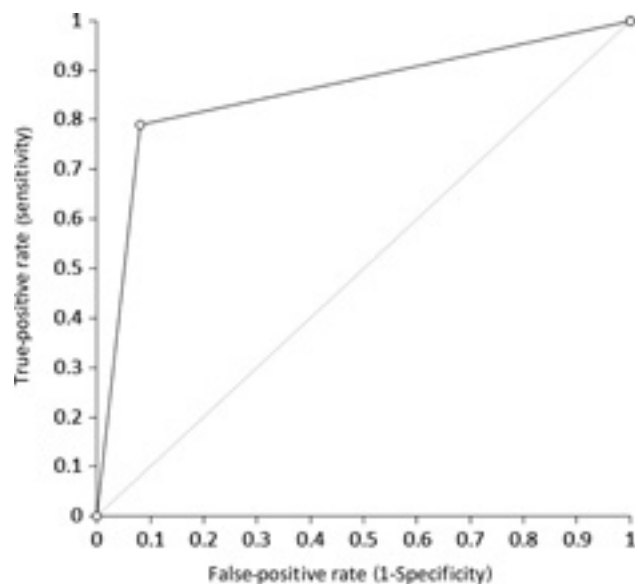


Figure 1: Receiver operating characteristic curve analysis of MAGLUMI 2019-nCoV IgM and IgG (either test positive) compared to Euroimmun Anti-SARS-CoV-2 IgA and IgG (either test positive). The overall concordance of either test positive is 90%.

Table 1: Rate of positivity of the different methods in the 48 patients in whom the date of symptom onset was available.

| Symptom onset | Euroimmun | | MAGLUMI | |
|---------------|--------------|--------------|--------------|--------------|
| | IgA | IgG | IgM | IgG |
| ≤5 days | 1/30 (3.3%) | 0/30 (0%) | 1/30 (3.3%) | 3/30 (10%) |
| >5–10 days | 4/13 (30.8%) | 2/13 (15.4%) | 2/13 (15.4%) | 7/13 (53.8%) |
| >10–21 days | 5/5 (100%) | 5/5 (100%) | 3/5 (60%) | 5/5 (100%) |

who reported that positivity for anti-SARS-CoV-2 IgM and IgG antibodies was 50% and 95% using different CLIAAs.

The results of this investigation thereby attest that the two currently available immunoassays for measuring anti-SARS-CoV-2 antibodies have a substantial degree of concordance, especially for IgG and for both immunoglobulins combined, exhibiting an overall 90% agreement at the respective cut-offs. A further refinement of diagnostic cut-offs may be advisable, however, as shown in this study and also highlighted by Padoan et al. in their separate investigation [5].

Importantly, the overall agreement with results of RT-PCR on upper respiratory specimens remains quite limited according to our findings. This obviously depends on the time passed between the onset of symptoms and blood and swab collection, but also on the fact that the clinical significance of these two test strategies is inherently different. In fact, although direct identification of SARS-CoV-2 on respiratory samples is used for etiological diagnosis, the identification of immune response against the virus, characterized by the appearance of IgA, IgM or IgG antibodies, remains a useful proof for epidemiological or surveillance purposes, but is not meant to replace RT-PCR testing, as evidenced by the time of detection of antibodies with respect to the onset of symptoms. It is also noteworthy that the appearance of IgM and IgA seems relatively tardy compared to other respiratory viruses. This enigmatic evidence paves the way to future investigations, considering that the delayed/lack onset of immune response may be one of the reasons underneath the heterogeneous pathogenicity of SARS-CoV-2 in COVID-19 patients, as recently underscored by Zhao et al. [10], whereby antibody titer was found to be directly correlated with disease severity in their work.

Some important limitations shall be disclosed in this study. First, the time passed between the onset of the symptoms and blood and swab collection was only available in a limited number of cases, so that the diagnostic performance of serological testing could not be clearly assessed. Then, the results of ELISA Euroimmun Anti-SARS-CoV-2 IgA and IgG tests were used as benchmarks vs. those of MAGLUMI 2019-nCoV IgM and IgG. Albeit

the Euroimmun tests cannot be considered the serological gold standard, they have been extensively validated ahead of commercialization and can hence be considered a reliable paragon. Finally, virus neutralization studies shall be planned to better understand the nature of antibodies to COVID-19 and define their potential protective role.

In conclusion, the results of this study complement those previous published by Padoan et al. [5], demonstrating that results of MAGLUMI 2019-nCoV IgM and IgG are well aligned with those of Euroimmun Anti-SARS-CoV-2 IgA and IgG, especially concerning the IgG and the cumulative immunoglobulin profile.

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