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# Analytical performances of a chemiluminescence immunoassay for SARS-CoV-2 IgM/IgG and antibody kinetics

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**Abstract**

**Background:** Coronavirus disease 2019, abbreviated to COVID-19, represents an emerging health threat worldwide as, after initial reports in China, it has continued to spread rapidly. The clinical spectrum of the disease varies from mild to severe acute respiratory distress syndrome (ARDS). Moreover, many patients can be asymptomatic, thus increasing the uncertainty of the diagnostic work-up. Laboratory tests play a pivotal role in the diagnosis and management of COVID-19, the current gold standard being real-time reverse transcription polymerase chain reaction (rRT-PCR) on respiratory tract specimens. However, the diagnostic accuracy of rRT-PCR depends on many pre-analytical and analytical variables. The measurement of specific COVID-19 antibodies (both IgG and IgM) should serve as an additional, non-invasive tool for disease detection and management.

**Methods:** The imprecision of the MAGLUMI™ 2000 Plus 2019-nCov IgM and IgG assays (Snibe, Shenzhen, China) was assessed by adopting the Clinical and Laboratory Standards Institute (CLSI) EP15-A3 protocol. Linearity of dilution and recovery was evaluated by means of mixes of high-level pools and low-level pools of serum samples. Immunoglobulin time kinetics were evaluated using a series of serum samples, repeatedly collected from COVID-19-positive patients at different times, from <5 days up to 26–30 days.

**Results:** Findings at the analytical validation of the assay carried out according to the CLSI EP15-A3 guideline demonstrated that imprecision and repeatability were acceptable (repeatability was <4% and <6% for IgM and IgG, respectively, whilst intermediate imprecision was <6%). In addition, results of dilution and recovery studies were satisfactory. The kinetics of COVID-19 antibodies confirmed previously reported findings, showing a rapid increase of both IgM and IgG after 6–7 days from the symptom onset. IgG had 100% sensitivity on day 12, whilst 88% was the higher positive rate achieved for IgM after the same time interval.

**Conclusions:** The findings of this study demonstrate the validity of the MAGLUMI 2000 Plus CLIA assay for the measurement of specific IgM and IgG in sera of COVID-19 patients, and for obtaining valuable data on the kinetics of both (IgM and IgG) COVID-19 antibodies. These data represent a pre-requisite for the appropriate utilization of specific antibodies for the diagnosis and management of COVID-19 patients.

**Keywords:** analytical performances; antibody kinetics; COVID-19; COVID IgG and IgM; rRT-PCR; SARS-CoV-2.

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## Introduction

Coronavirus disease 2019, abbreviated to COVID-19, is an emerging health threat and, on March 11, 2020, the Director-General of the World Health Organization (WHO) defined the spread of COVID-19 as a pandemic [1]. The responsible pathogen, a virus belonging to the Coronaviridae family, has been finally defined as "severe acute respiratory syndrome coronavirus 2" (SARS-CoV-2) for its high sequence identity (i.e. up to 80%) with the homologous virus that caused the 2003 SARS outbreak (i.e. SARS-CoV-1) [2].

After initial reports of disease outbreak in China, COVID-19 has spread worldwide, cases being identified in virtually all countries worldwide [3]. In Italy, after the first patient tested positive on admission to the intensive care unit (ICU) in Codogno Hospital (Lodi, Lombardia), within 14 days, numerous other cases of COVID-19, including a substantial proportion of critically ill patients,

were diagnosed in the surrounding area. A second cluster was simultaneously identified in the Veneto area and, since then, the number of COVID-19 patients has rapidly increased, mainly in Northern Italy, but all regions of the country have reported having patients being infected [4]. The clinical spectrum of SARS-CoV-2 infection can vary from mild up to onset of severe acute respiratory distress syndrome (ARDS). Moreover, many patients can be asymptomatic, thus increasing the uncertainty of the diagnostic work-up [5]. The timely and accurate diagnosis of COVID-19 infection is the cornerstone of appropriate treatment for patients, and crucial for limiting further spread of the virus, particularly as asymptomatic or mildly symptomatic subjects may be responsible for virus transmission [6]. Therefore, testing assumes critical relevance for ensuring an effective response to COVID-19 outbreak. The current gold standard for the etiological diagnosis of SARS-CoV-2 infection is (real-time) reverse transcription polymerase chain reaction (rRT-PCR) on respiratory tract specimens [7–9]. However, the quality of rRT-PCR testing remains of paramount importance in providing accurate and interpretable results, its diagnostic accuracy depending on many factors, including pre-analytical variables such as sample types and collection, transportation and storage conditions, as well as the quality and consistency of the PCR assays being used [10]. The collection of nasopharyngeal or throat swab specimens, a relatively invasive and almost uncomfortable procedure, can cause coughing and sneezing, thus generating aerosol, which constitutes a potential health hazard for healthcare workers [11].

The production of specific antibodies, particularly anti-SARS-CoV-2 IgM and IgG, should be used as an additional non-invasive method for detecting the disease, especially in patients who present late, with a low viral load. However, the timing of requests for serological assays and the interpretation of antibody results are pre-requisites of crucial importance in their efficacy. Therefore, the aim of this paper is to report an analytical validation of a novel chemiluminescent immunoassay (CLIA), available on an automatic platform, and to describe the kinetics of IgM and IgG antibodies in COVID-19 patients.

# Materials and methods

The MAGLUMI™ 2000 Plus (New Industries Biomedical Engineering Co., Ltd [Snibe], Shenzhen, China) is a chemiluminescent analytical system (CLIA), featuring high throughput (up to 180 tests/h). According to the manufacturer's inserts (271 2019-nCoV IgM, V2.0, 2020-03 and 272 2019-nCoV IgG, V1.2, 2020-02), the 2019-nCoV IgM cut-off is 1.0 AU/mL, while the 2019-nCoV IgG cut-off is 1.1 AU/mL. Manufacturers claimed that the calculated clinical sensitivities of IgM and IgG were 78.65% and 91.21%, respectively, while specificities of IgM and IgG were 97.50% and 97.3%, respectively.

## Evaluation of interferences in results due to gel separator tubes

According to the procedure recommended by Xiongyan et al. [12], viral activity could be inactivated before antibody determination by heating serum samples to 56 °C for 30 min. To ascertain whether 56 °C dry heat in the primary sample tube containing separator gel caused analytical interferences with respect to heated secondary aliquoted serum, we undertook an experimental series of comparisons in a total of 29 serum samples. In particular, for each primary sample tube with separator gel, an aliquot was prepared before viral inactivation, after which the primary sample tubes and the aliquots were heated together, and IgM and IgG results compared.

## Repeatability and intermediate precision evaluation

Precision was evaluated by using three human serum pools of samples with different values. Precision estimations were obtained by means of quintuplicate measurements of aliquots of the same pool, performed for a total of 5 consecutive days, following the Clinical and Laboratory Standards Institute (CLSI) EP15-A3 protocol [13]. The precision data claimed by the manufacturer were verified using three human serum pools, and specifications were estimated with the EP5-A3 protocol [14], by considering repeatability, and between-day variability. The results obtained for precision were compared to those claimed by the manufacturer using the procedure recommended by EP15-A3. Precision estimates were in accordance with the repeatability and intermediate precision conditions specified in the international vocabulary of metrology (VIM, JCGM 100:2012) for precision estimation within a 5-day period.

## Recovery assessment

Recovery was assessed using one pool of samples for IgM (2.18 AU/mL) and another pool for IgG (2.57 AU/mL), prepared using human serum samples. These pools were mixed with different amounts of low-level pools of samples (0.27 AU/mL for IgM and 0.093 AU/mL for IgG) in order to obtain different theoretical concentrations of IgM and IgG. Recovery was estimated according to the following formula:

$$\text{Recovery}\ (\%) = \frac{\text{Measured} - \text{Theoretical}}{\text{Theoretical}} \times 100$$

## Linearity assessment

Linearity was assessed by using a series of mixes of four sample pools, prepared with different IgM and IgG values, using serial dilution, as specified in the CLSI EP06 A: 2003 guideline (paragraph 4.3.1). In brief, two serum pools with a measured IgM antibody

value of 21.4 AU/mL and 2.27 AU/mL (high-level pools) were serially diluted with a low IgM antibody value serum pool (0.33 AU/mL). Likewise, two serum pools with a measured IgG antibody value of 73.72 AU/mL and 2.65 AU/mL (high-level pools) were serially diluted with two low IgG antibody value serum pools (0.086 AU/mL and 0.107 AU/mL, respectively). All measurements were performed in triplicate.

### Evaluation of IgM and IgG time kinetics

Through the time period between March 18 and March 26, from hospital wards with hospitalized COVID-19-positive (confirmed by positive rRT-PCR using nasopharyngeal swab samples) patients, a series of residual serum samples from routine laboratory testing were anonymized, aliquoted and stored at −80 °C. For the analyses, all samples were thawed and heat inactivated (see above) in batch. Samples were then evaluated using the MAGLUMI 2019-nCov IgM and 2019-nCov IgG (CLIA) systems, during the same analytical session. A total of 87 samples were collected from the 37 patients included in the study (one sample from each of 10 patients; two from each of six patients; three from each of 19 patients; four from each of two patients).

### Statistical analyses

The possibility to obtain viral activity inactivation in samples collected into serum blood tubes with a gel separator was assessed using Bland-Altman analyses. For evaluation of precision, ANOVA was used to estimate repeatability and intermediate precision. An in-house developed R (R Foundation for Statistical Computing, Vienna, Austria) script for implementing the CLSI EP15-A3 protocol was used for ANOVA and for calculating the upper verification limit. For time kinetic evaluation, the following strategy was used: for each sample included in the study, the collection date was matched with the corresponding date of symptom onset (i.e. fever) and antibodies measured. Using these data, the following time frames were defined (d, days): <5 d, 6–7 d, 8–9 d, 10–11 d, 12–13 d, 14–15 d, 16–17 d, 18–19 d, 20–25 d, 26–30 d. The GraphPad Prism version 8.4.1 for Windows was used to evaluate kinetic data. The mean IgM and IgG results (and standard errors) were plotted against the fever occurrence time categories (treated as continuous values). Smoothing splines with four knots were used to estimate a possible fit for the time kinetic curve.

# Results

## Evaluation of interferences in results due to gel separator tubes

Bland-Altman analyses findings demonstrated that the use of a heated primary tube with a gel separator and heated aliquoted serum generated comparable IgM and IgG results ($p = 0.122$ and $p = 0.548$, respectively) (Supplementary Figure 1).

## Repeatability and intermediate precision evaluation

Table 1 reports repeatability and intermediate precision, calculated using 5-day analysis according to the procedure suggested in CLSI EP-15-A3 [13], compared with the precision value claimed by the manufacturer (obtained using three samples at different concentrations measured in duplicate at three sites on 5 days, with three runs per day, according to the EP5-A3 protocol) [14]. The results obtained for the low and medium values were satisfactory, being lower than those reported by the manufacturer, while at the highest concentration they did not correspond with the manufacturer's specifications, also after UVL calculation, conducted as suggested by CLSI EP15-A3 [13].

**Table 1:** Precision results for 2019-nCov IgM and 2019-nCov IgG antibody assays expressed as coefficient of variation (CV) in percentage (%), obtained by using pools of samples.

| Measurand | Level, AU/mL | Design | Measured repeatability – CV, % | | Intermediate precision – CV, % | |
|---|---|---|---|---|---|---|
| | | | Laboratory evaluation[a] | Manufacturer's claims[b] | Laboratory evaluation[a] | Manufacturer's claims[b] |
| 2019-nCov IgM | 0.61 | 5 × 5 (CLSI Ep15-A3) | 3.06 | 6.71 | 5.05 | 6.95 |
| | 1.96 | | 1.84 | 2.06 | 3.31 | 3.59 |
| | 4.39 | | 4.05 | 2.23[c] | 5.06 | 2.65[c] |
| 2019-nCov IgG | 0.48 | 5 × 5 (CLSI Ep15-A3) | 5.69 | 8.76 | 5.70 | 8.80 |
| | 2.99 | | 3.86 | 6.08 | 3.87 | 6.25 |
| | 10.59 | | 3.18 | 1.62[c] | 3.96 | 1.82[c] |

[a]Calculated by including repeatability and between-day variability as specified in EP5-A3. [b]Obtained from the MAGLUMI™ 2019-nCov IgM (CLIA) insert, 271 2019-nCoV IgM-it, V2.0, 2020-03, and from MAGLUMI™ 272 2019-nCoV IgG-it, V1.2, 2020-02. [c]Indicates that the imprecision value was higher than that declared by manufacturers, also after the calculation of UVL as suggested by EP15-A3.

### Recovery evaluation

As a well-validated method was not available for comparison purposes, the recovery study was implemented to estimate possible proportional systematic error of the method. Recovery was calculated in ranges of values covering IgM and IgG cut-offs.

Our findings, as shown in Table 2, highlight overestimation for IgM (value range, 103%–123%), overestimation (103%–112%) for higher values and underestimation (63%–93%) for lower values in the case of IgG.

### Linearity assessment

Linearity data for IgM and IgG antibody MAGLUMI™ 2000 Plus CLIA are summarized in Figure 1. All tested mixes of sample pools deviated from linearity only at levels significantly higher than the IgM and IgG cut-off.

### Time kinetics

Figure 2 shows the kinetic results for the study patients at different days from fever onset, divided into time categories. Specifically, the graph shows average values and corresponding standard errors of IgM and IgG for each time category. Overlapping time kinetic trends are shown using spline interpolation.

Table 3 shows the number (and percentage) of positive test results of IgM and IgG for each time category. After the 11th day, all patients were found to be positive for IgG (100%), while the higher positivity of IgM (88%) was achieved only after the 13th day.

**Table 2:** Recovery results in percentages (%) for 2019-nCov IgM and 2019-nCov IgG.

| ID | Concentration, AU/mL | | Recovery, % |
|---|---|---|---|
| | Measured | Theoretical | |
| 2019-nCov IgM | | | |
| 1 | 2.05 | 1.99 | 103.0 |
| 2 | 1.93 | 1.80 | 107.3 |
| 3 | 1.84 | 1.60 | 114.8 |
| 4 | 1.62 | 1.41 | 114.7 |
| 5 | 1.46 | 1.22 | 119.8 |
| 6 | 1.24 | 1.03 | 120.4 |
| 7 | 1.03 | 0.84 | 122.5 |
| 8 | 0.78 | 0.65 | 120.0 |
| 9 | 0.56 | 0.46 | 123.4 |
| 2019-nCov IgG | | | |
| 1 | 2.41 | 2.32 | 104.0 |
| 2 | 2.33 | 2.07 | 112.5 |
| 3 | 1.89 | 1.82 | 103.3 |
| 4 | 1.40 | 1.58 | 88.9 |
| 5 | 1.24 | 1.33 | 92.9 |
| 6 | 0.90 | 1.08 | 83.2 |
| 7 | 0.60 | 0.83 | 72.3 |
| 8 | 0.37 | 0.59 | 62.6 |
| 9 | 0.22 | 0.34 | 65.8 |

Mean of measured concentrations is shown.

## Discussion

The rapid spread of COVID-19 represents a major challenge for all national healthcare systems worldwide. Although the degree of infection severity may vary from mild to severe, a considerable percentage of diseased patients need sub-intensive and intensive care with respiratory support, thus causing a real healthcare emergency [15]. Moreover, an increasingly serious issue is the frequency of COVID-19 infection in healthcare workers. In a report from the WHO-China Joint Mission on COVID-19, as many as 11,251 healthcare workers had become infected with COVID-19 by April 3, 2020, with a total of 112,401 cases of COVID-19 and 13,241 associated (11.7%) deaths [16].

Therefore, not only physicians, but also scientists, policymakers and administrators of all national healthcare systems, are focusing on the ongoing discussion on diagnostic tests for COVID-19 disease, even if only a few of those already developed have been extensively validated for clinical use, because this process is inherently time-consuming [17].

The current diagnostic method involves the identification of viral RNA in respiratory samples by means of rRT-PCR, though several pre-analytical and analytical limitations have recently been described to plague this technique [18]. First, it was demonstrated that the sensitivity of this test not only depends on the stage of disease (i.e. collection time), but also on the severity [19]. The overall throughput of RNA tests is also limited because it requires high workload, skillful operators for sample preparation and testing, and also requires expensive instrumentation and important biosafety measures. Therefore, less expensive and easy implementable serological tests are needed for detecting 2019-nCov antibodies, not only for diagnosing COVID-19, but also for characterizing the course of disease, as well as for epidemiological and vaccine evaluation studies.

We have hence carried out a study to investigate the analytical performance of the MAGLUMI™ 2000 Plus 2019-nCoV IgM and IgG chemiluminescence immunoassay and the kinetics of appearance of antibodies in COVID-19 patients. The first aspect we investigated was the possibility of using serum tubes with a gel separator

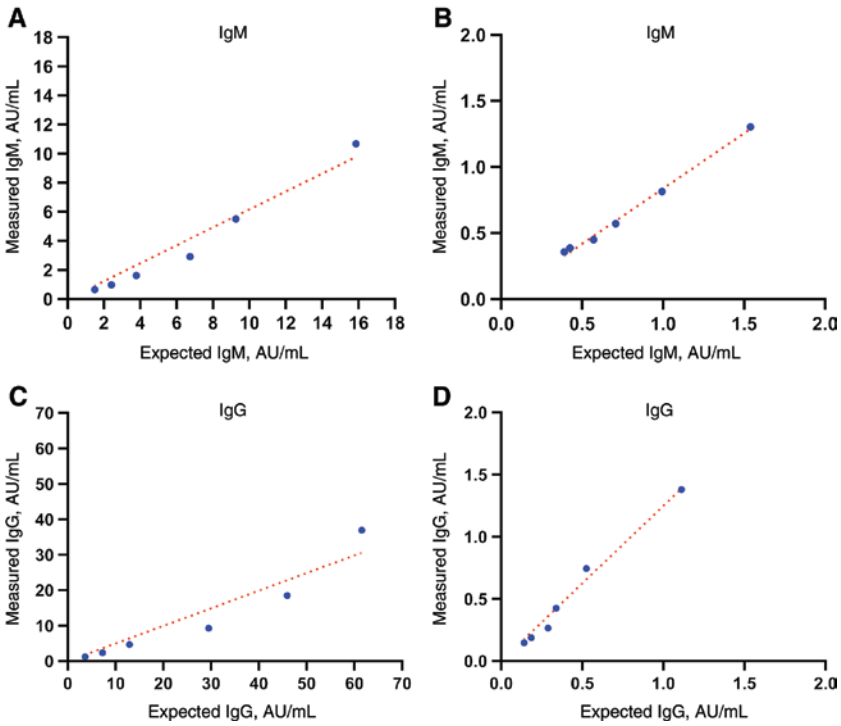


**Figure 1:** IgM and IgG antibody assay linearity results.
(A) IgM high-level pool at 21.04 AU/mL diluted with a low-level pool at 0.33 AU/mL; (B) IgM high-level pool at 2.27 AU/mL diluted with a low-level pool at 0.327 AU/mL; (C) IgG high-level pool at 73.72 AU/mL diluted with a low-level pool at 0.086 AU/mL; (D) IgG high-level pool at 2.65 AU/mL diluted with a low-level pool at 0.107 AU/mL. Averages of triplicate measurements are shown.

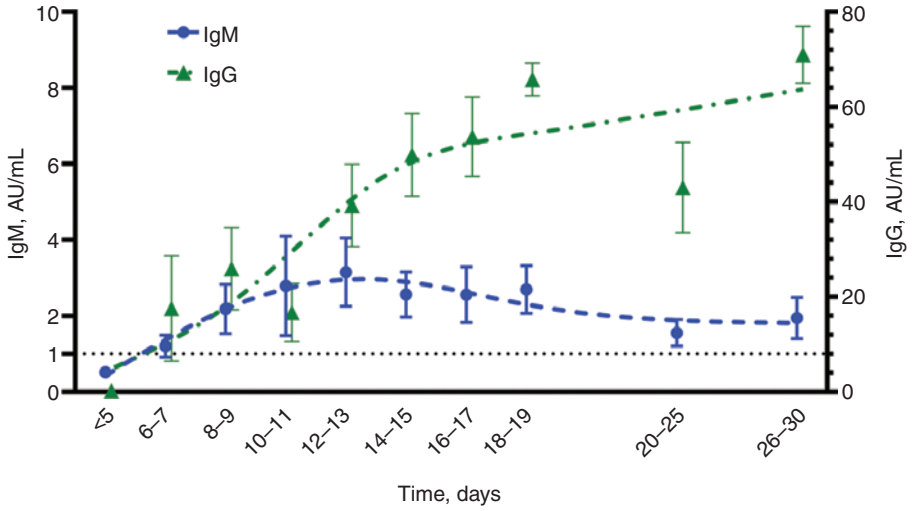


**Figure 2:** Time kinetic results for IgM (left axis, dashed line) and IgG (right axis, dashed-dotted line) at different days from the fever onset in the 37 study patients.
Different time frames are shown. Mean and standard errors are plotted. Smoothing splines were used to fit the time-trend lines for both immunoglobulins.

as primary samples for direct viral inactivation, without preparing secondary aliquots. This strategy is extremely important for operator safety as well as for effective workload management. The analyses performed on a series of 29 consecutive serum samples with a gel separator confirmed that this type of sampling tube could be suitably used for IgM and IgG measurement after dry heat for viral inactivation. Then, we assessed the imprecision profile of the assay using the CLSI EP15-A3 protocol [12].

Overall, our results show that MAGLUMI™ 2000 Plus has excellent precision characteristics. In fact, repeatability was <4% and <6% for IgM and IgG, respectively,

**Table 3:** Descriptive statistics of 2019-nCov IgM and 2019-nCov IgG for the studied patients, subdivided on the basis of each time point (calculated considering the initial onset of fever).

| Time from the onset of fever | IgM, AU/mL | IgG, AU/mL |
|---|---|---|
| ≤5 days | | |
| n | 4 | 4 |
| Mean ± SD | 0.513 ± 0.127 | 0.12 ± 0.17 |
| Median (IQR) | 0.488 (0.431–0.595) | 0.04 (0.03–0.22) |
| Min–max | 0.386–0.689 | 0.019–0.387 |
| n of Positive tests (%) | 0/4 (0%) | 0/4 (0%) |
| 6–7 days | | |
| n | 6 | 6 |
| Mean ± SD | 1.196 ± 0.725 | 17.50 ± 27.25 |
| Median (IQR) | 1.066 (0.558–2.01) | 7.64 (0.19–18.65) |
| Min–max | 0.381–2.097 | 0.154–70.74 |
| n of Positive tests (%) | 3/6 (50.0%) | 4/6 (66.7%) |
| 8–9 days | | |
| n | 12 | 12 |
| Mean ± SD | 2.174 ± 2.246 | 25.95 ± 30.7 |
| Median (IQR) | 1.489 (0.764–2.519) | 14.48 (1.10–39.99) |
| Min–max | 0.452–8.124 | 0.26–86.55 |
| n of Positive tests (%) | 7/12 (58.3%) | 9/12 (75.0%) |
| 10–11 days | | |
| n | 14 | 14 |
| Mean ± SD | 2.783 ± 4.894 | 16.67 ± 23.26 |
| Median (IQR) | 0.94 (0.41–2.11) | 2.34 (0.63–23.54) |
| Min–max | 0.38–18.04 | 0.94–74.35 |
| n of Positive tests (%) | 5/14 (35.7%) | 10/14 (71.4%) |
| 12–13 days | | |
| n | 9 | 9 |
| Mean ± SD | 3.15 ± 2.71 | 39.24 ± 26.54 |
| Median (IQR) | 1.91 (1.77–4.31) | 34.93 (31.22–43.25) |
| Min–max | 0.61–9.20 | 2.867–83.66 |
| n of Positive tests (%) | 7/9 (77.8%) | 9/9 (100%) |
| >13 days | | |
| n | 25 | 25 |
| Mean ± SD | 2.34 ± 1.79 | 55.20 ± 24.13 |
| Median (IQR) | 2.01 (1.16–3.14) | 59.47 (43.74–74.70) |
| Min–max | 0.384–7.68 | 1.32–87.61 |
| n of Positive tests (%) | 22/25 (88.0%) | 25/25 (100%) |

n, number of patients from whom sample was obtained within the specified time period.

whilst intermediate imprecision was <6%. Nevertheless, the results did not allow us to reproduce the manufacturer's claims for precision at the highest concentration level. Therefore, action was undertaken with the manufacturer, by asking for additional information underlying this difference in imprecision, which, however, does not impair clinical reliability.

Linearity of dilutions was also assessed, in order to evaluate the ability of the method to provide results directly proportional to the concentration of IgM and IgG in tested samples. We performed a series of serial dilutions using high-value pools diluted in low-value pools. The results obtained showed that within the 1.5 AU/mL–0.5 AU/mL range results are linear for both immunoglobulins, whilst linearity seems worse at the highest values, especially for IgG. As a well-validated method for comparing results is currently unavailable, we also performed recovery studies. The range inspected covered the range of values suggested by the manufacturer, and results showed that IgM was slightly overestimated, whereas IgG was overestimated for values above 1.9 AU/mL and slightly underestimated at values below 1.9 AU/mL. Overall, better recovery results were found for IgM, so simultaneous assessment of both IgM and IgG may be advisable.

The time kinetics of IgM and IgG was also evaluated during a time interval previously recommended [19]. Our

results showed that both IgM and IgG rapidly increased after the onset of fever. Considering the cut-offs suggested by the manufacturer (i.e. 1.0 AU/mL for IgM and 1.1 AU/mL for IgG), the immunoglobulin rise could be considered as significant 6–7 days after fever onset. These findings are in agreement with those recently reported by others. For example, using an ELISA in-house developed method, Zhang et al. found that the increase of antibody against the virus was clearly visible in almost all patients after 5 days of symptom onset, a time period that was usually considered as a transition from an early to a late period of infection [19]. Likewise, using a commercial ELISA kit from Livzon Diagnostics (China), Tan et al. found a marked increase of immunoglobulins 7 days after the onset of symptoms, particularly in patients with severe disease [20]. Table 3 reports in detail the kinetics of IgM and IgG, showing that IgG requires at least 12 days to attain 100% sensitivity, whilst the highest positive rate achieved for IgM was 88% throughout the study period. Interestingly, three patients had IgM values of 0.811 AU/mL, 0.909 AU/mL and 0.863 AU/mL, thus remaining below the cut-off. This suggests that further cut-off refinement would be necessary for increasing IgM sensitivity.

The present study has some notable limitations. For example, no reliably validated method was available for comparison studies, nor were cross-reactivities of the assays tested. Furthermore, the criterion for assessing the time kinetics of IgM and IgG antibodies was the time of fever onset. We used this symptom because: (a) was available for all patients included in our study; (b) is usually accurately recorded by both patients and physicians; (c) has also been used in many other studies. For example, Lauer et al. estimated that the median incubation period to fever onset was 5.7 days (95% CI: 4.9–6.8 days) for COVID-19 patients [5]. Another aspect could be that IgM and IgG kinetics shall be assessed over a longer period in order to estimate the entire trend of humoral immune response to COVID-19 infection.

In conclusion, the findings of this study show that MAGLUMI™ 2000 Plus CLIA may be a reliable immunoassay for assessing the immunological response in sera of COVID-19 patients. Our results also confirm that simultaneous measurement of IgM and IgG can be helpful, especially from the early phase of infection.

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**Ethical approval:** The local Institutional Review Board deemed the study exempt from review.

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