Andrea Padoan*, Ilaria Talli, Chiara Cosma, Stefania Moz, Giulia Furlan, Filippo Navaglia, Lucio Marchioro, Martina Zaninotto, Daniela Basso and Mario Plebani

Analytical and clinical evaluations of SNIBE Maglumi chemiluminescent immunoassay for the detection of SARS-CoV-2 antigen in salivary samples

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Abstract

Objectives: In this study, we describe the analytical and clinical performances of the SNIBE Maglumi SARS-CoV-2 antigen fully-automated chemiluminescent immunoassay (MAG-CLIA) on salivary samples.

Methods: Limit of detection (LOD), linearity and precision were tested for values close to or below the declared LOD. Clinical performance of MAG-CLIA was evaluated on left-over salivary samples from the healthcare workers (HCW) surveillance program, at the University-Hospital of Padova. Salivary samples were analyzed by Lumipulse G SARS-CoV-2 Ag, and in case where the values exceeded 0.41 ng/L, further testing was conducted using TaqPathTM COVID-19 RT-PCR (Applied Biosystems, Thermo Fisher Scientific).

Results: The estimated MAG-CLIA LOD was 3 ng/L, with repeatability of 7.5 %. Good linearity was demonstrated by diluting two samples at 52.7 ng/L and 211.4 ng/L. Of the 228 HCW samples, 59/228 (25.9 %) were positive, 169/228 (74.1 %) were negative. MAG-CLIA SARS-CoV-2 sAg median level (and interquartile range [IQR]) was 5.03 ng/L (<0.001–35.8 ng/L) for positive and <0.001 ng/L (<0.001 ng/L) for negative samples. MAG-CLIA AUC was 0.795 (95 % CI: 0.720–0.871). Using the best cut-off, 3.5 ng/L, sensitivity and specificity were 57.1 % (95 % CI: 42.2–71.2 %) and 97.0 % (95 % CI: 93.2–99.0 %), respectively. The agreement with the molecular assay was 88.1 % (Cohen's kappa 0.606 [SE=0.066, p<0.001]).

Conclusions: The analytical performances of MAG-CLIA are satisfactory, also when values below LOD were tested. In saliva samples, although specificity was elevated, clinical performance was not comparable with that on nasopharyngeal swabs (NPS).

Keywords: SARS-CoV-2 antigen; saliva; COVID-19; chemiluminescent assay; Maglumi

***Corresponding author: Andrea Padoan**, PhD, Department of Medicine (DIMED), University of Padova, Via Giustiniani 2, 35128 Padova, Italy; Laboratory Medicine Unit, University-Hospital of Padova, Via Giustiniani 2, 35128 Padova, Italy; and QI.LAB.MED., Spin-off of the University of Padova, Padova, Italy, E-mail: andrea.padoan@unipd.it. https://orcid.org/0000-0003-1284-7885

Ilaria Talli, Department of Medicine (DIMED), University of Padova, Padova, Italy; and Laboratory Medicine Unit, University-Hospital of Padova, Padova, Italy

Chiara Cosma and Daniela Basso, Department of Medicine (DIMED), University of Padova, Padova, Italy; Laboratory Medicine Unit, University-Hospital of Padova, Padova, Italy; and QI.LAB.MED., Spin-off of the University of Padova, Padova, Italy. https://orcid.org/0000-0001-8745-6171 (D. Basso)

Stefania Moz and Filippo Navaglia, Laboratory Medicine Unit, University-Hospital of Padova, Padova, Italy

Giulia Furlan, QI.LAB.MED., Spin-off of the University of Padova, Padova, Italy

Lucio Marchioro and Martina Zaninotto, Laboratory Medicine Unit, University-Hospital of Padova, Padova, Italy; and QI.LAB.MED., Spin-off of the University of Padova, Padova, Italy

Mario Plebani, Department of Medicine (DIMED), University of Padova, Padova, Italy; Laboratory Medicine Unit, University-Hospital of Padova, Padova, Italy; QI.LAB.MED., Spin-off of the University of Padova, Padova, Italy; and Clinical Biochemistry and Clinical Molecular Biology, School of Medicine, University of Padova, Padova, Italy. https://orcid.org/0000-0002-0270-1711

Introduction

After more than 3 years, on May 5th, the World Health Organization (WHO) declared the end of coronavirus disease 2019 (COVID-19) as a public health emergency [1, 2]. The most significant turning point in the pandemic was the worldwide distribution of vaccines [3], whose efficacy in establishing long-term immune and cellular immunity to SARS-CoV-2 infection [4, 5] was demonstrated by several studies for both adults [6] and children [7]. However, the epidemiological indicators of the European Center for Disease Prevention and Control website on August 18th 2023 clearly showed that COVID-19 testing is still massively ongoing since the SARS-CoV-2 virus has not disappeared [8]. As a consequence, the national efforts for controlling viral spread have been

reduced in many countries, not only by limiting the period of isolation to 5 days, as recommended by the WHO guidelines released in January 2023 [9], but also by discussing and promoting the end of the isolation period. On the other hand, screening for SARS-CoV-2 is still active only in a few specific settings that have demonstrated high effectiveness in limiting viral spread. Examples include pre-surgery testing to identify asymptomatic infections before health procedures and healthcare screening to reduce the spread of the virus from physicians and nurses to hospitalized patients. Further, testing strategies are still needed in specific settings that include fragile patients, such as pediatric children with immunosuppressive status [10, 11]. Although molecular amplification of viral nucleic acids (NAAT), such as revert real-time PCR (rRT-PCR) remains the gold standard method for diagnosis [12], other testing procedures can be used to determine the infection at more rapid times, while always considering sensitivity and specificity for each assay. In particular, laboratory-based chemiluminescent antigen testing has been widely used for COVID-19 testing, since the diagnostic performance was quite better than rapid antigen testing [13] and, in addition, they might allow using saliva as a sample matrix, improving patient compliance [14]. Further, laboratory-based antigen tests reduce testing time, since they evaluate SARS-CoV2 antigens without viral nucleic acid amplification [11, 15]. In order to address such issues, the aim of the study was to evaluate the clinical performance of the chemiluminescent MAGLUMI SARS-CoV-2 Ag (MAG-CLIA) (Snibe Co., Shenzhen, China) for detecting SARS-CoV-2 antigen in salivary samples (SARS-CoV-2 sAg), for the assessment of its analytical precision at low SARS-CoV-2 antigen concentrations.

Materials and methods

Samples included in the analysis

For the aim of the study, 228 leftover salivary samples were randomly selected from the healthcare workers (HCW) ongoing routine screening program at the University-Hospital of Padova, Italy, between March 15th 2023 and May 9th 2023. Asymptomatic or symptomatic HCW, working in all the hospital wards, were enrolled in the screening by self-collecting a salivary sample every 2–3 weeks. Salivary samples were self-collected using Salivette® (Sarstedt, Germany), centrifuged for 5 min at 4000 *g*, and then tested for SARS-CoV-2 sAg for the screening program. Then samples were stored at 4 °C overnight. The day after, they were analyzed using MAGLUMI SARS-CoV-2 Ag (CLIA) (Snibe Co., Shenzhen, China) (REF 1302 19026M, insert 282 CoV-2 Ag-it, V1.0, 2020–12), which is a chemiluminescent assay for the detection of SARS-CoV-2 able to detect the viral antigens in nasopharyngeal (NPS) patients' samples, while it has not been evaluated for SARS-CoV-2 Ag detection in salivary samples.

For the screening, salivary samples were analyzed through the use of Lumipulse G SARS-CoV-2 Ag assay (Fujirebio, Japan) [16]. Samples giving a result equal to or higher than 0.41 ng/L are considered positive, and confirmed through the nucleic acid amplification procedure (NAAT), a procedure executed on the same sample as described elsewhere [17]. Briefly, the extraction of the viral RNA was performed through MagNA Pure 96 DNA and Viral NA Small Volume Kit (REF 06543588001 version 09) (Roche, Switzerland). After that, samples undergo amplification using TaqPathTM COVID-19 RT-PCR Kit (Applied Biosystems, Thermo Fisher Scientific, Massachusetts, USA). To confirm the reliability of the Lumipulse G SARS-CoV-2 Ag assay, in a subgroup of 50 negative salivary samples randomly selected, negative results were confirmed by NAAT.

Estimation of the limit of detection (LOD) of SNIBE Maglumi SARS-CoV-2 sAg

The limit of detection was estimated by serially diluting the calibrator (50 ng/L) in duplicate in a pool of negative salivas, until obtaining a final dilution of 1 to 512. For each dilution, the RLU was recorded and compared to the expected values in ng/L. A dot-plot containing the expected concentrations and the RLU was made, overlapped with a polynomial curve. LOD was calculated by using the results of the samples with the series of the highest dilutions, encompassing the flat part of the curve, using the formula LOD = mean + 3SD.

Precision of SNIBE Maglumi SARS-CoV-2 sAg

Precision was evaluated by using several samples. Aliquots of one negative sample (with SARS-CoV-2 Ag levels around 4 ng/L) and one positive sample (with SARS-CoV-2 Ag levels of 22 ng/L) were repeatedly measured three times, for a total of three consecutive days. Furthermore, in a series of three negative samples, with SARS-CoV-2 Ag levels below the LOD (such as declared by the manufacturer at 4 ng/L), repeatability was evaluated using the raw instrumental readings (i.e. relative light units [RLU]) by performing at least five replicates within the same analytical run.

Linearity assessment of SNIBE Maglumi SARS-CoV-2 sAg

In order to evaluate the linearity of the assay for SARS-CoV-2 sAg, two samples with different viral antigen concentrations were serially diluted in a pool of negative salivas, as specified in the CLSI EP06 A: 2003 guideline (paragraph 4.3.1), until reaching a dilution of 1/128, in order to evaluate the results given by the instrument in terms of concentration and RLU. Each measurement was repeated twice in the same analytical session.

Statistical analyses

Statistical analyses were performed using Stata v16.1 (Statacorp, Lakeway drive, TX, USA). Median and interquartile ranges (IRQ) were used as descriptive statistics. Non-parametric Kruskal–Wallis test was used to assess differences in measurements across groups. Receiving operating characteristic curve (ROC) evaluations were performed by non-parametric analyses. Diagnostic performance was evaluated using the "diagt" tool in Stata.

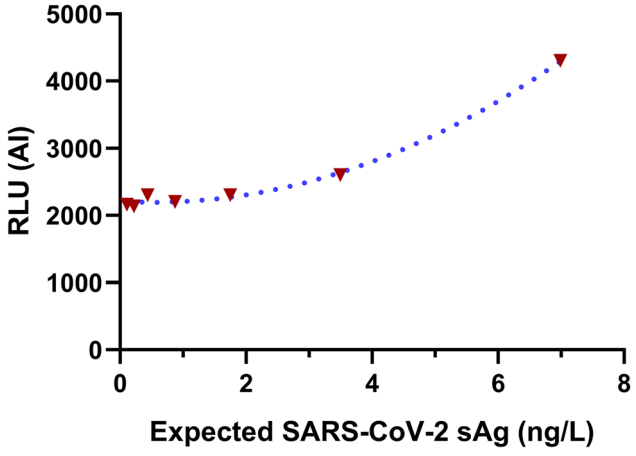
Ethical statement

The study was conducted in accordance with the Declaration of Helsinki, and the Institutional Review Board of the University of Padova (protocol no. 27444).

Results

Estimation of the limit of detection of SNIBE Maglumi SARS-CoV-2 sAg

The curve reporting the expected concentration of SARS-CoV-2 sAg and the measured RLU is reported in Figure 1. The best-interpolated curve resulted in a second-order polynomial curve, with R^2 equal to 0.994. Considering the results, RLU from 2160 to 2304 belonged to the initial flat part of the curve, in which variations of sample concentrations caused negligible variations in instrumental RLU. Thus, the calculated LOD was 2500 RLU, corresponding to an interpolated concentration value of 3 ng/L. These data support the reliability of values below the LOD, accordingly to the study of Pighi et al. [18].



Dilution	Expected SARS-CoV-2 sAg (ng/L)	RLU (AI)
1/8	6.9875	4300
1/16	3.4937	2600
1/32	1.7468	2304
1/64	0.8734	2200
1/128	0.4367	2304
1/256	0.2183	2136
1/512	0.1091	2160

Figure 1: Dot plot of the expected concentrations of SARS-CoV-2 sAg and the measured RLUs of a series of dilutions of the calibrator (50 ng/L) in a pool of negative salivas. For each dilution, measurements were performed in duplicate, and results averaged. A second order polynomial curve was overlapped to the plot. Dilution factors, expected concentration of SARS-CoV-2 sAg and RLU in arbitrary units (AI) are shown in the table below the Figure.

Precision of SNIBE Maglumi SARS-CoV-2 sAg

By evaluating 3 replicates for 3 days, the first at 2520 RLU (3.1 ng/L) and the second at 7589 RLU (25.7 ng/L), repeatability and intermediate precision were found to be 7.5 and 22.0 % for the sample with the lowest value, respectively, and 4.2 and 5.6 % for the sample with the highest value, respectively. Using the samples with concentration values below LOD, the precision resulted in 18.3 % at a mean RLU value of 1740, in 16.9 % at 2160 RLU, and in 15.5 % at 2100 RLU. Manufacturer's precision results (repeatability 2.25 % and reproducibility 2.70 %) were claimed for SARS-CoV-2 Ag values above 100 ng/L, thus not comparable with the concentrations used in our findings.

Linearity of SNIBE Maglumi SARS-CoV-2 sAg

Figure 2 reported linearity results of MAG-CLIA SARS-CoV-2 sAg. The first sample analysis presented a mean concentration of SARS-CoV-2 sAg of 52.7 ng/L, with an RLU of 14592. The second sample had a mean concentration of 211.4 ng/L with an RLU of 50108. Both samples were diluted with a pool of negative saliva (mean RLU 1800, concentration<0.001 ng/L). Both experiments demonstrated a good linearity of the assay, while a slight but not significant deviation (defined as a non-significant second-order polynomial coefficient) was identified in the sample with the lowest concentration.

Performance of SNIBE Maglumi SARS-CoV-2 sAg

Over the 228 evaluated salivary samples, 59/228 (25.9 %) were positive for SARS-CoV-2 sAg and confirmed by rRT-PCR, and 169/228 (74.1 %) were negative. Considering rRT-PCR results, samples median Cycle threshold (Ct) value was 27.4, with an interquartile range (IQR) from 24.7 to 30.4. Among rRT-PCR positive samples (Ct<40), SNIBE Maglumi SARS-CoV-2 sAg median level (and IQR) was 5.03 ng/L (<0.001 ng/L to 35.8 ng/L), while median RLU (and IQR) was 3348 (1936–9948); for rRT-PCR negative samples, the median value was <0.001 ng/L with a corresponding IQR<0.001 ng/L, while median RLU was 1644 (IQR: 1376–2076). Statistically

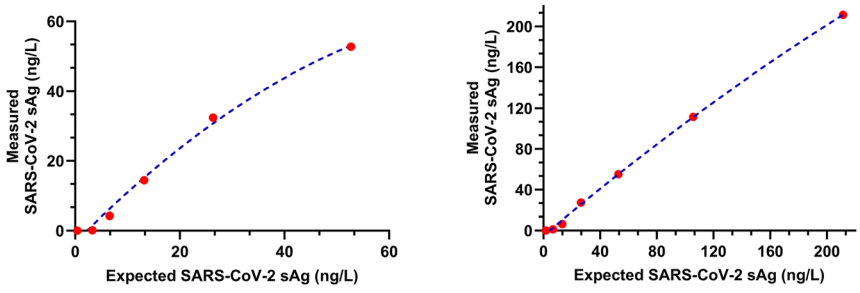


Figure 2: Dot-plots of the linearity results obtained for two samples (left panel, sample at 52.7 ng/L, with an RLU of 14592; right panel, sample at 211.4 ng/L with an RLU of 50108) were serially diluted in a pool of negative salivas. Second order polynomial regressions were overlapped.

significant differences were found both for SNIBE Maglumi SARS-CoV-2 sAg (χ^2=39.9, p<0.001) and for RLU (χ^2=40.2, p<0.001).

Overall diagnostic performance was then calculated by means of ROC analyses, which revealed an area under the curve (AUC) of 0.795 (95 % CI: 0.720–0.871) for SARS-CoV-2 sAg, with almost overlapping results using the measured instrumental RLU. Using a cut-off of 4 ng/L, which corresponds to the manufacturer's declared LOD, sensitivity and specificity (and their corresponding 95 % CI) were 56.1 % (95 % CI: 40.2–69.3 %) and 97.6 % (95 % CI: 94.1–99.4 %), respectively, with a positive and a negative likelihood ratio of 23.3 (95 % CI: 8.6–63.3) and 0.46 (95 % CI: 0.34–0.63). With 4 ng/L as the cut-off, the agreement with the molecular test was 88.1 %, with a Cohen's kappa of 0.613 (SE=0.066, p<0.001). Using the cut-off derived by the Youden's index, 3.5 ng/L, sensitivity and specificity (and their corresponding 95 % CI) were 57.1 % (95 % CI: 42.2–71.2 %) and 97.0 % (95 % CI: 93.2–99.0 %), respectively, with a positive and a negative likelihood ratio of 19.3 (95 % CI: 7.9–47.4) and 0.44 (95 % CI: 0.32–0.61). With this last cut-off of 3.5 ng/L, the agreement with the molecular test was 88.1 %, with a Cohen's κ of 0.606 (SE=0.066, p<0.001). Two further analyses were performed. In the first, positive samples with rRT-PCR Ct<26 (n=21/59, 35.6 %) and negative samples were considered. In the second, rRT-PCR Ct<30 (n=43/59, 72.9 %) and negative samples were used. For samples with rRT-PCR Ct<26, ROC analyses for SARS-CoV-2 sAg underlined an AUC of 0.995 (95 % CI: 0.989–1.000), and using a cut-off of 4 ng/L sensitivity and specificity were 100 % (95 % CI: 81.5–100.0 %) and 97.5 % (95 % CI: 93.8–99.3 %), respectively. For samples with rRT-PCR Ct<30, for SARS-CoV-2 sAg AUC was 0.904 (95 % CI: 0.836–0.971), sensitivity and specificity were 73.5 % (95 % CI: 55.6–87.1 %) and 96.6 % (95 % CI: 92.8–98.8 %), respectively. Figure 3 reports the SARS-CoV-2 sAg and RLU with respect to rRT-PCR Ct.

Discussion

The public attention to SARS-CoV-2 is undeniably reducing, especially due to the mitigation of viral pathogenicity over time and the success of vaccination campaigns; however, COVID-19 still remains a relevant disease for fragile people, such as immunocompromised patients and older individuals vulnerable to respiratory infection [19, 20]. In addition, determining SARS-CoV-2 positivity for patients programmed to undergo to surgery or relevant healthcare procedures is of utmost importance, as well as controlling the viral spread and transmission. Indeed, a study using big data has recently demonstrated that the increase in COVID-19 hospitalized patients influences the mortality risk of critically ill non-COVID-19 patients [21].

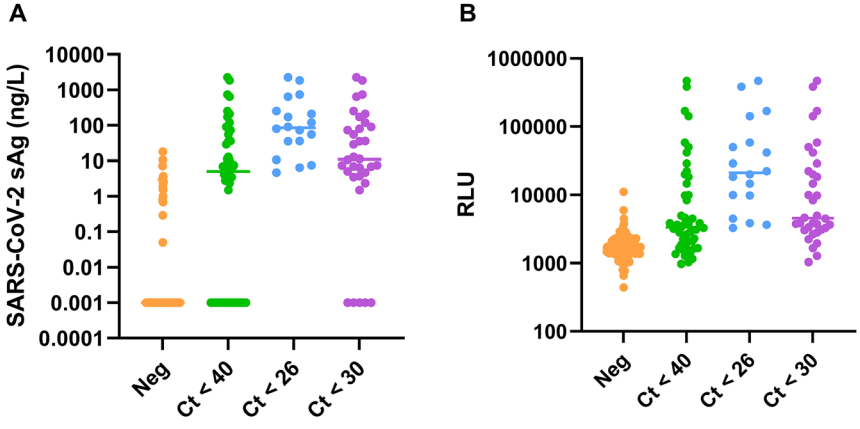


Figure 3: Dot plots showing the SARS-CoV-2 sAg (A) and the measured RLU (B), considering negative samples (rRT-PCR results of Ct≥40), all positive samples (rRT-PCR results of Ct<40), positive samples with rRT-PCR results of Ct<26 and positive samples with rRT-PCR results of Ct<30. Log_{10} scales were used.

To date, many laboratory tests for coronavirus disease 2019 (COVID-19) diagnosis have been developed. Although NAAT assays (e.g. rRT-PCR targeting multiple sequences of SARS-CoV-2) are still considered the gold standard for diagnosis of COVID-19, chemiluminescent, laboratory-based SARS-CoV-2 Ag tests were documented to be valid cost-effective alternatives, with good sensibility and specificity in detecting infection [17, 22, 23]. In this study, the analytical and clinical performance of the chemiluminescent SNIBE Maglumi (MAG-CLIA) SARS-CoV-2 sAg was evaluated using salivary samples. Notably, saliva presents several advantages over nasopharyngeal swab (NPS), being the former an unpainful test, with the possibility of standardized self-collection; further, it has been demonstrated to offer reliable results, with excellent performance for chemiluminescent assays [16, 24, 25]. We first verified that with salivary samples, the MAG-CLIA allowed reaching an LOD of 3 ng/L, quite inferior to the manufacturer's declared LOD (8 ng/L). Since SARS-CoV-2 viral particles are less concentrated in saliva than in NPS, the reduction of the limit of detection could improve diagnostic performance. In light of these results, the MAG-CLIA precision and linearity were assessed at very low concentration levels. Precision results at 3.1 ng/L demonstrated that repeatability was excellent, whilst intermediate precision, estimated in 3 consecutive days was around 22 %. On the other hand, at more elevated concentrations precision results were excellent. The linearity of the assay, obtained using serial dilutions of replicated samples was very impressive, including the concentration below 20 ng/L. Thus, overall, these findings underpin the utilization of MAG-CLIA for value below the declared cut-off for NPS (25 ng/L); moreover, the evaluations performed by using RLU seem to support the fact that the curve SARS-CoV-2 sAg concentration/RLU, might theoretically be suitable for detecting values less than LOD, even if an improvement of the assay is required to reach very low RLU values, for example by using a different calibration curve, embracing also lowest Ag values. Nevertheless, measured clinical performance of the assay was limited. By ROC analyses, sensitivity did not exceed 60 % for all the possible cut-offs, and the agreements with rRT-PCR, calculated by Cohen's kappa were not elevated. These results are in contrast with findings obtained using the same assay in NPS by Pighi et al. [18], who found a sensitivity of 88.4 %, with a specificity of 95 % by recalculating the cut-off using the RLU instead of SARS-CoV-2 sAg. Di Wang et al. [26] tested also NPS and found a sensitivity of 95.7 and 98.3 % with respect to an RT-qPCR assay, using a cut-off of 0.64 ng/L. Therefore, it could be speculated that the analytical sensitivity of MAG-CLIA is not enough to detect SARS-CoV-2 Ag equally well in NPS and saliva. However, taking into consideration samples with high viral load (rRT-PCR Ct results<26), MAG-CLIA sensitivity and specificity were excellent.

This study has some limitations. Firstly, the number of evaluated samples is limited, especially considering the number of positive samples. Secondly, demographic and clinical features were unavailable. In addition, neither viral culture nor paired NPS were used to confirm the MAG-CLIA performance. At last, not all negative samples were confirmed by NAAT. However, to our knowledge, this is the first study evaluating the MAG-CLIA analytical performance in salivary samples.

Conclusions

MAG-CLIA SARS-CoV-2 Ag detection is fast enough to ensure a rapid diagnosis of infected subjects, still displaying high sensitivity and specificity in subjects with high viral concentrations. However, when comparing SARS-CoV-2 salivary Ag concentration with NAAT, limited sensitivity and specificity were found, especially for samples with low viral load. Carefully conducted pre-analytical procedures, including sample handling, storage, and centrifugation may improve not only the precision of the results, but also the accuracy of the results on salivary samples with low Ag concentration, and thus an analytical improvement of the method might be achieved by lowering analytical sensitivity and LOD.

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Research ethics: The study was conducted in accordance with the Declaration of Helsinki, and the Institutional Review Board of the University of Padova (protocol no. 27444).
Informed consent: Not applicable.
Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.
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