Clinical assessment of the DiaSorin LIAISON SARS-CoV-2 Ag chemiluminescence immunoassay

Gian Luca Salvagno[1,2], Gianluca Gianfilippi[3], Giacomo Fiorio[3], Laura Pighi[1], Simone De Nitto[1], Brandon M. Henry[4], Giuseppe Lippi[1]

[1] Section of Clinical Biochemistry, University of Verona, Verona, Italy

[2] Service of Laboratory Medicine, Pederzoli Hospital, Peschiera del Garda, Italy

[3] Medical Direction, Pederzoli Hospital, Peschiera del Garda, Italy

[4] The Heart Institute, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA

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Corresponding author:
Prof. Giuseppe Lippi
Section of Clinical Biochemistry
University Hospital of Verona
Piazzale L.A. Scuro, 10
37134 Verona
Italy
Phone: 0039-045-8122970
Fax: 0039-045-8124308
E-mail: giuseppe.lippi@univr.it

ABSTRACT

Background

Due to the large volume of tests needed in a relatively short time for screening and diagnosing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, antigen immunoassays may provide a potential supplement to molecular testing. This study was aimed to assess the clinical preference of DiaSorin LIAISON SARS-CoV-2 Ag chemiluminescence immunoassay.

Methods

An upper respiratory specimen was collected in a series of patients referred to the Laboratory Medicine service of Pederzoli Hospital (Peschiera del Garda, Verona, Italy) for screening or diagnosis of SARS-CoV-2

infection. Nasopharyngeal samples were assayed with DiaSorin LIAISON SARS-CoV-2 Ag test and Altona Diagnostics RealStar® SARS-CoV-2 RT-PCR Kit.

Results

The final study population consisted of 421 patients (median age, 48 years; 227 women), 301 (71.5%) with positive result of molecular testing, and 126 (29.9%) with cycle threshold (Ct) values of both *E* and *S* genes <29.5, thus reflecting higher infectivity. The area under the curve of DiaSorin LIAISON SARS-CoV-2 Ag test 0.82 (95% CI, 0.79-0.86) for sample positivity and 0.98 for higher sample infectivity (95% CI, 0.97 to 0.99). The optimal cut-off for sample positivity was 82 $TCID_{50}$/mL (0.78 sensitivity, 0.73 specificity and 77% diagnostic accuracy), whilst that for identifying samples associated with a high infective risk was 106 $TCID_{50}$/mL (0.94 sensitivity, 0.96 specificity and 95% diagnostic accuracy).

Conclusion

The performance of this chemiluminescence immunoassay would not permit it to replace molecular testing for diagnosing SARS-CoV-2, but may enable rapid and efficient detection of subjects with high SARS-CoV-2 viral load, who are responsible for the largest proportion of infectious clusters.

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INTRODUCTION

Irrespective of the need for timely identification, isolation and/or treatment patients with active severe acute respiratory coronavirus 2 (SARS-CoV-2) infection, targeted population screening may represent a valuable resource for purposes of preventing or containing COVID-19 outbreaks [1,2]. Recent guidance provided by both the World Health Organization (WHO) [3] and the Task Force on COVID-19 (coronavirus

disease 2019) of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) [4] has endorsed the potential usage of antigen immunoassays, which are specifically designed to detect various SARS-CoV-2 antigens (especially those of the nucleocapsid protein) in biological materials, mostly nasopharyngeal samples and saliva.

The more clinically valuable and safe applications of SARS-CoV-2 antigen immunoassays encompass the screening of subjects with highly suggestive signs or symptoms of infection, those entering high risk workplaces or other crowded settings (e.g., long term care homes, healthcare facilities, schools, airports and stations, factories, offices and theatres, among others), as well as subjects with known exposure to SARS-CoV-2 infected individuals. In all these cases, a positive result of a SARS-CoV-2 antigen immunoassay is associated with a higher probability of active SARS-CoV-2 infection, whilst a negative test result cannot straightforwardly rule out the possibility of an ongoing infection characterized by low nasopharyngeal and/or saliva viral load [3-5]. This aspect has hence persuaded both the WHO and the IFCC Task Force on COVID-19 to emphasize the importance of the diagnostic performance characteristics in assay selection, thus recommending the use of SARS-CoV-2 antigen immunoassays with high specificity (i.e., preferably ≥0.97) and acceptable sensitivity (i.e., advisably ≥0.80).

Besides the vast array of the so-called antigen rapid detection tests (Ag-RDTs) currently available on the diagnostic market, as recently reviewed by the Cochrane COVID-19 Diagnostic Test Accuracy Group [6], some immunoassays fully suited for central laboratory automation are becoming increasingly available by the in vitro diagnostic industry. There are many potential advantages to these tests compared to Ag-RDTs, thus including higher analytical sensitivity, generation of quantitative results, larger

throughput and possibility of full interface with the laboratory information system (LIS), thus enabling to permanently store test results for longitudinal patient monitoring and/or for guiding clinical decision making, since higher viral loads have been convincingly associated with enhanced risk of clinical deterioration and/or development of severe/critical illness in patient with COVID-19 [7]. Therefore, this study was aimed to assess the clinical performance of the novel DiaSorin LIAISON SARS-CoV-2 Ag chemiluminescence immunoassay.

MATERIALS AND METHODS

Study population

The study population included a series of patients referred to the Laboratory Medicine service of the Pederzoli Hospital (Peschiera del Garda, Verona, Italy) between March 29 and April 18, 2021, for diagnosis or screening of SARS-CoV-2 infection. An upper respiratory specimen (Virus swab UTM™, Copan, Brescia, Italy) was collected upon hospital admission from each patient by a skilled healthcare operator and was then used for both SARS-CoV-2 antigen and molecular testing. All specimens were analyzed within 1 hour from collection.

DiaSorin LIAISON SARS-CoV-2 Ag

The novel DiaSorin LIAISON SARS-CoV-2 Ag (DiaSorin, Saluggia, Italy) is a fully-automated chemiluminescence immunoassay developed for quantitative assessment of SARS-CoV-2 nucleocapsid (N) antigen protein in nasal or nasopharyngeal swabs eluted in universal transport medium (UTM) or viral transport media (VTM). According to the manufacturer, the target usage of the assay should be when reference molecular tests are unavailable, when the long turnaround time of molecular testing may preclude timely patient management, as well as for permitting to analyze large volumes of specimens

during outbreaks and/or for screening asymptomatic people with the purpose of identifying potential SARS-CoV-2 (super)spreaders.

Briefly, anti-SARS-CoV-2 nucleocapsid rabbit polyclonal antibodies are coated onto magnetic particles linked with an isoluminol derivative. During a first incubation step, the nucleocapsid antigen eventually present in the test sample binds to the conjugate. After a subsequent incubation, the solid phase reacts with viral antigens bound to the conjugate, and the unbound material is then removed by washing. The starter reagents are then added, triggering a flash chemiluminescence reaction, whose light signal is proportional to the concentration of SARS-CoV-2 nucleocapsid antigen present in the test sample.

According to the manufacturer's declaration, the time to first test result performed on the fully automated chemiluminescence analyzer LIAISON® XL (DiaSorin, Saluggia, Italy) is 42 min, the throughput is 136 tests/hour, the analytical sensitivity is 22 $TCID_{50}$/mL, the upper limit of quantitation is 100,000 TCID50/mL, whilst the total imprecision ranges between 2.9% to 18.4%. The values of the local total imprecision, calculated on daily performance of quality controls, were almost overlapping.

Altona Diagnostics SARS-CoV-2 molecular testing

Altona Diagnostics RealStar® SARS-CoV-2 RT-PCR Kit (Altona Diagnostics GmbH, Hamburg, Germany) is a real-time reverse transcription polymerase chain reaction (rRT-PCR) assay for detecting SARS-CoV-2 RNA in a vast array of clinical specimens, including nasopharyngeal swabs. This technique entails two separate amplification and detection steps of SARS-CoV-2 genes, the first targeting the *E* gene sequence and the second the *S* gene. The test also uses a set of probes and primers for amplifying an

internal control, aimed at sorting out possible rRT-PCR inhibition. The assay was perfumed using a Bio-Rad CFX96™ Deep Well Dx Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA). Test results were reported both as quantitative and qualitative measures. In the former case, results were provided as cycle threshold (Ct) value of both E and S genes, whilst qualitative data were reported as positive/negative at test cut-off (i.e., Ct <45 or ≥45) or at the infectivity threshold defined Gniazdowski et al. (i.e., Ct <29.5 or ≥29.5) [8]. The assay has a total run time of around 2-3 hours.

Statistical analysis

The diagnostic efficiency of DiaSorin LIAISON SARS-CoV-2 Ag was assessed by comparing test results with those obtained with molecular testing, using Spearman's correlation, by constructing receiver operating characteristic (ROC) curves, and calculating the diagnostic accuracy, sensitivity and specificity at the two diagnostic thresholds of rRT-PCR positivity (i.e., Ct value <45) and association with potential infectivity (i.e., Ct value <29.5).

Statistical analysis was performed with Analyse-it software (Analyse-it Software Ltd, Leeds, UK). Quantitative data were reported as median mean and interquartile range (IQR).

The investigation was performed as part of routine clinical laboratory operations, using pre-existing specimens collected for systematic SARS-CoV-2 diagnostic screening and testing at the local facility, and thereby patient informed consent and Ethical Committee approval were unnecessary.

All test results were anonymized prior to statistical analysis. The study was conducted in accordance with the Declaration of Helsinki, under the terms of relevant local legislation.

RESULTS

The final study population consisted of 421 patients (median age, 48 years and IQR, 31-59 years; 227 women, 53.9%), 301 (71.5%) with positive result of rRT-PCR (i.e., Ct values of both E and S genes <45), and 126 (29.9%) with Ct values of both E and S genes <29.5, thus reflecting potential association with higher infectivity according to Gniazdowski et al. [8]. In the 301 rRT-PCR positive samples, the Spearman's correlation between antigen concentration and Ct values of the E and S gene was r = -0.85 (95% CI, -0.88 to -0.82; p<0.001) and r = -0.84 (95% CI, -0.87 and -0.81; p<0.001), respectively.

The distribution of DiaSorin LIAISON SARS-CoV-2 Ag values in nasopharyngeal samples with Ct values above or below the diagnostic thresholds of rRT-PCR positivity or association with higher infectivity is shown in figure 1. The median values in rRT-PCR positive samples was 94.8 (IQR, 82.9-1466.9) $TCID_{50}$/mL compared to 78.2 (IQR, 75.2-84.2) $TCID_{50}$/mL in those testing negative (i.e., Ct values >45; p<0.001), whilst that in samples associated with high infectivity risk was 3819.1 (IQR, 198.6-28061.3) $TCID_{50}$/mL compared to 82.0 (IQR, 76.7-88.4) $TCID_{50}$/mL in those with lower infectivity risk (i.e., Ct values >29.5; p<0.001).

The diagnostic performance of the DiaSorin LIAISON SARS-CoV-2 Ag immunoassay versus molecular testing is shown in figure 2. Briefly, the area under the ROC curve (AUC) for rRT-PCR positivity and association with potential infectivity was 0.82 (95% CI, 0.79-0.86; p<0.001) and 0.98 (95% CI, 0.97 to 0.99; p<0.001), respectively. The optimal cut-off for identifying rRT-PCR positivity was 82 $TCID_{50}$/mL, which was associated with 0.78 sensitivity (95% CI, 0.73 to 0.83), 0.73 specificity (95% CI; 0.64 to 0.80) and 77% diagnostic accuracy (95% CI, 73 to 81%). For comparison, the 200 $TCID_{50}$/mL cut-off suggested by the manufacturer was associated

with 0.31 sensitivity (95% CI, 0.25 to 0.36), 1.00 specificity (95% CI; 0.97 to 1.00) and 50% diagnostic accuracy (95% CI, 45 to 55%), whilst the 100 $TCID_{50}$/mL cut-off suggested by Häuser et al [9] was associated with 0.45 sensitivity (95% CI, 0.39-0.51), 0.99 specificity (95% CI, 0.95-1.00) and 61% accuracy (95% CI, 56 to 65%). The optimal cut-off for identifying samples with higher risk of infectivity, which may hence characterize the so-called super-spreaders, was 106 $TCID_{50}$/mL, which was associated with 0.94 sensitivity (95% CI, 0.88 to 0.98), 0.96 specificity (95% CI; 0.93 to 0.98) and 95% diagnostic accuracy (95% CI, 93 to 97%).

DISCUSSION

The impact of SARS-CoV-2 diagnostics on laboratory medicine, requiring the expedient implementation of rapid and accurate diagnostic techniques for decision making regarding patient diagnosis, isolation, and/or treatment, has been so dramatic that it has been defined more or less as an "Armageddon" in the pages of this journal [10]. In fact, it is unquestionable

Figure 1	Test results (median and interquartile range) of DiaSorin LIAISON SARS-CoV-2 Ag chemiluminescence immunoassay in nasopharyngeal samples positive at molecular testing (i.e., cycle threshold values of both SARS-CoV-2 *S* and *E* genes <45), and in nasopharyngeal specimens associated with higher infectious risk (i.e., cycle threshold values of both SARS-CoV-2 *S* and *E* genes <29.5).
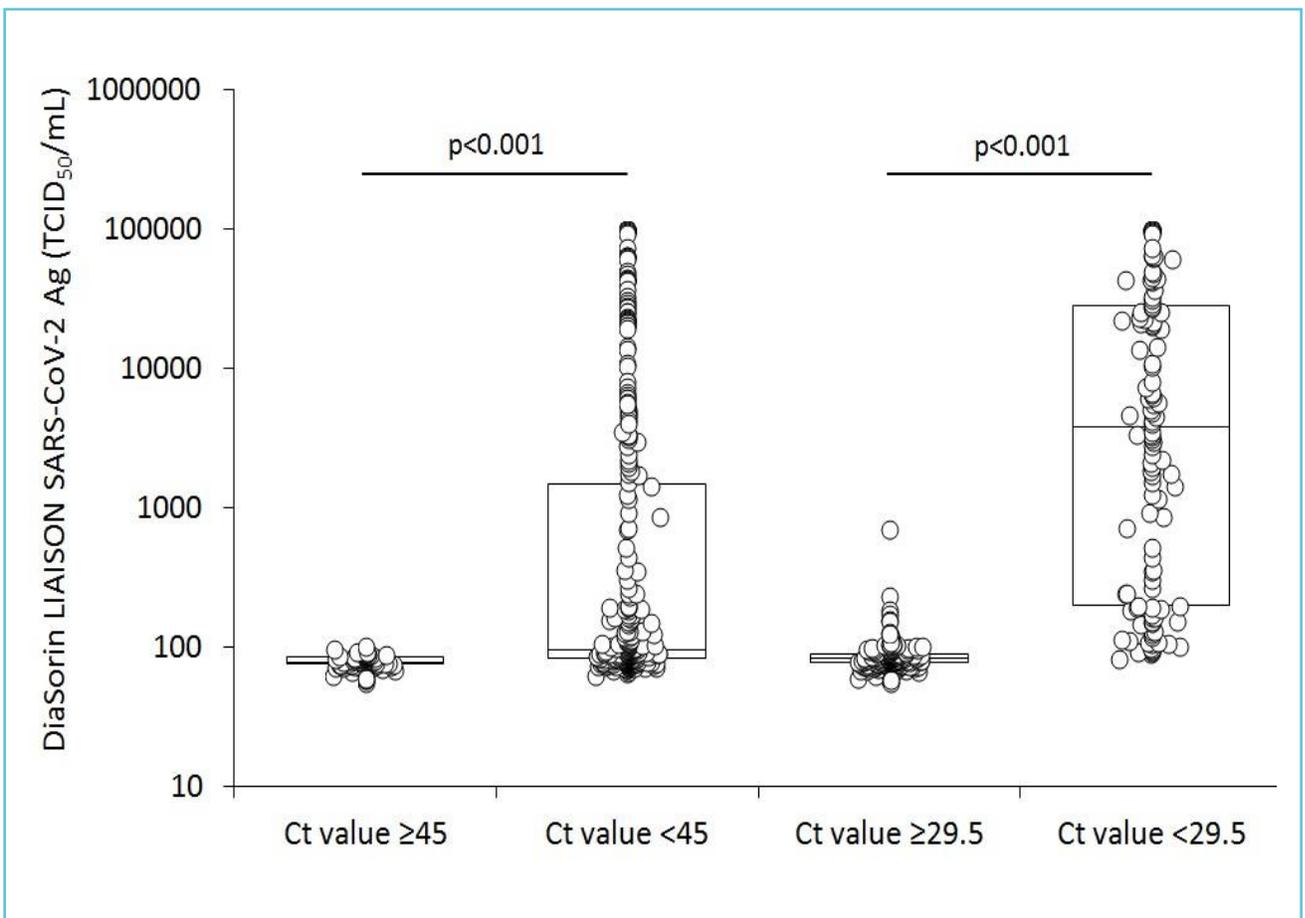
Figure 2   Receiver operating characteristic (ROC) curve of DiaSorin LIAISON SARS-CoV-2 Ag chemiluminescence immunoassay for:
(a) identifying positive nasopharyngeal samples at molecular testing (i.e., cycle threshold values of both SARS-CoV-2 S and E genes <45); or (b) discriminating nasopharyngeal specimens associated with higher infectious risk (i.e., cycle threshold values of both SARS-CoV-2 S and E genes <29.5).
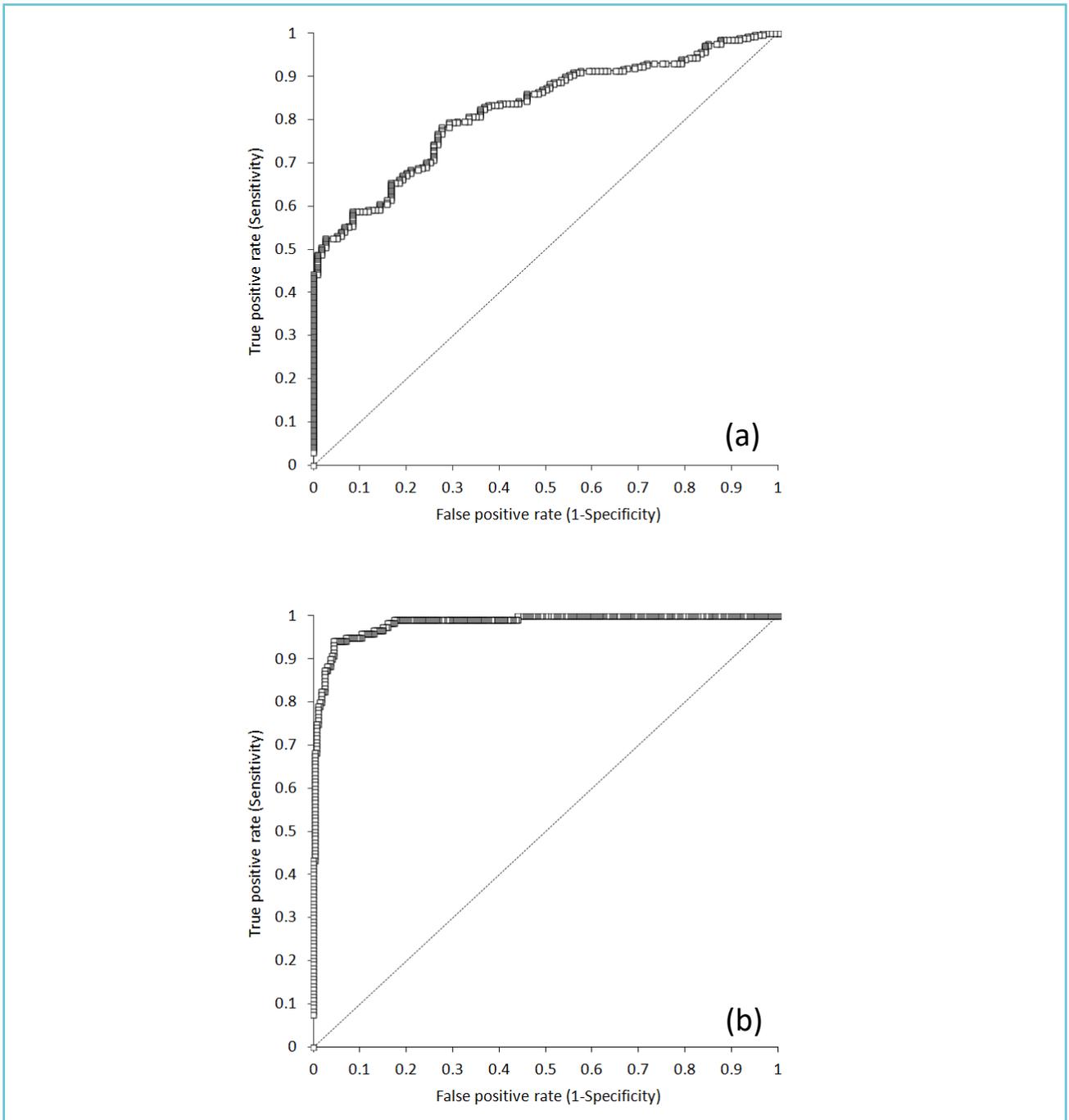
that many diagnostic facilities have virtually collapsed, and many others are still struggling with ongoing shortages of human and analytical resources, as recently underpinned by the results of a global survey promoted by the American Association of Clinical Chemistry [11]. In this rapidly evolving situation, the availability of high throughput and accurate techniques for screening and/or diagnosing SARS-CoV-2 infections, especially for identifying the so-called super-spreaders, a limited number of persons but among which nearly 90% of the viral particles circulating in the community are carried [12], should be regarded as a top priority. The use of rapid and high throughput SARS-CoV-2 antigen immunoassays has been proposed as a potential solution to this urgent matter, provided that their clinical performance are validated in real-life scenarios.

The results of our clinical assessment of the novel DiaSorin LIAISON SARS-CoV-2 Ag chemiluminescence immunoassay suggest that this technique has excellent performance for detecting nasopharyngeal samples with high viral load (i.e., Ct values <29.5), though its cumulative sensitivity appears considerably lower when all specimens are considered. These results are well-aligned with those recently presented by Häuser and colleagues [9]. Briefly, these authors also found that when applying the manufacturer-recommended cut-off of 200 $TCID_{50}$/mL, the specificity was 1.00, as in our cohort, however, the diagnostic sensitivity was only 0.40, which is very similar to the 0.31 sensitivity that we found in our study. When the cut-off was lowered to 100 $TCID_{50}$/mL, Häuser et al. found the sensitivity increased to 0.50 while only marginally decreasing the specificity to 0.98. Interestingly, these values are very similar to those found in our study using that same diagnostic threshold (i.e., 0.45 sensitivity and 0.99 specificity, respectively). Nonetheless, in our cohort, the optimal cut-off for identifying

sample positivity was even lower, 82 $TCID_{50}$/mL, which yielded 0.78 sensitivity, 0.73 specificity and 77% diagnostic accuracy.

In a separate investigation, Lefever et al. also evaluated the diagnostic performance of DiaSorin LIAISON SARS-CoV-2 Ag immunoassay versus a reference NAAT [13], reporting 0.68 sensitivity and 1.00 specificity at the 200 $TCID_{50}$/mL manufacturer's cut-off. Regardless of which cut-off is used, both our data and previously reported data [9,13] would hence suggest that the diagnostic accuracy of this chemiluminescence immunoassay appears still insufficient to completely replace NAAT as the reference technique for diagnosing SARS-CoV-2 infection, since neither in our cohort or in previous studies, the minimum required sensitivity recommended by the WHO and the IFCC Task Force on COVID-19 (i.e., ≥0.80) could be reached.

That said, the diagnostic performance of DiaSorin LIAISON SARS-CoV-2 Ag for identifying samples associated with higher infectivity (i.e., with Ct values <29.5) was excellent, exhibiting an AUC of 0.98, and displaying 0.94 sensitivity, 0.96 specificity and 95% diagnostic accuracy at the 106 $TCID_{50}$/mL cut-off. These values are aligned to, or even better than the minimum performance requirements currently recommended by both the WHO and the IFCC Task Force on COVID-19 [3,4].

Importantly, the data published by Lefever and colleagues are consistent with our findings, since they also found 0.83 sensitivity in nasopharyngeal specimens with a high and potentially infective SARS-CoV-2 viral load (i.e., Ct values of *N* gene <30) [13]. This would imply that this fully-automated chemiluminescence antigen immunoassay could be especially suited, and thereby reliably used, for rapidly and efficiently detecting subjects bearing high SARS-CoV-2 viral load (i.e., the so-called super-spreaders), who are responsible for the vast majority of infectious clusters [14,15].

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REFERENCES

1. Lippi G, Horvath AR, Adeli K. Editorial and Executive Summary: IFCC Interim Guidelines on Clinical Laboratory testing during the COVID-19 Pandemic. Clin Chem Lab Med 2020;58:1965-9.

2. Lippi G, Henry BM, Sanchis-Gomar F. Potential drawbacks of frequent asymptomatic coronavirus disease 2019 (COVID-19) testing. Infect Control Hosp Epidemiol. 2020 Oct 29:1-2. doi: 10.1017/ice.2020.1305. Epub ahead of print.

3. World Health Organization. Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays. September 11, 2020.

4. Bohn MK, Lippi G, Horvath AR, Erasmus R, Grimmler M, Gramegna M, et al. IFCC interim guidelines on rapid point-of-care antigen testing for SARS-CoV-2 detection in asymptomatic and symptomatic individuals. Clin Chem Lab Med. 2021 Apr 27. doi: 10.1515/cclm-2021-0455. Epub ahead of print.

5. Mattiuzzi C, Henry BM, Lippi G. Making sense of rapid antigen testing in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) diagnostics. Diagnosis (Berl). 2020 Nov 26:dx-2020-0131. doi: 10.1515/dx-2020-0131. Epub ahead of print.

6. Dinnes J, Deeks JJ, Berhane S, Taylor M, Adriano A, Davenport C, et al. Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection. Cochrane Database Syst Rev. 2021 Mar 24;3:CD013705.

7. Pujadas E, Chaudhry F, McBride R, Richter F, Zhao S, Wajnberg A, et al. SARS-CoV-2 viral load predicts COVID-19 mortality. Lancet Respir Med 2020;8:e70.

8. Gniazdowski V, Morris CP, Wohl S, Mehoke T, Ramakrishnan S, Thielen P, et al. Repeat COVID-19 Molecular Testing: Correlation of SARS-CoV-2 Culture with Molecular Assays and Cycle Thresholds. Clin Infect Dis. 2020 Oct 27:ciaa1616. doi: 10.1093/cid/ciaa1616. Epub ahead of print.

9. Häuser F, Sprinzl MF, Dreis KJ, Renzaho A, Youhanen S, Kremer WM, et al. Evaluation of a laboratory-based high-throughput SARS-CoV-2 antigen assay for non-COVID-19 patient screening at hospital admission. Med Microbiol Immunol. 2021 Apr 15:1–7. doi: 10.1007/s00430-021-00706-5. Epub ahead of print.

10. Gruson D, Ko G, Luu D. COVID-19: Armageddon before Light? EJIFCC 2020;31:103-5.

11. American Association of Clinical Chemistry. Coronavirus Testing Survey. Available at: https://www.aacc.org/science-and-research/covid-19-resources/aacc-covid-19-testing-survey. Last accessed, May 3, 2021.

12. Yang Q, Saldi TK, Lasda E, Decker CJ, Paige CL, Muhlrad D, et al. Just 2% of SARS-CoV-2-positive individuals carry 90% of the virus circulating in communities. medRxiv [Preprint]. 2021 Mar 5:2021.03.01.21252250. doi: 10.1101/2021.03.01.21252250.

13. Lefever S, Indevuyst C, Cuypers L, Dewaele K, Yin N, Cotton F, et al. Comparison of the quantitative DiaSorin Liaison antigen test to RT-PCR for the diagnosis of COVID-19 in symptomatic and asymptomatic outpatients. J Clin Microbiol. 2021 Apr 13:JCM.00374-21. doi: 10.1128/JCM.00374-21. Epub ahead of print.

14. Walsh KA, Jordan K, Clyne B, Rohde D, Drummond L, Byrne P, et al. SARS-CoV-2 detection, viral load and infectivity over the course of an infection. J Infect 2020;81:357-71.

15. Goyal A, Reeves DB, Cardozo-Ojeda EF, Schiffer JT, Mayer BT. Viral load and contact heterogeneity predict SARS-CoV-2 transmission and super-spreading events. Elife 2021;10:e63537.