Comparative assessment of Maglumi SARS-CoV-2 antigen test and nucleic acid amplification test

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ABSTRACT

Background: Nucleic Acid Amplification Test (NAAT) can detect the SARS-CoV-2 at low viral load, but the risk of isolating unnecessarily people who are no longer infectious may preclude its use as screening test. Immunoassay can be an alternative screening tool to detect viral antigens, thanks to their high throughput, feasibility and practicability. In this study, we compare the results obtained by a chemiluminescent immunoassay against the molecular test.
Methods: 105 positive NAAT nasopharyngeal swabs (NPS) with threshold cycle (Ct) between 39 and 13, and 15 negative samples were analyzed with two different methods based on chemiluminescent technology (CLIA) targeted to nucleocapsid SARS-Cov-2 antigen: Maglumi (SNIBE diagnostic Shenzen, China), and Liaison XL (DiaSorin s.p.a., Saluggia, Italy).
Results: all 15 negative NPS (Ct >40) were confirmed as negative with both CLIA assays; 31 positive NPS out of 105 positive (Ct ≤40) were confirmed positive on Maglumi and Liaison (29.5%). The agreement between the two immunoassays was 100%.
Conclusions: as expected, CLIA tests demonstrated to be less sensitive to the viral presence than NAAT. In particular, Maglumi and Liaison can detect a positivity when the virus is detected at a Ct <25. The range between 24 and 25 Ct was a grey area, where NAAT and CLIA were not in perfect agreement. It seems that antigen tests reach satisfactory sensitivities when infected people are more likely to be contagious; this characteristic makes them a useful tool for screening the population, especially during the pandemic

Parole chiave: *SARS-Cov-2, molecular test, CLIA antigen test*

INTRODUCTION

The gold standard for the virus presence for the diagnosis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), named COVID-19, is the molecular test because it is the most sensitive method. The samples utilized to diagnose the virus cover a vast array of clinical specimens (i.e., nasopharyngeal and/or nasal swabs, nasal wash/aspirates) and of different specific transport media [Viral Transport Medium (VTM), Universal Transport Medium (UTM)] (1). It has been widely recognized that molecular tests require specialized equipment and training, long processing time, limiting contact tracing and rapid epidemiological decisions for public health (2).

During the pandemic, therefore, the search for faster and more rapid diagnostic tests for SARS-CoV-2 has become urgent to perform rapidly large volumes of molecular tests, which has far exceeded the capacity of clinical laboratories in our Country and worldwide (3). Even today, the capacity to support huge amounts of diagnostic testing for SARS-CoV-2 represents a major health challenge for the prevention and containment of COVID-19. The American Association for Clinical Chemistry (AACC), in order to define the state of the art for COVID-19, conducted a still ongoing survey to define the rationale for planning and validating alternative strategies to help overcome the long testing time and scarcity of molecular tests (4).

As soon as new immunoassays targeting viral antigen became commercially available, laboratories added them to molecular test because they better met the need

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to provide rapid and accurate results. Results accuracy is defined considering several factors, such as the appropriateness of the sample collection technique, the sample suitability for the specific assay technique, and the time since exposure (4). Indeed, testing strategies using rapid antigen testing to detect ongoing infection have the potential to accelerate the detection of infection and provide clinical information for public health management decisions to reduce virus transmission (5). Currently, the proposed tests available for the detection of SARS-CoV-2 are those based on nucleic acids, and those based on antigens or antibodies as well (6).

The World Health Organization (WHO) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) have recently published guidelines and recommendations about the possibility of using antigen tests for population screening and epidemiological purposes (7).

A good diagnostic test must satisfy specific analytical requirements, such as an adequate lower limit of detection, which is the ability to detect patients with low viral load, in the case of qualitative diagnostic tests. However, the main limitation of immunoassays is their lower sensitivity compared with molecular COVID-19 tests (8).

In addition, nasal swab used in antigenic tests are less invasive, and thus much more tolerable by patients, than the nasopharyngeal swab used for molecular testing. For this reason, the nasal swab allows for easier and more frequent collections, especially for the management of hospitalized patients who need to be screened a few days apart (9).

In this study, the performance of a new Maglumi chemiluminescent assay for the detection of SARS-CoV-2 in nasopharyngeal swabs, has been evaluated in comparison to the nucleic acid amplification assay and to the chemiluminescent assay performed in full automation on Liaison XL, already in use in our laboratory. In addition, the consistency of results between molecular and antigenic tests has been investigated.

METHODS

Study design

A retrospective, observational study was performed in the Ospedale Civile, Laboratory Medicine of Modena, Italy, between March 1st and May 1st, 2022.

105 nasopharingeal swabs with a threshold cycle (Ct) between 39 and 13, were obtained from consecutive patients tested for COVID-19 for diagnostic purpose as they were symptomatic or had been in close contact with SARS-CoV-2 positive subjects; 15 samples with a Ct value of >40 were selected as negative samples.

Molecular tests on nasopharyngeal swabs were performed on the same day they were delivered to the laboratory; the semples were frozen at -80°C immediately after the molecular test. Once stratified by result, samples were analyzed for SARS-CoV-2 antigen blindly on the two different chemiluminescence instruments on the same day.

Assay Methods

RT qPCR: the molecular test for SARS-CoV-2 RNA detection was a real-time reverse transcription-polymerase chain reaction (rT-PCR) technique involving concurrent double amplification of the SARS-CoV-2 N and RdRp genes (Alinity m SARS-CoV-2 AMP Kit, by Abbott Molecular, USA). A NAAT result was positive when one or both target genes were amplified. The assay, including automated nucleic acid extraction and amplification and target sequence detection was performed on Alinity M. The turnaround time for 12 molecular samples is about 2 hours and 15 minutes. The limit of detection (LOD) observed for positive samples was 100 virus copies/mL. The total precision from the producer was 2.4 %.

Maglumi X8: according to the manufacturer's statement, Maglumi X8 (SNIBE diagnostic Shenzen, China) is a fully automated chemiluminescent analyzer with high processivity. The time to the first result is 15 minutes, productivity is a maximum of 600 tests/hour. The analytical sensitivity for antigen COVID-19 test is 4 pg/mL, and the upper limit of quantification is 10 000pg/mL. The total precision from the producer was 2.19%. The number of sample positions was 300.

Liaison XL: according to the manufacturer's statement, the time to obtain the first test result performed on the fully automated chemiluminescence analyzer LIAISON® XL (DiaSorin s.p.a., Saluggia, Italy) is 42 minutes, the productivity is 136 tests/hour, the analytical sensitivity was 22 TCID50/mL, the upper limit of quantification is 100 000 TCID50/mL. The total precision from the producer was 15.2%. The number of sample positions is 144.

The swabs were analyzed directly on Maglumi X8 and on Liaison XL platforms without the need of pretreatment and according to the method described by the manufacturer. Briefly, both methods are based on direct immunoassay sandwich reaction. Polyclonal rabbit anti-SARS-CoV-2 antibodies coated on magnetic particles bound with an isoluminol derivative. During the initial incubation step, any nucleocapsid antigen present in the test sample binds to the conjugate. After a subsequent incubation, the solid phase reacts with the conjugate-bound viral antigens, and the unbound material is removed by washing. Starting 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.

Ethics

The institutional authority of laboratory approved the study as it had the following features:

- it was retrospective with a low evaluation complexity
- the additional test, likely the test initially required for the patient, was targeted to the same clinical question (positivity/negativity to SARS-CoV2 virus)
- it was not feasible to reach every single patient to ask for informed consent

RESULTS

Comparison between molecular and antigen test

Overall, 31 samples were positive at molecular test rT-PCR and with the two immunoassays; both Maglumi and Liaison confirmed as negative the 15 NAAT negative swabs with Ct ≥40. The remaining 74 swabs positive with the gold standard resulted negative with the immunoassays.

Concerning the positive swabs, 31 swabs with Ct between 13 and 25 (29.5%) were confirmed positive by both immunoasays, while the remaining 74 positive swabs with Ct between 24 and 39 (66.3%) resulted negative on Maglumi and Liaison analyzers.

All samples with Ct >25 were labeled negative by both methods, while all samples with Ct <24 were detected positive by CLIA assays.

The gray area of immunoassays corresponded to a Ct range between 24 and 25; indeed the same 7 and 4 samples of a total of 11 samples with Ct in the range 24 - 25 were recognized positive and negative respectively by both immunoassays.

Based on these results, the certain sensitivity limit of the immunoassays corresponds to a NAAT Ct <25, while the sensitivity of the molecular assay is Ct ≤40 (Table 1).

Comparison between Maglumi X8 and Liaison XL

The agreement of results for positive and negative samples obtained with the two CLIA analyzers Maglumi and Liaison was 100%. They both recognized positivity when Ct was <25. Regarding the full automated analyzers productivity, we estimate the time to obtain results for 1 000 samples considering the available number of sample positions, the time to the first result, and the time to the following results. As shown in Table 2, this study highlights that the time for processing 1 000 swabs of Maglumi was on average 30% lower to that of Liaison.

DISCUSSION

It is well known that rT-PCR positivity can persist for several weeks after the onset of the disease and the disappearance of symptoms, a phase when the viral load may be very low and infectivity no longer persistent (10).

The long duration of the tail of the positive molecular test suggests that the majority of infected subjects were identified positive to SARS-CoV-2 after the infectious period has overcome. This also means that people can be unnecessarily quarantined many for days because of a positive rT-PCR test, despite having already overcome the transmissible phase of the infection (11).

The molecular test can give clinical questionable positive analytical results because of its extreme sensitivity, being able to amplify even fragments of the viral nucleic acid that persist in the nasopharyngeal tract (12). In addition, false positives may occur due to contamination during manual processing, but could be also due to the large number of samples processed by automated instruments (13). The high sensitivity of the molecular test is an advantage and a disadvantage at the same time because it can cause unnecessary

Table 1
The percentage of agreement between rT-PCR results obtained by Alinity M and the two CLIA assays, Maglumi X8 (Snibe) and Liaison XL (Diasorin). Viral load levels are defined as a function of the threshold cycle

Ct and viral load for COVID-19				
Ct	N° of samples	Viral load	Maglumi X8 percentage of agreement versus rT-PCR	LiaisonXL percentage of agreement versus T-PCR
	24	High viral load	100% (24/24)	100% (24/24)
24-25	11	High viral load	63.6% (7/11)	63.6% (7/11)
26-30	35	Medium viral load	0/35	0/35
31-40	35	Low viral load	0/35	0/35
>40	15	Negative	100% (15/15)	100% (15/15)

Ct, threshold cycle.

Table 2
Comparison of sample load capacity and processing velocity of Maglumi X8 Snibe and Liaison XL DiaSorin, inferred by our experimental data

Clia productivity parameters		
Parameters	Maglumi x8	Liaison xl
Number of sample positions	300	144
Time to the first result	25 minutes	40 minutes
Time the following result	Every 18^{sec} after first result	Every 22^{sec} after first result
Time to process 1000 samples*	5 hours	7 hours

** time necessary to process 1000 samples rounded up to the hour*

isolation of subjects, even when they were no longer infectious (14), where it may be helpful when patients are immunodepressed or they need to be admitted in critical settings as oncology, or hematology areas. Several studies have shown that higher Ct values of SARS-CoV-2 correspond to non-replicative or non-infectious viral RNA as determined by viral culture (15). Our results confirmed that the antigen assay positivity to the virus may coincide with the maximum infectivity period, which lasts 2 weeks after the onset of symptoms. Therefore, today the diagnosis of SARS-CoV-2 infection can be made using antigen testing according to WHO guidelines (4,16).

Very few reports have been published on Maglumi and Liaison performances in comparison to molecular tests. Hauser et al. reported for Liaison a specificity of 100% and a sensitivity of 91.2% when the Ct was <25, with an overall sensitivity of 40.2% taking into consideration higher positive Ct values (17).

In another study, Lefever et al. found that the sensitivity of the Liaison antigen test in symptomatic and asymptomatic outpatients with a high viral load, was 100% without false positive results. The sensitivity in the asymptomatic patients was lower because of the lower viral load of this group (18).

Wang et al. report the sensitivity of Maglumi was 91.46% for samples with Ct values between 30 and 40 (19).

In the present study, in order to exclude the possibility of false positive NAAT, the swabs with Ct >37 were tested twice and all the repetitions were in agreement with the first result. These data demonstrate that Maglumi and Liason methods, both targeted to the nucleocapsid protein antigen are in total agreement. They were less sensitive to viral presence than the molecular test during the first and last stages of infection, when the number of viral copies is very low and consequently the expression of the antigen is low too; the sensitivity of the two CLIA tests is 100% when the Ct is <24. In this phase of the infection there is the greater state of disagreement between the two assays technologies, as shown in Figure 1, where, according to our findings, our hypothesis is represented graphically: the results of rT-PCR and antigenic CLIA assay to detect SARS-CoV-2 infections may vary between them in strict dependence on the onset of symptoms. We approximated the time intervals of detection of SARS-CoV-2 infection, which is presented qualitatively: molecular test detects the viral load starting from shortly before the symptoms onset to the following 4 weeks. Instead, the antigen tests can detect positivity in a narrower time window lasting roughly 2 weeks (20).

In a systematic review in 2022, Lippi et al. suggested that due to the higher accuracy of SARS-CoV-2 antigen testing in the presence of high viral loads, antigenic tests could be used to identify the so-called “superviral carriers,” responsible for most cases of disease transmission, because of the higher probability of dissemination of viral particles. The high number and heterogeneity of interpersonal contacts, together with the high viral load in the upper and/or lower respiratory tract, are currently considered to be the main determinants of superdiffusion events, in such a way that it has been estimated that accurate and timely identification of these supercarriers would prevent up to 90% of all secondary SARS-CoV-2 infections (2).

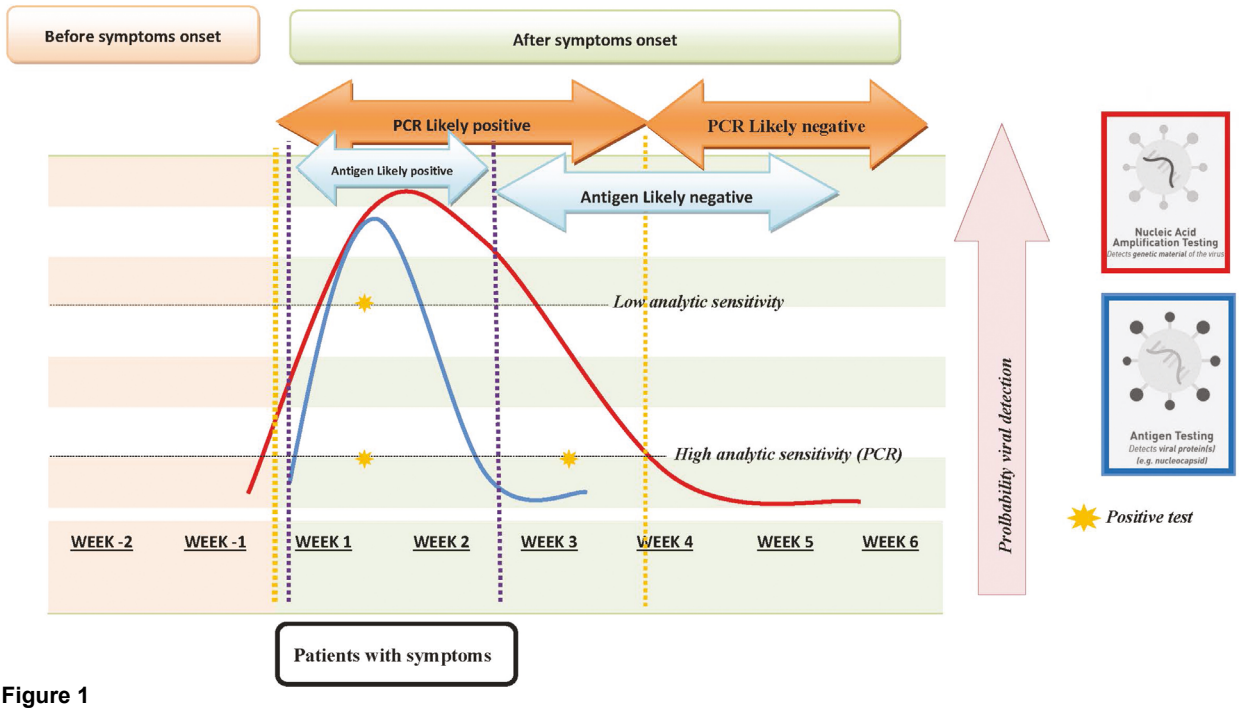

Figure 1

Trend in estimated change over time in the sensitivity of diagnostic tests: rt-PCR (red line) and antigenic test (blue line) for detecting SARS-CoV-2 infection, compared to the onset of symptoms. Both tests detect infection (yellow circles): the low-sensitivity immunassays detects positivity only in patients with symptoms, while the polymerase chain reaction (PCR) identifies positivity in a larger window, including patients with symptoms and asymptomatics, despite having already passed the transmissible phase of infection.

Therefore, detection of SARS-CoV-2 Ag CLIA represents an effective and alternative approach for rapid diagnosis and an extremely useful tool for population screening, especially during a pandemic (21).

The unstoppable emergence and spread of new variants of SARS-CoV-2 characterized by different virulence and infectivity are creating major problems for all clinical laboratories around the world which are increasingly struggling to support public prevention measures based on rapid tests and accurate trait tracing that enable processing of high volumes of samples (e.g. during rallies, in public transport, at schools, and so on).

The limit of our study was the relatively small number of samples tested due to the difficulty of providing reagents and diagnostic kits to carry out the experimental phase. Furthermore, the sensitivity of the tests to the virus could be dependent on the characteristics of the sample, such as collection, storage and transport conditions. The turnaround time of the molecular test has been reported by several studies to be greater than one hour (22), on the contrary the CLIA analyzers have a shorter processing time, and in particular Maglumi can process 1000 samples in 5 hours, so that it may be suitable for managing the laboratory emergency in the event of a pandemic.

CONCLUSIONS

According to the obtained results, the antigenic test is a useful screening tool especially on large populations because its short turnaround time and low cost in comparison to rT-PCR. Moreover, the lower sensitivity in comparison to the gold standard allows for identification of positive individuals during the period of the highest virulence and with the highest transmission capacity. Further investigations are obviously needed to confirm these preliminary results.

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CONFICT OF INTEREST

None.

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