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Performance evaluation of an automated SARS-CoV-2 Ag test for the diagnosis of COVID-19 infection on nasopharyngeal swabs

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Abstract

Objectives: The detection of SARS-CoV-2 in infected people is a key tool to help in controlling COVID-19 pandemic. Like rapid antigenic tests, automated antigen tests, that present the advantage of a higher throughput flow, may be of interest. The LIAISON® SARS-CoV-2 Ag test was evaluated for the quantification of SARS-CoV-2 nucleocapsid antigen in nasopharyngeal swabs by comparison to RT-PCR.

Methods: The study involved 378 nasopharyngeal samples (UTM® and FLOQSwab™, Copan Diagnostics), including 46 swabs positive for SARS-CoV-2 by RT-PCR. These samples came from asymptomatic (n=99, 26.2%) or symptomatic people (n=279, 73.8%), at different times from symptom onset. The samples were analyzed on LIAISON® XL.

Results: The overall specificity was 99.4% (CI95% [98.6–100]). The negative predictive value reached 100% in asymptomatic people. Among the 46 positive samples, the overall sensitivity was 84.8% (CI95% [74.4–95.2]), reached 91.9% (CI95% [83.1–100]) in the first fourth days after symptoms onset and was 100% for Cq values ≤25. Antigen was not detected in samples with Cq values >25. Similar results were observed on nasopharyngeal swabs coming from patients infected with the 20I/501Y.V1 variant or the 20H/501Y.V2 variant.

Conclusions: According to technical performances, the LIAISON® SARS-CoV-2 Ag test may be a useful tool for COVID-19 diagnosis, especially during the first four days of symptoms.

Keywords: antigen; automation; diagnosis; nasopharyngeal swab; SARS-CoV-2.

Introduction

In December 2019, a novel coronavirus (severe acute respiratory syndrome coronavirus 2 – SARS-CoV-2) emerged in Wuhan, China [1]. This new pathogen responsible for COVID-19 has rapidly spread across the globe and resulted in a pandemic, still hard to control one year after. In March 2021, the balance sheet is up to 135 million of infected people worldwide and 3 million of deaths [2]. The fight against the pandemic includes mass screening, with the aim to isolate infected people and break the chain of contagion.

The gold standard for virological COVID-19 confirmation is reverse-transcription PCR (RT-PCR) performed on nasopharyngeal swab or lower respiratory track in the case of severe symptoms. Diagnosis using RT-PCR is however relatively slow (3–4 h) and required skilled technicians. To optimize the mass screening, pooling strategy in combination with RT-PCR have been proposed [3], but with the risk to lead to a lower sensitivity.

To increase the screening capacity, antigen (Ag) tests have been proposed to join the panel of methods used for COVID-19 diagnosis. They consist of direct tests allowing the detection of viral proteins (mainly the capsid proteins). Among them, diagnostic tests based on immunochromatography methods are rapid (15–20 min) and can be performed outside biological laboratories. Nevertheless, as unitary tests, they are hardly usable for mass screening. Furthermore, the well described lack of sensitivity of unitary antigen tests may lead to false negative results, which may compromise the mass screening strategy.

The use of sensitive automatized antigen tests is thus a helpful alternative. Even if the analysis time is longer compared to immunochromatographic tests (i.e. 45 min), they require little staffs and allow serial analysis. Here, we evaluate a newly developed automated test (LIAISON®...
SARS-CoV-2 Ag) based on chemiluminescence enzyme immunoassay (CLEIA) used for the quantitative determination of SARS-CoV-2 antigen in nasopharyngeal swab. To evaluate the specificity and the sensitivity of the test, results from antigen level were compared to the gold standard quantitative RT-PCR.

**Materials and methods**

**Patients and samples**

The study included 378 nasopharyngeal swabs from the same number of patients, collected in the context of mass screening at the University Hospital of Nancy, France (DC-2020-4006 Covi-Lor collection). All samples were obtained using FLOQSwabs™ and viral transport media (2 mL) in UTM® (Copan Diagnostics, CA, USA).

RT-PCR was used as the gold standard to determine if individuals were infected or not. Therefore, the cohort consisted of 46 SARS-CoV-2 infected patients and 332 non-infected. Among them, 99 were asymptomatic (26.2%), 221 presented symptoms (i.e. fever, cough, headache, asthenia, anosmia or ageusia) for less than 4 days (58.5%), 30 between 5 and 7 days (7.9%), and 15 for more than 8 days (4.0%) (Table 1).

**SARS-CoV-2 genome detection**

Nucleic acid was extracted from nasopharyngeal swab using Virus DNA/RNA Extraction Kit (ref 100021043, MGI Tech Co. Ltd, Shenzhen, China) on MGISP-960 device, following the manufacturer recommendations (200 µL of sample extracted to 30 µL of elution buffer). A one step RT-qPCR was then performed according to the protocol developed by the Pasteur Institut looking for two targets, the IP2 and IP4 regions of the RdRp gene [4]. The human GAPDH gene was also targeted as an internal control associated to the presence of epithelial cells. Analyses were re-runed in case of discordant results (Cq values) between IP2 and IP4 targets.

**SARS-CoV-2 nucleocapsid antigen detection**

SARS-CoV-2 antigen detection was performed using the LIAISON® SARS-CoV-2 Ag assay. As recommended by the provider and after brief vortexing, 1 mL of the nasopharyngeal swab transport medium was collected less than 12 h after sampling, added to 1 mL-inactivation buffer tube provided by the manufacturer and stored at room temperature for 2 h. Samples were then stored at 4 °C (5 days maximum) or at −20 °C until the analysis. Inactivation buffer tubes were centrifuged (4000×g, 10 min) prior to their analysis on LIAISON® XL platform.

The LIAISON® SARS-CoV-2 Ag assay is a direct two-step sandwich chemiluminescence enzyme immunoassay (CLEIA). Specific rabbit polyclonal antibodies to SARS-CoV-2 nucleocapsid antigen are used for coating magnetic particles (solid phase) and linked to an isoluminol derivative (isoluminolantibody conjugate). During the first incubation, SARS-CoV-2 viral antigen present in samples binds to the conjugate. During the second incubation, the solid phase reacts with SARS-CoV-2 viral antigen already bound to the conjugate. After second incubation, the unbound material is removed with a wash cycle. Subsequently, the starter reagents are added and a flash chemiluminescence reaction is thus induced.

The assay data performed by the manufacturer mentioned a limit of detection of 22.0 TCID50/mL, a sensitivity of 98.6% (CI95% [92.5–99.7]) on samples positive for RT-PCR within 10-days onset symptoms, and a specificity of 99.5% (CI95% [97.4–99.9]). The intra and inter-laboratory precision were 2.7, 1.7, 2.0, 1.4, 1.2% for low, medium and high antigen levels respectively.

**Statistical analyses**

Statistical analyses were performed using Prism 5 (GraphPad Software, CA, USA) and Excel software (Microsoft Corp., WA, USA). Receiver operating characteristic (ROC) curve analyses were performed using Prism 5 (GraphPad Software, CA, USA), to determine the optimal cutoff antigen level allowing the distinction of SARS-CoV-2 infection status. The ROC curve was created by plotting the true positive rate (sensitivity) against the false positive rate (1 – specificity) at various threshold settings. The non-parametric Kruskal-Wallis test was used to compare SARS-CoV-2 Ag and genome levels. To estimate the genome levels, quantification cycle values obtain by RT-qPCR (Cq) were convert to PCR units, considering that a Cq value of 38 corresponds to 1 PCR unit (i.e. limit of detection) as follow: Cq value of 38 = 1 PCR unit, Cq value of 37 = 2 PCR units, Cq value of 36 = 4 PCR units, etc.

**Results**

**Sensitivity and specificity of the automated LIAISON® SARS-CoV-2 antigen test**

SARS-CoV-2 Ag detection was performed on 378 nasopharyngeal swabs, including 46 positive samples determined by
RT-qPCR (mean Cq value: 19.4 ± 4.8; median 18.8; IQR=4.4) and 332 negative samples. Analytical performance on site were evaluated on positive control and were in line with provider data (intra-assay variability: CV=9.90 vs. 10.1%)

Among the 46 positive samples, 39 were positive for SARS-CoV-2 Ag detection according to the manufacturer interpretation rules (i.e. cutoff of 200 TCID50/mL), meaning an overall sensitivity of 84.8% (CI95% [74.4–95.2]). Among the seven false negative samples, one is located in the 100–200 TCID50/mL gray zone defined by the manufacturer (161.9 TCID50/mL), while three are close (95.9, 94.1 and 88.8 TCID50/mL). Among the 332 negative samples, 330 were negative for SARS-CoV-2 antigen detection, meaning an overall specificity of 99.4% (CI95% [98.6–100]).

The positive predictive value reached 95.1% and the negative predictive value reached 97.9%. Taking into account the virus prevalence commonly observed at different epidemic time (i.e. 2% as a basal rate, 5% on average and 15% during the epidemic peak), the positive predictive value reaches 74.2, 88.1 and 96.1% respectively, while the negative predictive value remains higher than 97%.

The median level of SARS-CoV-2 Ag among the 46 RT-qPCR positive samples was 6,433.2 TCID50/mL (range <22 to >1,000,000 TCID50/mL) and that of the RT-qPCR negative samples was 26.7 TCID50/mL (range <22 to 341.5 TCID50/mL) (Figure 1A). The mean antigen level of the PCR-positive samples was significantly higher than that of the PCR-negative samples (p<0.001, Student’s t-test). A ROC curve analysis was performed to determine the optimal cutoff antigen level allowing the distinction of SARS-CoV-2 infection status. The accuracy of the test was the highest when a cutoff of 57 TCID50/mL was used, leading to a sensitivity of 95.8% (CI95% [93.0–97.7]) and a specificity of 95.2% (CI95% [85.2–99.5]) (Figure 1B).

**Antigen detection and time onset symptoms**

The samples were classified according to the time between sampling and day of the symptom’s onset (Table 1). Samples were collected the day before or the first day of symptoms (n=17; 4.5%), in the 2–4th days (n=204; 53.97%), in the 5–7th (n=30; 7.94%), in the 8–14th (n=12; 3.17%), and in the 15–28th days (n=3; 0.8%) after the symptoms. The information was missing for 13 samples, and 99 samples were collected from asymptomatic volunteers.

![Figure 1: Comparison of the results between SARS-CoV-2 antigen detection (Ag) and RT-qPCR results.](image-url)
When focused on the first four days (D-1 to D+4), the sensitivity of the SARS-CoV-2 Ag detection reached 91.9% (CI95% [83.1–100.0]), with a median level of 11,040.0 TCID50/mL for RT-qPCR positive samples (range 58.2 to >100,000 TCID50/mL) (Figure 1C). The accuracy of the test was the highest when a cutoff of 82 TCID50/mL was used, increasing the sensitivity to 98.4% (CI95% [95.3–99.7]) for a specificity of 97.3% (CI95% [85.8–99.9]) (Figure 1D). The sensitivity then decreased when regarding samples from D-1 to D+7 to reach 89.7% (CI95% [80.2–99.3]) and until 85.4% (CI95% [74.6–96.2]) when regarding samples from D-1 to D+14. Concerning asymptomatic patients, the negative predictive value of the SARS-CoV-2 Ag detection reached 100%, since no sample was found to be positive.

### Comparison of antigen test results and RT-qPCR

The sensitivity was evaluated according to the Cq value obtained by targeting the IP4 region by RT-qPCR. The sensitivity of the SARS-CoV-2 Ag detection was 100% when considering samples with a Cq<25 (n=39). The median level of SARS-CoV-2 Ag was 6,689.3 TCID50/mL (range 366.6 to >100,000 TCID50/mL).

Seven samples with Cq values higher than 25 were negative for SARS-CoV-2 Ag detection. For those, sampling was performed 2–4 days post infection (n=3), 5–7 days post infection (n=1) or after the 8th day (n=3) (Table 2).

No correlation was observed regarding the SARS-CoV-2 Ag level and the viral load estimated by PCR units (r2=0.068 when considering all positive samples, r2=0.405 when considering Cq values higher than 15, data not shown). However, for both parameters, levels were significantly higher for positive samples collected during the first 4 days after the symptom’s onset (Figure 2, Table 3). Hence, even in the absence of a strict correlation, the higher antigen levels are associated to the higher genome quantities (p<10⁻⁴, Table 2).

### Detection of SARS-CoV-2 variants

Cell culture supernatant of VERO-E6 cells infected with the original SARS-CoV-2 strain (isolated in March 2020 in France and confirmed by whole genome sequencing, n=2), the 20I/501Y.V1 "UK" variant, lineage B1.1.7 (n=1) [5], the 20H/501Y.V2 “South African” variant, lineage B1.351 (n=2) [6] and the 19B/501Y strain, lineage A.27 (n=1) were tested. The SARS-CoV-2 antigen was detected in all samples.

### Table 2: Antigen detection according to the RT-qPCR Cq values (SD = standard deviation).

<table>
<thead>
<tr>
<th>Cq value range</th>
<th>n</th>
<th>Positive</th>
<th>Negative</th>
<th>Sensitivity</th>
<th>Cumulative sensitivity</th>
<th>Mean level</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;18</td>
<td>21</td>
<td>21</td>
<td>0</td>
<td>100.0%</td>
<td>100.0%</td>
<td>48,744.56</td>
<td>37,264.97</td>
</tr>
<tr>
<td>18–22</td>
<td>17</td>
<td>17</td>
<td>0</td>
<td>100.0%</td>
<td>100.0%</td>
<td>11,229.01</td>
<td>20,015.70</td>
</tr>
<tr>
<td>22–26</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>33.3%</td>
<td>95.1%</td>
<td>358.95</td>
<td>406.29</td>
</tr>
<tr>
<td>26–30</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>0.0%</td>
<td>84.8%</td>
<td>59.31</td>
<td>35.43</td>
</tr>
</tbody>
</table>
Furthermore, nasopharyngeal swabs coming from patients infected with the 20I/501Y.V1 variant (n=14) or the 20H/501Y.V2 variant (n=4) were tested. Again, all samples with Cq value <25 (n=17/18) were positive for SARS-CoV-2 Ag detection.

**Discussion**

Since the emergence of the new coronavirus, SARS-CoV-2, testing infected people is the key point to help in breaking the pandemic. RT-PCR on nasopharyngeal sample is still the gold standard assay, considering sensitivity and specificity. However, other strategies have been proposed, based on the analysis of different sample type (e.g. saliva easier to sample, self-collected nasal swab [7]), or using other methods of detection such as the antigenic tests. Antigen tests are globally easier to handle, without requiring skilled laboratory technicians or specific devices. This is almost true for most of antigen tests, meaning unitary rapid direct test which have recently been discussed by the International Federation for Clinical Chemistry and Laboratory Medicine (IFCC). Advantages implementation include rapid turnaround-time, potential low cost equipment and preventative case identification [8]. First studies revealed however that rapid antigen test could be $10^2$–$10^3$ fold less sensitive than RT-PCR [9, 10] with sensitivity close to 58.1% when focusing on asymptomatic patients [8]. Automated antigen tests are also currently available. They have to be performed in a specialized laboratory, but are attractive by their rapidity (more than 100 samples/h), low cost and easy to use [11]. They deserve thus to be also evaluated, by comparison to RT-PCR, the gold standard method.

The evaluation of the LIAISON® SARS-CoV-2 Ag test was realized on 378 nasopharyngeal swabs collected from asymptomatic and symptomatic patients, at different time from the onset of symptoms. They were classified as positive for SARS-CoV-2 infection based on RT-PCR results [4].

For the method evaluated here, the overall specificity was evaluated to be of 99.4% (CI95% [98.6–100]) in symptomatic and asymptomatic patients. The two positive SARS-CoV-2 Ag subjects but negative regarding RT-PCR reported here had presented symptoms for 2–4 days before sampling. The nasopharyngeal samples have been retested using another molecular method (Xpress SARS-CoV-2, GeneXPeri®, Cepheid), that confirmed the first negative RT-PCR result. Such unspecific reactions are punctually observed and inherent in the immunological methodology used [12]. Other antigenic method, either rapid antigen tests or automated methods, have previously been evaluated. The specificity of automated antigen methods reached usually 96–100% according to the device [11, 13]. The specificity of rapid antigen tests is close to 98–99% [14, 15].

According to the provider information, the threshold to distinguish positive sample is settled to 200 TCID$_{50}$/mL, with a gray zone ranging from 100 to 200 TCID$_{50}$/mL. ROC analyses defined that the accuracy of the test was the highest when a cutoff of 57 TCID$_{50}$/mL was used when considering all samples, or a cutoff of 82 TCID$_{50}$/mL when considering early sampling after onset symptoms (≤4 days).

Recently, another automated method was evaluated for its sensitivity. In one study, the lumipulse presto SARS-CoV-2 Ag assay showed a sensitivity of 75.7% and a correlation of 0.74 between antigen concentration and RNA level [11]. In another one, the sensitivity evaluated on 58 samples coming from 11 infected patients reached 55.2% [13]. In this last work, a kinetic study demonstrated that antigen level becomes negative before day 8 after onset symptoms, as observed in our evaluation. The rapid unitary direct tests have for their part lower and unequal sensitivity, ranging from 30.77% [14] to 68.6% [15] or 80.2% [16]. Consistently, the sensitivity was higher during the first days from the onset of the symptoms [15, 16], as observed in our study.

Moreover, the antigen detection is more sensitive when viral load is high, corresponding to low RT-PCR Cq values [11, 13]. Concerning the LIAISON® SARS-CoV-2 antigen test evaluated here, the sensitivity reached 100% for samples with Cq values lower than 25. This “cut off” was also commonly observed for rapid unitary direct test [8, 14, 15]. Even if the detection of SARS-CoV-2 antigen cannot be assimilated to the detection of infectious virus and risk of transmission [17], the transmission rate tends however to be higher for high viral loads, when the sensitivity of antigen method is maximal, and although Cq thresholds are assay-specific, values of 30 might be considered as a cut-point to define infection in patient specimen [18]. Automated antigen detection method should thus be an effective tool to detect positive people at risk of transmitting SARS-CoV-2.

The limit of the study is that no positive asymptomatic people and no samples with Cq values higher than 30 were tested. The selection of the patients was however in agreement with the population of a COVID-19 screening center, where automated antigen methods could be implanted. Another point is that the presence of symptoms was self-reported by volunteers and the type of symptoms were not collected.

Antigen tests are designed for the detection of spike or nucleocapsid SARS-CoV-2 protein. The outline of the pandemic has changed during the last months, due to the emergence of new viral variants. The well-known variants
of concerns, meaning 20I/501Y.V1, 20H/501Y.V2 and 20J/501Y.V3 show characterized mutations on spike protein that can lead to immune escape, especially the A484K mutation present in V2 and V3 [5, 6]. Antigen tests based on spike protein detection can thus fail to detect these variants and their use is no longer recommended. The SARS-CoV-2 antigen test evaluated here is designed on the nucleocapsid protein and has been validated by the provider on UK strains (data not shown). However, mutations can also impact the nucleocapsid gene, as the D3L and S235F mutations found in 20I/501Y.V1 [5], the T205I mutation found in 20H/501Y.V2 [6] and the P80R mutation found in 20J/501Y.V3 [19]. First, to confirm the ability of the LIAISON® SARS-CoV-2 Ag test to detect the viral proteins in spite of these mutations, cell supernatant from SARS-CoV-2 culture as well as nasopharyngeal samples collected from people that were infected by SARS-CoV-2 variants have been tested. The antigenic proteins were detected for all the strains, meaning the 20I/501Y.V1, the 20H/501Y.V2 as well as the variant of interest 19B/501Y. The variant of concern 20J/501Y.V3 was not tested due to the lack of sample.

The characteristics of the LIAISON® SARS-CoV-2 antigen method are in accordance with the WHO guidelines. The sensitivity is optimal for symptomatic people, especially during the first 4 days after the onset of the symptoms, when RT-PCR Cq values are lower (<25 Cq). The method is less sensitive that RT-PCR but more sensitive than rapid antigen test, with a better workflow throughput. The 2 h sample incubation in inactivation buffer prior the analysis is however cumbersome and slow processing in the context of mass screening, even if the delay is reduced to 30 min when using a dry swab without transport medium. It could however constitute an additional tool for COVID-19 screening campaigns within a range of methods including RT-PCR or antigen detection, in saliva, nasopharyngeal swab or self-collected nasal swab. Concerning the juggling between the different diagnostic and/or sample types, the IFCC specifies that nasopharyngeal specimen have demonstrated superiority in terms of sensitivity and that tests results have to be carefully interpreted considering clinical context, especially when using antigenic tests [8]. Hence, taking into account their lower sensitivity, a negative antigenic test result does not definitively exclude the presence of SARS-CoV-2 and impose a confirmation by conventional RT-PCR in individuals presenting clinical symptoms [8].

**Informed consent:** Informed consent was obtained from all individuals included in this study.

**Ethical approval:** Not applicable.

**References**


