Application of ultrasensitive assay for SARS-CoV-2 antigen in nasopharynx in the management of COVID-19 patients with comorbidities during the peak of 2022 Shanghai epidemics in a tertiary hospital

https://doi.org/10.1515/cclm-2022-0661
Received July 8, 2022; accepted November 29, 2022; published online December 9, 2022

Abstract

Objectives: Various comorbidities associated with COVID-19 add up in severity of the disease and obviously prolonged the time for viral clearance. This study investigated a novel ultrasensitive MAGLUMI® SARS-CoV-2 Ag chemiluminescent immunoassay assay (MAG-CLIA) for diagnosis and monitoring the infectivity of COVID-19 patients with comorbid conditions during the pandemic of 2022 Shanghai.

Methods: Analytical performances of the MAG-CLIA were evaluated, including precision, limit of quantitation, linearity and specificity. Nasopharyngeal specimens from 232 hospitalized patients who were SARS-CoV-2 RT-qPCR positive and from 477 healthy donors were included. The longitudinal studies were performed by monitoring antigen concentrations alongside with RT-qPCR results in 14 COVID-19 comorbid participants for up to 22 days. The critical antigen concentration in determining virus infectivity was evaluated at the reference cycle threshold (Ct) of 35.

Results: COVID-19 patients were well-identified using an optimal threshold of 0.64 ng/L antigen concentration, with sensitivity and specificity of 95.7% (95% CI: 92.2–97.9%) and 98.3% (95% CI: 96.7–99.3%), respectively, while the Wondfo LFT exhibited those of 34.9% (95% CI: 28.8–41.4%) and 100% (95% CI: 99.23–100%), respectively. The sensitivity of MAG-CLIA remained 91.46% (95% CI: 83.14–95.8%) for the samples with Ct values between 35 and 40. Close dynamic consistence was observed between MAG-CLIA and viral load time series in the longitudinal studies. The critical value of 8.82 ng/L antigen showed adequate sensitivity and specificity in evaluating the infectivity of hospitalized convalescent patients with comorbidities.

Conclusions: The MAG-CLIA SARS-CoV-2 Ag detection is an effective and alternative approach for rapid diagnosis and enables us to evaluate the infectivity of hospitalized convalescent patients with comorbidities.

Keywords: antigen detection; chemiluminescent immunoassay; COVID-19 diagnosis; COVID-19 monitoring; SARS-CoV-2; with comorbidity.

Introduction

Omicron subvariant BA.2, one of the variant of concerns (VOCs) of SARS-CoV-2, has now become dominant in many regions of the world, including this epidemic in Shanghai since late February, 2022 [1]. It has been demonstrated that Omicron BA.2 has an increased transmissibility and immune escape capability when compared to other variants [2, 3]. The prevalence of the virus has led to a dramatic increase number of COVID-19 patients with comorbidity, which brings challenges to hospitals. The clearance time of SARS-CoV-2 in these patients was significantly prolonged, causing conflicts between the treatment of underlying diseases and COVID-19, and increasing the healthcare...
burden [4, 5]. Time- and cost-effective assessment of virus clearance may provide guidance to the clinical determination of treatment and reduce the waste of healthcare resources. SARS-CoV-2 nucleic acid test in upper respiratory samples is currently the most routine way to assess the clearance of the viral [6]. However, factors such as long turnaround time, complicated experimental process, poor RNA extract efficiency, susceptibility of RNA degradation and the inconsistency of results across RT-PCR platforms limit its application [7, 8]. Therefore, valid markers that can be used for rapid and accurate diagnosis and monitoring of SARS-CoV-2 infection have become increasingly essential to healthcare strategies for effective COVID-19 management [9–11].

Antigen-detecting lateral flow tests (LFTs) have been introduced by the World Health Organization (WHO) to achieve high coverage and quick turnaround of testing [12]. LFTs offer rapid results at low costs [13], yet the performance of such tests is controversial. Studies suggest that the sensitivities for antigen-detecting LFTs are low, especially when the tests are applied to asymptomatic or convalescent individuals [13, 14].

The quantitative ultrasensitive SARS-CoV-2 antigen test is another valid marker in screening for the COVID-19 patients [15–17]. Yet for hospitalized individuals under comorbid conditions, there is still a lack of dynamic evaluation on the viral loads along with the antigen concentrations. In addition, researches on viral transmissibility of sustained low viral load for the comorbidities are limited. Recently, a novel ultrasensitive quantitative assay, namely MAGLUMI® SARS-CoV-2 Ag chemiluminescent immunoassay (MAG-CLIA), has received the in vitro diagnostics device (IVDD) CE mark. It costs approximately 1/4 of RT-qPCR with less than 2.5 USD, and provides a high-throughput, relatively compact and automated process for the detection of SARS-CoV-2 nucleocapsid antigen. The MAG-CLIA can process a maximum of 600 tests in 60 min and is scalable to up to 2,400 tests per hour when four modules are combined. Here in this study, we evaluated this assay in 232 individual COVID-19 patients with a variety of comorbidities during epidemic peak of COVID-19 (April 1st – May 31st, 2022) in a tertiary hospital in Shanghai, in comparison to RT-qPCR and LFT. We explored the dynamic performances of the assay in viral load time courses, the influence of comorbidities on the time kinetics, as well as the cutoff value of antigen concentration in monitoring virus infectivity, which can be determined by the Ct value of 35, as the previous viral culture studies have shown the non-contagious for samples with Ct values above 35 [18]. Moreover, we demonstrated that MAG-CLIA could be an adequate approach to evaluate the infectivity of convalescent patients, to facilitate the cost-effective and prompt detection of individuals carrying infectious viruses, and to provide risk determination of the present WHO discharge guidelines.

Materials and methods
MAGLUMI® SARS-CoV-2 Ag assay

Automated quantitative detection of the SARS-CoV-2 nucleocapsid antigen was performed using MAGLUMI® SARS-CoV-2 Ag chemiluminescent immunoassay (hereafter referred to as MAG-CLIA) on a MAGLUMI X8 analyzer. This method has been specifically developed for detecting and quantifying the SARS-CoV-2 nucleocapsid antigen in human nasopharyngeal and oropharyngeal swabs. In brief, pretreated nasopharyngeal or oropharyngeal swab samples, magnetic microbeads coated with anti-SARS-CoV-2 nucleocapsid protein monoclonal antibody, and N-(4-aminobutyl)-N-ethylisoluminol (ABEI) labeled with another anti-SARS-CoV-2 nucleocapsid protein monoclonal antibody are mixed and incubated thoroughly to form sandwich complexes. After precipitation in a magnetic field, the solid phase is washed and subsequently initiated for a chemiluminescent reaction. The light signal is measured by the specified instrument as relative light units (RLUs). The signal is proportional to the concentration of SARS-CoV-2 nucleocapsid protein present in the test sample. The laboratory turnaround time for the assay is within 60 min for 600 tests.

Analytical performance studies

The analytical performance of the MAG-CLIA was evaluated, including precision, limit of quantitation (LoQ), linearity and analytical specificity. In brief, precision was evaluated by following the CLSI EP05-A3 [19]. Within-run precision study was performed with 20 replicates of low (approximately 2.51 ng/L) and high (approximately 8.68 ng/L) mixed SARS-CoV-2 positive nasopharyngeal swab samples in 1 complete run. Between-day precision was assessed using the same mixed SARS-CoV-2 positive nasopharyngeal swab samples daily over 20 days (Supplementary Table 1). For LoQ, five samples in low concentrations were measured in replicates and multiple runs according to the CLSI EP17-A2 [20]. The LoQ was subsequently determined by plotting the coefficient of variation (CV) of each sample against the corresponding mean concentrations. To assess linearity, a nasopharyngeal sample in high SARS-CoV-2 antigen concentration was serially diluted with one in low concentration. Diluted sample pools were measured in triplicate and linear regression analysis was performed by following CLSI EP06 [21]. To study the cross-reactivity, 48 commercialized microbial specimens (Supplementary Table 2) were added into healthy human nasopharyngeal samples and were measured via the MAG-CLIA (spiked-in samples). In addition, 10 non-COVID-19 respiratory specimens from clinical patients with upper respiratory virus infection were also measured (clinical samples). To assess the detection performance on different virus variants, five heat-inactivated SARS-CoV-2 variants (ZeptoMetrix, LLC., USA) were evaluated, respectively. For more detailed materials and methods, please refer to the Supplementary Data.
RT-qPCR and antigen-detecting lateral flow test (LFT)

SARS-CoV-2 nucleic acid tests were performed on a MA-6000 Real-Time Quantitative Thermal Cycler (Sansure Biotech Inc.) using the COVID-19 Coronavirus Real Time PCR Kit (Bioperfectus Technologies Co., Ltd.). Viral loads in respiratory specimens were estimated from cycle threshold (Ct) values. The limit of detection of the assay has been verified to be 500 copies, which is corresponding to the Ct value of 40. Specimens were judged positive when the measured Ct value for either ORF1ab gene or N gene was 40 or less. For further analyses, Ct values of ORF1ab gene was used as the reference unless specified. The Wondfo 2019-nCoV Antigen Test kit (Wondfo Biotech Co., Ltd.) was used as LFT in this study. The test was performed per package insert.

Study design and sample collection

This study was conducted from April 1st to May 31st 2022 at the Department of Laboratory Medicine of Huashan Hospital affiliated to Fudan University, Shanghai, China. The protocol of the current study was approved by the Huashan Hospital Institutional Review Board (HIRB) (NO. 2022-571). A total of 709 individuals were included in the study, consisting of 232 COVID-19 patients (median age of 71 years with interquartile range [IQR] from 59 to 83) and 477 healthy donors (median age of 69 years with IQR from 56 to 79) who had never been diagnosed as COVID-19 positive either before or during our study. Among 232 COVID-19 patients, 48 were asymptomatic, 145 mild, 32 moderate and seven severe (Table 1). There were 196 patients with comorbidities, mainly including hypertension, diabetes, nephropathy, coronary heart disease, and cerebrovascular infarction. Nasopharyngeal samples from patients were collected upon the admission to the hospital (unless specified) and preserved in Tris-HCl buffer (2 mL). Similarly, nasopharyngeal samples from healthy donors were collected. The same heat inactivated specimen was used for RT-qPCR and MAG-CLIA SARS-CoV-2 Ag assessment. A minimum of 500 μL specimens was required to complete the tests. All tests were performed within a maximum of 2 h after sample collection. To ensure the sample quality, the internal control Ct value for RT-qPCR was limited to 30. The LFTs were conducted at bedside immediately. In longitudinal studies, we monitored the antigen concentrations, in parallel with RT-qPCR results, in 14 patients for up to 22 days. The study initiated from the first nucleic acid positive test and terminated after the test showed negative.

Statistical analyses

Data analyses were performed using GraphPad Prism (version 9.00, GraphPad Software, La Jolla, CA, USA) and MedCalc statistical software (version 18.2.1, MedCalc Software Ltd., Ostend, Belgium). GraphPad Prism was used for regression analyses and for plotting the diagnostic performance of CLIA and LFT. Receiver operating characteristic (ROC) curve analyses were performed in MedCalc. The ROC curve was created by plotting the true positive rate (sensitivity) against the false positive rate (1-specificity) at various threshold settings. Youden index was used to estimate the best thresholds. Statistical significance of the difference between groups was performed using the Kruskal-Wallis test. The difference is statistically significant when p<0.05.

Results

Analytical performance of MAGLUMI® SARS-CoV-2 Ag tests

The total imprecision of the CLIA quantitative assay was less than 8% (Supplementary Table 1), with the limit of

<table>
<thead>
<tr>
<th>Covariateb</th>
<th>COVID-19 patients (n=232)</th>
<th>Healthy individuals (n=477)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>71 (59–83)</td>
<td>69 (56–79)</td>
<td>0.378</td>
</tr>
<tr>
<td>Gender (female)</td>
<td>116 (50%)</td>
<td>233 (48.85%)</td>
<td>0.200</td>
</tr>
<tr>
<td>Antigen concentrationc</td>
<td>16.64 (3.28–285.37)</td>
<td>&lt;0.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Ct value for ORF1ab gene</td>
<td>33.015 (28.19–36.07)</td>
<td>&gt;40</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Ct&lt;25</td>
<td>35 (15.09%)</td>
<td>0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>25≤Ct&lt;30</td>
<td>39 (16.81%)</td>
<td>0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>30≤Ct&lt;35</td>
<td>76 (32.76%)</td>
<td>0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>35≤Ct&lt;40</td>
<td>80 (34.48%)</td>
<td>0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Ct=40</td>
<td>2 (0.86%)</td>
<td>477 (100%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Days from first positive test</td>
<td>8 (5–12)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Symptom-free</td>
<td>48 (20.69%)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Mild symptom</td>
<td>145 (62.5%)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Moderate symptom</td>
<td>32 (13.79%)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Severe symptom</td>
<td>7 (3.02%)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>With comorbidity(ies)</td>
<td>196 (84.48%)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Without comorbidity</td>
<td>36 (15.52%)</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

*SARS-CoV-2 viral loads were estimated via cycle threshold (Ct) of ORF1ab gene. Continuous variables reported as median (interquartile range) and categorical variables reported as n (percentage). Antigen concentration is shown in ng/L. NA, not applicable.*
quantitation (LoQ) at 0.4 ng/L (Figure 1A) and linear range from 0.4 to 3,184.00 ng/L (Figure 1B). No interference was detected in 48 commercial microbial specimens and 10 routine clinical virus specimens (Figure 1C). There is a remarkable linear correlation between the antigen concentrations vs. titers of tissue culture infectious doses (TCID\textsubscript{50}/mL) for five different SARS-CoV-2 VOCs, respectively (Figure 1D). For detailed results, please refer to the Supplementary Data.

### Characteristics of the subjects

Nasopharyngeal samples from 709 individuals were included in this study. Table 1 reports the demographic characteristics of the study subjects. The majority of the patients had relatively high Ct values (32.76% for 30–35 Ct and 34.48% for 35–40 Ct) corresponding to low viral loads, while 35 of 232 (15.09%) patients had high viral loads with Ct<25. The median antigen concentration in patients was 16.64 ng/L (IQR 3.28–285.37) and in healthy individuals was negligible (less than 0.1 ng/L). Overall, the SARS-CoV-2 antigen levels of patients and that of healthy subjects differed significantly (p<0.001).

### Diagnostic performance

A receiver operating characteristic (ROC) curve was plotted to determine the optimal cutoff value of the SARS-CoV-2 antigen, which allows the distinction of SARS-CoV-2 infection from healthy status (Figure 2A). By comparing the antigen results between SARS-CoV-2 positive patients (Ct value ≤ 40) and healthy individuals, results provided an area under the ROC curve (AUC) of 0.987, with 95% confidence interval (CI) ranging from 0.976 to 0.994.
the Youden index calculation, the sensitivity and the specificity of the test reached 95.7% (95% CI: 92.2–97.9%) and 98.7% (95% CI: 96.7–99.3%) respectively, when a cutoff of 0.640 ng/L was used (Table 2).

The diagnostic performance of the assay was next evaluated by stratifying the results according to Ct values of RT-qPCR (Table 3). The performance of the Wondfo 2019-nCoV Antigen Test (an LFT) was evaluated in parallel (Figure 2B, C). Compared with RT-qPCR, the MAG-CLIA SARS-CoV-2 Ag test was positive and showed 100% concordance for samples with RT-qPCR Ct<33. For samples with Ct values between 35 and 40, the sensitivity of the test remained 91.46% (95% CI: 83.14–95.8%). Contrarily, the sensitivity of the LFT decreased gradually and remarkably when Ct values increased. The LFT showed 87.8% sensitivity in detecting samples with Ct<30. The sensitivity dropped to 66.4% for samples with Ct<33, and to only 34.9% for samples with Ct≥40.

**Dynamic level of SARS-CoV-2 antigen**

To explore the dynamic performance of MAG-CLIA SARS-CoV-2 Ag in the viral load time courses, and to explore the influence of comorbidities on the time kinetics, we performed longitudinal studies in 14 COVID-19 positive participants (Figure 3). Among the 14 participants, two of them had bacterial infection (patient 9, 13), two with diabetes (patient 7, 10), three hypertensions (patient 2, 4, 5), three nephropathies (patient 6, 8, 14), one fungal infection (patient 3) and the other three (patient 1, 11, 12) without any underlying disease. Figure 3 shows modeled SARS-CoV-2 viral RNA (as seen by Ct values of ORF1ab gene) trajectories together with the viral antigen measured for individuals. As the antigen concentration decreased, a decrease of SARS-CoV-2 viral loads was observed over time, as indicated by the increase of Ct values. This trend was observed for the majority of the participants. Moreover, as exemplified in patients 6, 8, 13 and 14, the fluctuation of SARS-CoV-
2 Ag was found to be closely and dynamically consistent with that of the viral nucleic acid loads in the time courses. Notably, the underlying comorbidities prolonged the median negative conversion time (the time Ct value returned to above 35) for viral nucleic acid loads (7 days for patients without comorbidity vs. 12 days for those with comorbidities, \( p=0.0247 \)).

**Correlation between SARS-CoV-2 antigen and Ct values**

MAG-CLIA targets for the N-terminal domain of N protein in SARS-CoV-2, which wraps coronavirus RNA through non-covalent bonds to form the nucleocapsid. This should result in a significant positive correlation between viral load and antigen concentration. Therefore, we examined correlations between SARS-CoV-2 antigen concentration and viral load determined by Ct values of RT-qPCR. Indeed, a significant (\( p<0.0001 \)) inverse correlation between the Ct values and \( \log_{10} \) antigen levels was observed (correlation coefficient \( R^2=0.747 \); Figure 4A). It is also noteworthy that the correlation between Ct values and \( \log_{10} \) antigen levels varied among some individuals, as shown in the scatter plot in Figure 4A.

**Evaluation of COVID-19 transmission with CLIA quantification of SARS-CoV-2 antigen**

Recent studies observed a strong correlation between SARS-CoV-2 viral loads and transmission [24], and reported no cases of COVID-19 transmission with SARS-CoV-2 viral RNA loads \(<4 \log_{10} \text{copies/mL} \) [18]. Given that, \( \log_{10} \) viral load was then estimated from the Ct value using the empirical formula \( 14.543 - (Ct \times 0.3018) \) for the MA-6000 Real-Time Quantitative Thermal Cycler system, and the viral load of \( 4 \log_{10} \) copies/mL was verified to correspond to the 35 Ct value of the current system. This was in accordance with previous viral culture studies which showed that SARS-CoV-2 is no longer contagious for samples with Ct values \( \geq 35 \) [22, 23]. Moreover, it is suggested by the National Health Commission of the PRC in the Diagnosis and Treatment Protocol for COVID-19 (Trial Version 9) that isolation can be discontinued when a Ct value \( \geq 35 \) is observed. The antigen concentration corresponding to 35 cycles was then calculated to be 8.71 ng/L using the inverse linear regression model shown in Figure 4A. In addition, we also observed a consistent average antigen concentration of 8.82 ng/L in nasopharyngeal swab samples of another 50 COVID-19 patients on the day when RT-qPCR Ct values first returned to above 35. Thus, we hypothesized that 8.82 ng/L might be a promising antigen concentration in differentiating contagious patients from the recovering (Table 2).

To further assess the diagnostic ability of the ultra-sensitive SARS-CoV-2 antigen test with the concentration of 8.82 ng/L in differentiating contagious patients from the recovering, an ROC curve was then plotted by classifying results into Ct values less than 35 and Ct values over 35 (and including 35) (Figure 4B). The ROC curve in identifying infectious patients showed an AUC of 0.921 (95% CI: 0.890–0.946). When 8.82 ng/L was selected as the critical value, the sensitivity and the specificity of the test were 84.5% (95% CI: 78.2–89.5%) and 85.0% (95% CI: 79.6–89.4%), respectively.

To explore the time nodes of detection, we tracked the RT-qPCR results from over 1,000 COVID-19 cases between

**Table 3: The sensitivity of two antigen detection assay according to the Ct values from RT-qPCR.**

<table>
<thead>
<tr>
<th>Ct value</th>
<th>MAGLUMI® SARS-CoV-2 Ag assay</th>
<th>Wondfo 2019-nCoV antigen test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( n (&gt;0.64 \text{ ng/L}) )</td>
<td>( n (\leq 0.64 \text{ ng/L}) )</td>
</tr>
<tr>
<td>(&lt;30)</td>
<td>74</td>
<td>74</td>
</tr>
<tr>
<td>(&lt;31)</td>
<td>89</td>
<td>89</td>
</tr>
<tr>
<td>(&lt;32)</td>
<td>101</td>
<td>101</td>
</tr>
<tr>
<td>(&lt;33)</td>
<td>113</td>
<td>113</td>
</tr>
<tr>
<td>(&lt;34)</td>
<td>131</td>
<td>130</td>
</tr>
<tr>
<td>(&lt;35)</td>
<td>150</td>
<td>147</td>
</tr>
<tr>
<td>(&lt;36)</td>
<td>173</td>
<td>169</td>
</tr>
<tr>
<td>(&lt;37)</td>
<td>186</td>
<td>179</td>
</tr>
<tr>
<td>(&lt;38)</td>
<td>206</td>
<td>197</td>
</tr>
<tr>
<td>(&lt;39)</td>
<td>223</td>
<td>213</td>
</tr>
<tr>
<td>(\leq40)</td>
<td>232</td>
<td>222</td>
</tr>
</tbody>
</table>
Figure 3: The longitudinal data of 14 individual COVID-19 positive patients. The log₁₀ antigen concentrations and Ct values are plotted against days since first positive RT-qPCR. The time kinetics for antigen are shown in red, and for viral loads (indicated in Ct values of RT-qPCR) in blue.
April 1st and 10th May 2022 in our laboratory to describe the kinetic evidence of the SARS-CoV-2 Omicron subvariant BA.2 in Shanghai, China (Figure 4C). Overall, the viral load in nasopharynx reduced over time, as seen by the increase of Ct values. The Ct value reached 35 after a median of 9 days since the first observation of positive RT-qPCR test. Furthermore, after 12 days (the median value) since the first positive RT-qPCR results, SARS-CoV-2 was no longer detected in these hospitalized patients’ nasopharynxes (Ct>40).

**Discussion**

RT-qPCR is currently the most routinely used diagnostic testing method for COVID-19. However, the long turnaround time of RT-qPCR leads to diagnostic delay. Moreover, Ct values obtained by RT-qPCR are inversely related to the relative viral RNA levels. They are not standardized to give quantitation of viral concentration across RT-PCR platforms [25]. Antigen-based LFTs, which have been developed recently, can generate results within 20 min and outside of a laboratory. Studies have shown that the sensitivity of LFTs varies massively, ranging from 0 to 96% with an average of 56% [25, 26]. Our study also demonstrates that one of the LFTs, the Wondfo 2019-nCoV Antigen Test, has sensitivities of 35.2–87.8% based on different RT-qPCR Ct values, which is in agreement with a recent study [27].

In the present study, we evaluated a novel CLIA-based quantitative SARS-CoV-2 antigen detection assay, the MAGLUMI® SARS-CoV-2 Ag test. A good correlation (R²=0.747, p<0.0001) was found between SARS-CoV-2 antigen concentrations and Ct values, which is similar to the findings of multiple literature reports [15–17]. The sensitivity of the assay (cutoff value at 0.64 ng/L) is 100% till Ct values of 33 (equivalents to the viral load of 10¹⁵ copies), and it still remains at a high sensitivity of 95.7% with the corresponding Ct values ≤40. Recent studies have validated the performance of multiple ultrasensitive and
quantitative antigen assays, such as single molecule array assay (SIMOA) (Quanterix) and SARS-CoV-2 S-PLEX, MesoScale Diagnostics (MSD) [28, 29]. Results suggested that the screening sensitivity for SIMOA was 70.6% and S-PLEX offered a sensitivity between that of RT-PCR and rapid antigen tests. In addition, the sensitivity reduced in high-Ct-value samples. MAG-CLIA has demonstrated excellent screening performance among currently registered CLIA systems, with the sensitivity of these CLIAs ranging from 70.0 to 84.8% [30, 31]. Furthermore, the MAGLUMI® SARS-CoV-2 Ag test shortens turnaround time, is cost effective, minimizes the chance of missing positive cases and has a similar diagnostic window of SARS-CoV-2 infection as RT-qPCR. Since global mobility has been gradually resumed, population-dense places such as customs, airports and concert halls may benefit from the rapid screening of MAG-CLIA to avoid the outbreak of COVID-19.

The SARS-CoV-2 Ag was shown to be closely and dynamically consistent with the viral load time series in the longitudinal studies. In addition, some comorbidities remarkably prolonged the time for both viral nucleic acid loads and antigen concentrations to return to negative. These provide evidence that MAG-CLIA SARS-CoV-2 Ag assay could be a good surrogate of molecular testing for monitoring COVID-19 patients.

Some recent studies have reported little chance of COVID-19 transmission with SARS-CoV-2 viral RNA loads <4 log_{10} copies/mL [18, 32], and some viral culture studies have shown that SARS-CoV-2 is no longer contagious for samples with Ct values ≥35 [23, 33]. Moreover, it is suggested by the National Health Commission of the PRC in the Diagnosis and Treatment Protocol for COVID-19 (Trial Version 9) that isolation can be discontinued when a Ct value ≥35 is observed. Meanwhile, the 35 Ct value of the current system is verified to correspond to a viral load of 4 log_{10} copies/mL. Therefore, patients with Ct values below 35 in this study are assumed to be infectious. A consistent average antigen concentration of 8.82 ng/L in nasopharyngeal swab samples of 50 COVID-19 patients was observed on the day when RT-qPCR Ct values first returned to above 35. We further assessed the diagnostic ability of the MAG-CLIA with the concentration of 8.82 ng/L in differentiating contagious patients from the recovering and showed that the sensitivity and specificity was 84.5 and 85.0%, respectively. Given that the majority of the COVID-19 cases obtain a Ct value ≥35 after 9 days since the first positive RT-qPCR result (Figure 4C) and the comorbid condition prolonged the median negative conversion time for viral nucleic acid loads (7 days for patients without comorbidity vs. 12 days for those with comorbidities, \( p=0.0247 \)), we recommend to monitor antigen levels during the treatment, and determine isolation strategies on day 7 (without comorbidity) and 12 (with comorbidities) via the SARS-CoV-2 antigen concentration to facilitate the cost-effective screening and to provide discharge risk assessment.

Furthermore, Pekosz et al. have demonstrated that an antigen qualitative test has a higher positive predictive value than RT-qPCR when compared to viral contagiousness determined by viral culture [34]. Lai and Lam also suggested that Ct values from RT-qPCR are not standardized to give quantitation of viral concentration due to huge deviations between different platforms [25]. These provide evidences that CLIA SARS-CoV-2 antigen, which is traceable to international units, has an advantage in determining the risk of transmissibility over RT-qPCR. Due to the poor culturability of most clinical specimens, we did not perform viral culture to assess the infectivity of antigen positive samples. However, it is reasonable to believe that the value of the quantitative MAGLUMI® SARS-CoV-2 Ag (CLIA) test may be much higher than that of a nucleic acid test in judging the infectivity of the patients. Thus, the antigen concentration of 8.82 ng/L could be a recommended threshold for judging the infectivity of the COVID-19 patients.

Nevertheless, individual variations lead to inconsistencies between nucleic acids and antigens. This was exemplified in an immunocompromised patient (patient 3 in Figure 3). This 59-year-old male participant had been diagnosed with cryptococcal meningitis in May 2021, and since then has been receiving fluconazole antifungal therapy. He was found to be SARS-CoV-2 positive on April 26th, 2022. We have closely monitored the Ct values and the antigen concentrations in his nasopharyngeal samples from his admission (April 28th) until the nucleic acids were shown negative (May 20th). On the 15th day since positive, his antigen concentration dropped down to the baseline (<8.82 ng/L), while the Ct value remained below 30. As such, even in the acute infection window, there might be no good correlation between antigenic dynamics and nucleic acid dynamics among these immunocompromised individuals. At this time, which criteria should be selected to assess infectivity remains controversial and needs to be further studied.

In conclusion, the MAGLUMI® SARS-CoV-2 Ag (CLIA) test correlates well with the Ct values determined by RT-qPCR and has shown very good diagnostic efficiency in the distinction of SARS-CoV-2 infection. Given that it facilitates low cost, scalable and rapid diagnosis, we suggest that this ultrasensitive SARS-CoV-2 antigen test can be used as an important tool in a stepwise strategy with nasopharyngeal samples in real-life mass screening settings. Furthermore,
MAG-CLIA SARS-CoV-2 antigen assay could also provide a good risk assessment of viral contagiousness. Closely monitoring the antigen concentration could be a preferable approach for monitoring the disease and directing clinical isolation strategies. MAG-CLIA is an effective and alternative approach for rapid diagnosis and enables us to evaluate the infectivity of hospitalized convalescent patients with comorbidities.

Research funding: This study was supported by Shanghai Municipal Science and Technology Major Project (grant no. HS2021SHZX001), three-year action plan for the construction of Shanghai Public Health System (2020–2022) (grant no. GWV-10.1-XK4), and the project of Huashan Hospital North, Fudan University (grant no. HSBY201920).

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: We declare that none of the work contained in this manuscript has been published in any language or is currently under consideration at any other journal, and there are no conflicts of interests to declare.

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

Ethical approval: All procedures were in accordance with the Helsinki Declaration. The protocol of the current study was approved by the Huashan Hospital Institutional Review Board (HIRB) (NO. 2022-571).

References


Supplementary Material: The online version of this article offers supplementary material (https://doi.org/10.1515/ccml-2022-0661).