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Note

Time-course evaluation of the quantitative antigen test for severe acute respiratory syndrome coronavirus 2: The potential contribution to alleviating isolation of COVID-19 patients

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Introduction: The automated quantitative antigen test (QAT), which detects severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is suitable for mass screening. However, its diagnostic capability differentiated by time from onset and potential contribution to infectivity assessment have not been fully investigated.

Methods: A retrospective, observational study using nasopharyngeal swab specimens from coronavirus disease (COVID-19) inpatients was conducted using Lumipulse® SARS-CoV-2 antigen test. Diagnostic accuracy was examined for the early (up to 10 days after onset) and late (over 10 days after onset) stages. Time-course QAT changes and reverse-transcription quantitative polymerase chain reaction tests results were displayed as locally estimated scatterplot smoothing curve, and receiver operating characteristic curve (ROC) analysis was used to determine the appropriate cutoff value for differentiating the early and late stages.

Results: We obtained 100 specimens from 68 COVID-19 patients, including 51 early-stage and 49 late-stage specimens. QAT sensitivity and specificity were 0.82 (0.72–0.90) and 0.95 (0.75–0.99) for all periods, 0.93 (0.82–0.98) and 1.00 (0.99–1.00) for the early stage, and 0.66 (0.48–0.82) and 0.93 (0.69–0.99) for the late stage, respectively. The ROC analysis indicated an ideal cutoff value of 6.93 pg/mL for distinguishing early-from late-stage specimens. The sensitivity, specificity, positive predictive value, and negative predictive value for predicting the late stage were 0.76 (0.61–0.87), 0.76 (0.63–0.87), 0.76 (0.61–0.87), and 0.76 (0.63–0.87).

Conclusions: QAT has favorable diagnostic accuracy in the early COVID-19 stages. In addition, an appropriate cutoff point can potentially facilitate rapid identification of noncontagious patients.
Moreover, some studies have implied that the RAT is a potentially superior predictor of positive viral culture results to RT-qPCR \[6,7\], implying that RAT may contribute to the identification of specimens with disease transmissibility. In this study, we aimed to investigate the following: (1) the chronological changes in QAT accuracy in the early and late stages of COVID-19 and (2) the potential contribution of QAT to the estimation of disease transmissibility.

This was a retrospective, observational study of COVID-19 patients admitted to the National Center for Global Health and Medicine (NCGM) with a confirmed RT-qPCR diagnosis prior to admission. Their samples were stored, with written consent having been obtained in a previous prospective study (NCGM-G-003472-02), and ethical consideration was made by way of secondary use with an opt-out option. A universal transport medium (1 mL or 3 mL; COPAN Diagnostics Inc., USA) was used as the viral transport medium (VTM). If the VTM volume was less than 1 mL, it was diluted with 2 mL of saline, of which 500 μL was used for analysis. Patients who had nasopharyngeal swab specimens collected during storage between March 14, 2020, and June 12, 2020, were included.

Two-hundred microliter samples were used to obtain 60 μL of nucleic acid extract using the QIAasymprome DSP virus/Pathogen Mini Kit (QIAGEN, Hilden, Germany). Five microliters of nucleic acid extract was used for RT-qPCR. RT-qPCR was performed using the Applied Biosystems 7500 Real Time PCR System (Applied Biosystems, Foster City, CA, USA) or QuantStudio® 5 (Applied Biosystems). Using the calculated cycle threshold value and PCR amplification efficiency of the N2 primer set, a correlation equation for the Ct value and the number of RNA copies was determined. The Ct value obtained from the reference material (SeraCare, AccuPlex™ SARS-CoV-2 Reference Material Kit) was used to perform a correction to calculate the number of RNA copies. For the samples with negative RT-qPCR results using the National Institute of Infectious Diseases (NIID) method \[8\], RT-qPCR retesting was performed using a standardized-assay kit (SARS-CoV-2 Direct Detection RT-qPCR Kit; Takara Bio, Japan).

Lumipulse® SARS-CoV-2 Ag testing was performed according to the manufacturer’s instructions (Fujirebio Inc.). Briefly, samples were centrifuged at 2,000 × g for 10 min, and the supernatant was used for the following test. We used 100 μL of thawed samples to measure antigen levels with a Lumipulse® G1200 automated machine (Fujirebio Inc.), which is based on the CLEIA method. When the antigen level was not measured because it exceeded the detection limit, we tested the diluted sample and calculated the antigen level of the original sample.

In this study, specimens from confirmed patients were used, and the extraction value of 1.34 pg/mL was used to determine QAT positivity. The sensitivity and specificity (95% confidence intervals [CI]) with RT-qPCR results were calculated for the early (up to 10 days after onset) and late (over 10 days after onset) stages. The validity of the results was examined using Cohen’s kappa and Gwet’s AC1 statistic (AC1). Time-series changes in antigen levels and RT-qPCR results were displayed as the locally estimated scatterplot smoothing (LOESS) curve, and receiver operating characteristic (ROC) curve analysis was conducted with the cutoff value defined as the least distance from the top-left corner of the box. Match-rate and time-series analyses were performed using SAS software, version 9.4 (SAS Institute, Cary, NC). All other analyses were performed using SPSS Statistics (version 26; IBM Corp., Armonk, NY, USA).

This study was conducted in compliance with the guidelines of the Declaration of Helsinki after receiving ethical review approval from the Ethics Committee of the NCGM (NCGM-G-003586-00). A total of 100 specimens were obtained from confirmed COVID-19 patients, including 51 early-phase and 49 late-stage specimens, with a median time from onset (interquartile range) of 8 (6–9) and 16 (13–19) days, respectively. There were 77 mild cases (no oxygen demand), 14 moderate cases (oxygen in demand except for ventilator), and 9 severe cases (ventilator in demand). The majority of patients were immunocompetent except for one case of metastatic cancer and three cases of human immunodeficiency virus infection in mild disease and two cases of solid organ transplantation in severe disease. The antiviral agents used were Hydroxychloroquine (32 cases; 18 mild, 6 moderate, and 8 severe), inhaled Ciclesonide (11 cases; 10 mild and 1 moderate), Favipiravir (6 cases; 3 mild and 3 moderate), and Lopinavir-Ritonavir (1 moderate case). Among the collected VTM specimens, 70 of 100 specimens were positive, while 30 were negative, as determined by the NIID assay. Of these negative specimens, 10 were confirmed positive by the Takara assay, resulting in 80 positive and 20 negative specimens by RT-qPCR. For all specimens, QAT sensitivity and specificity for all periods were 0.82 (0.72–0.90) and 0.95 (0.75–0.99), respectively (Table 1). QAT sensitivity and specificity for the early phase were 0.93 (0.82–0.98) and 1.00 (0.39–1.00) and those for the late stage were 0.66 (0.48–0.82) and 0.93 (0.69–0.99), respectively.

LOESS curve was used to compare QAT and RT-qPCR along the time series (Fig. 1). The correlation coefficients between QAT and SARS-CoV-2 RNA in all periods, in the early stage, and in the late stage were 0.883, 0.882, and 0.794, respectively (Supplementary Figure 1). The ROC analysis for samples in all periods indicated that the most appropriate cutoff value to distinguish early-phase from late-stage specimens was 6.93 pg/mL (Fig. 2). Using new cutoff values, the sensitivity, specificity, positive predictive value, and negative predictive value for predicting

Table 1

Concordance of the QAT with the RT-qPCR in different time periods.

(A) QAT vs RT-qPCR: all periods

| Antigen test | RT-qPCR | Total |
|--------------|---------|-------|
| Positive     | Positive Positive Negative Total |
| Negative     | Negative Positive Negative Total |
| 66           | 1       | 67    |
| 14           | 19      | 33    |

(B) QAT vs RT-qPCR: from day 1 to day 10 after onset (the early stage)

| Antigen test | RT-qPCR | Total |
|--------------|---------|-------|
| Positive     | Positive Positive Negative Total |
| Negative     | Negative Positive Negative Total |
| 44           | 0       | 44    |
| 3            | 4       | 7     |

(C) QAT vs RT-qPCR: over 10 days after onset (the late stage)

| Antigen test | RT-qPCR | Total |
|--------------|---------|-------|
| Positive     | Positive Positive Negative Total |
| Negative     | Negative Positive Negative Total |
| 22           | 1       | 23    |
| 11           | 15      | 26    |

QAT, SARS-CoV-2 quantitative antigen test; RT-qPCR, reverse-transcription quantitative polymerase chain reaction; SD, standard deviation.
the LOESS curves and the 95% confidence intervals for RT-qPCR and Lumi-
was isolated for up to 9 days in COVID-19 patients with typical symp-
patient is within 10 days or later after onset, and it seems reasonable that
This was a positive criterion that did not consider the time of onset. In
-terms of antigen levels shown by Aoki et al. only one sample
early stages, in the late stages when using QAT. However, it is more
important to reliably distinguish patients in the early stages of the dis-
long-term shedding of the infectious virus has been reported in severe
were no longer infectious. RT-qPCR provides a diagnosis based on the
cycle threshold value. The problem with this diagnostic method is that
the cycle threshold cutoff value is not validated among diagnostic re-
agents [15]. The same 10-day quarantine is prescribed for asymptomatic
pathogen carriers and those with slightly mild disease, although the time
from onset is sometimes ambiguous [13]. However, a uniform response
potentially overestimates quarantining large numbers of people who are
no longer a public health threat.

Previous studies have suggested that RAT is a potentially superior
predictor of viral culture results to RT-qPCR. Pekosz et al. found
concordance between SARS-CoV-2 VeroE6 TMPRSS2 culture, RT-qPCR,
and rapid antigen testing and revealed that RAT had a higher positive
predictive value than RT-qPCR (90% vs. 70%) [6]. Kohmer et al. re-
ported that the antigen-detecting rapid diagnostic test correlated better
with cell culture infectivity in vitro than RT-qPCR (61.8%–82.4% vs
51.6%) [7]. Our previous study also implied the utility of qualitative
antigen testing in predicting the appropriate duration of quarantine
[16]. Qualitative antigen tests generally tend to be less sensitive, and
false-negative results in contagious patients have been a challenge. In
contrast, Lumipulse® SARS-CoV-2 Ag allows quantitative evaluation of
antigens, which may minimize the problem of false negatives in the early
stages of infection. In addition, this test may be used to differentiate
critically ill or contagious patients by setting a value other than the original
cutoff for a positive result.

Finally, the optimal strategy for preventing the spread of infection
could depend on the disease prevalence in the community. Even testing
with suboptimal accuracy, such as rapid antigen testing, can be useful in
situations where a pandemic is spreading rapidly. Mathematical models
showed that frequent testing and strict isolation of patients using RATs
could contribute to pandemic control at justifiable cost by reducing local
transmission of the virus and its mortality [17,18]. Although RT-PCR is a
diagnostic method with high sensitivity and specificity, it requires more
financial and human resources. Other convenient diagnostic methods
could ensure efficient use of limited resources. QAT, with its relatively
high diagnostic accuracy, might contribute to the control of the current
pandemic.

There are some limitations in our study. First, we set 10 days from
the COVID-19 onset as the criterion for relaxation of isolation. However,
long-term shedding of the infectious virus has been reported in severe
disease and immunocompromised cases [19,20]. These specific cases
should be considered separately from our study. Second, although viral
culture serves as a surrogate marker of viral infectivity, we did not perform
viral culture in our study. Therefore, we lack a strict determination of
infectivity for individual cases. Discontinuation of isolation using our
cutoff requires caution, and its use may be limited to cases with mild-to-moderate disease.

In conclusion, Lumipulse® SARS-CoV-2 Ag is a rapid test with
favorable diagnostic accuracy in the early stages of COVID-19. The use
of an appropriate cutoff value potentially facilitates the rapid identifi-
cation of noncontagious patients.

Authorship statement

Concept and design: H.N, K.Y.
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Data collection: M.S, N.K.
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Declaration of competing interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jiac.2021.08.015.

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