

Application Note

Development of highly sensitive and rapid antigen detection assay for diagnosis of COVID-19 utilizing optical waveguide immunosensor

Ever since its outbreak, the COVID-19 pandemic caused by SARS-CoV-2 has been spreading rapidly causing a global health crisis. Accurate diagnosis and instituting appropriate intervention to relevant individuals are essential not only to slow down the spread of this pandemic but also to manage our resources efficiently. To date, testing for SARS-CoV-2 infection mostly relies on reverse transcription–quantitative polymerase chain reaction (RT–qPCR) on a nasopharyngeal or saliva specimen and remains the gold standard for diagnosis as it exhibits high sensitivity and specificity. However, RT–qPCR requires expensive equipment, and the centralized testing format practically requires ~24 h to obtain the results warranting more than one visit to the healthcare facility (Brendish et al., 2020). Moreover, a unique drawback that the RT–qPCR faces in COVID-19 diagnosis is that it cannot specifically distinguish between the infectious viruses shed from the respiratory tract and the persisting non-transmissible dead viruses or genetic fragments (Cento et al., 2020).

Rapid antigen detection tests (Ag-RDTs) for SARS-CoV-2 directly detect the viral proteins in respiratory specimen and offer multiple benefits in comparison to RT–qPCR, such as shorter turnaround time, lower cost, decentralized point-of-care testing, and ease of use (Li and Li, 2021). However, most of the

currently available Ag-RDTs lose out to RT–qPCR in terms of sensitivity. This could be due to either the inherent low sensitivity of the assay platform or the apparent reduction in sensitivity when pitched against the RT–qPCR later in course of the infection. Ag-RDTs may also show cross reactivity to other-related viruses based on the quality of the detector antibodies used.

In the present study, we improved the sensitivity of SARS-CoV-2 Ag-RDT by utilizing the optical waveguide-based biosensor technology (Uematsu et al., 2016). We further abolished the problem of cross-reactivity by using a pair of highly specific monoclonal antibodies (mAbs) targeting SARS-CoV-2 nucleocapsid protein (NP) antigen (Yamaoka et al., 2021b). Thus, the developed Rapiim SARS-CoV-2-N assay reported in this study possessed a detection limit of 9.3×10^4 copies/ml and exhibited no cross-reactivity with related viruses. This specific and sensitive assay has a high potential for COVID-19 diagnosis as well as for rapid identification of transmission competent individuals.

Rapiim SARS-CoV-2-N was developed based on antigen-sandwich principle using a pair of mAbs (capture antibody conjugated with light scattering particle and detector antibody immobilized on the surface of an optical waveguide film) that specifically detect SARS-CoV-2 NP antigen while binding to different epitopes of the antigen without any spatial interference (Figure 1A; Supplementary Figure S1). Upon addition of the sample to the processing solution, the capture antibody conjugate binds to the SARS-

CoV-2 NP antigen to form larger immune complexes. This solution is added to the cartridge where the detector antibody binds to the incoming immune complexes near the waveguide film. In the analyzer, incident light on the waveguide undergoes total internal reflection at the surface of the waveguide and is emitted out as the outgoing light. During this process, some of the light seeps out of the surface of the film as the evanescent light, which is scattered proportional to the number of immune complexes present on the surface. Evanescent light scattering attenuates the intensity of the outgoing light, which is detected by the optical sensor. The light attenuation rate is analyzed with calculation algorithm within the analyzer and a positive or negative result is displayed on the screen (Figure 1A). With this technique, it is possible to obtain non-subjective, reliable results in a single step as early as 4–15 min.

To check for cross-reactivity, we tested viral fluids from 19 respiratory virus particles and six recombinant antigens of related coronaviruses in Rapiim SARS-CoV-2-N and found that none of the viruses or recombinant antigens were flagged positive, denoting the exclusive specificity of the test for SARS-CoV-2 (Figure 1B; Supplementary Table S1). To identify the minimum detection sensitivity of the kit in detecting viable viruses, we used serial dilutions of culture supernatants of two differently isolated SARS-CoV-2 namely, SARS-CoV-2 JPN/TY/WK-521 (accession ID: LC522975.1), isolated at the National Institute of Infectious Diseases in January 2020, and

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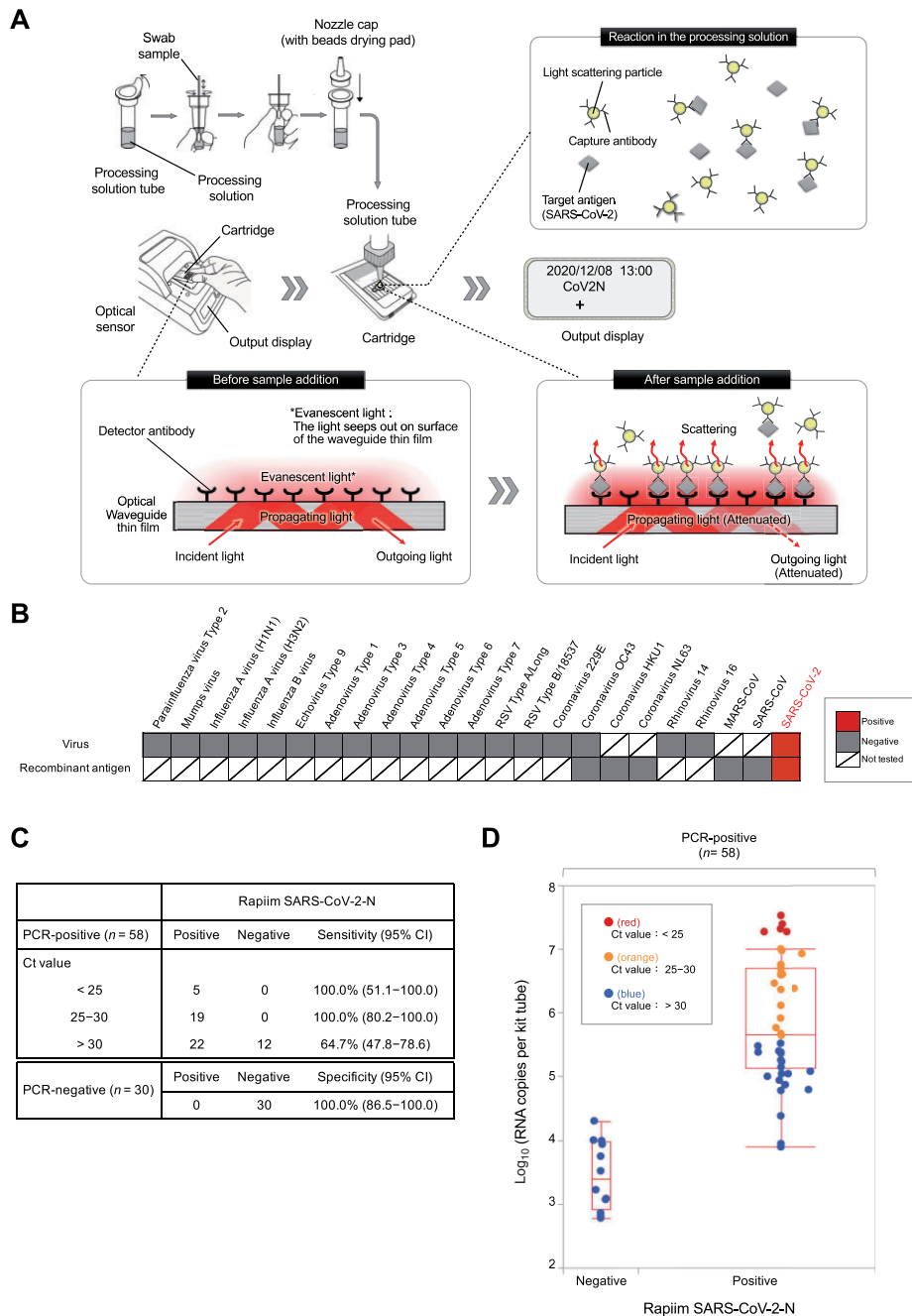


Figure 1 Development of highly sensitive and rapid antigen detection assay for diagnosis of COVID-19. **(A)** Schematic illustration of the principle of Rapiim SARS-CoV-2-N. The processing solution contains light scattering particles conjugated with a highly specific anti-SARS-CoV-2 NP antibody (capture antibody), while the cartridge contains an optical waveguide thin film embedded with another highly specific anti-SARS-CoV-2 NP antibody (detector antibody). Upon addition of a positive swab sample, the two antibodies capture SARS-CoV-2 antigen to form larger immune complexes (light scattering particle-capture antibody-antigen-detector antibody) on the surface of the optical waveguide thin film. In the analyzer, the scattering particles attenuate the outgoing light proportional to the number of immune complexes formed. The attenuated outgoing light is detected by the optical sensor and interpreted by the algorithm to finally provide a positive or negative result on the display screen. **(B)** Cross-reactivity test. Either the whole virus or recombinant NP antigen of the mentioned viruses was tested by the Rapiim SARS-CoV-2-N assay. **(C)** Performance of Rapiim SARS-CoV-2-N in detecting PCR-positive samples stratified by viral load. Positive PCR specimens were further classified according to the Ct values, and PCR concordance rates of the Rapiim SARS-CoV-2-N assay were compared for high viral titer (Ct < 25), medium viral titer (Ct 25–30), and low viral titer (Ct > 30). **(D)** Concentration distribution of viral load-stratified PCR-positive clinical specimens detected by the Rapiim SARS-CoV-2-N assay. Distribution of the results of the Rapiim SARS-CoV-2-N assay against the virus concentration in the vertical axis. PCR-positive samples with high (Ct < 25) or medium (Ct 25–30) viral titer are flagged positive while those of low viral titer (Ct > 30) are invariably flagged negative by Rapiim SARS-CoV-2-N.

SARS-CoV-2 Japan/YCU01/2020 (GISAID accession ID: EPI_ISL_693298), isolated at Yokohama City University in July 2020. In both cases, the limit of detection (LOD) was 9.3×10^4 copies/ml suggesting that the SARS-CoV-2 antigen from different mutants could be equally detected by Rapiim SARS-CoV-2-N (Supplementary Table S2). Indeed, the epitope regions targeted by mAb1 and mAb2 of the two different virus strains used were identical and conserved from mutations (Supplementary Table S3). Also, Rapiim SARS-CoV-2-N was able to detect SARS-CoV-2 at a much lower LOD than other commercially available rapid antigen detection kits suggesting its superiority over conventional lateral flow immunoassay (LFIA)-based Ag-RDTs (Supplementary Table S4).

We then evaluated Rapiim SARS-CoV-2-N for its minimum detection sensitivity in 58 PCR-positive and 30 PCR-negative nasopharyngeal swabs (Supplementary Table S5). Positive PCR specimens were further classified according to the Ct values, and PCR concordance rates of the two antigen detection assays were compared for high viral titer (Ct < 25), medium viral titer (Ct 25–30), and low viral titer (Ct > 30) (Supplementary Figure S2). We found that Rapiim SARS-CoV-2-N was able to detect all samples with high and medium viral titers, while it could detect 64.7% (95% confidence interval: 47.8%–78.6%) samples in the low virus titer cohort (Figure 1C). The distribution of tested samples with the virus concentrations in the treated solution was plotted as the vertical axis, showing that the concentration of virus in the specimen that causes Rapiim SARS-CoV-2-N to test negative for true positive specimens is low and remains within a narrow distribution (Figure 1D). This result indicates that Rapiim SARS-CoV-2-N can test positive for true positive specimens with lower virus concentration (Figure 1D). Notably, Rapiim SARS-CoV-2-N was completely concordant in PCR-negative specimens having 100% specificity (Figure 1C).

We examined the relationship between the numerical value of the attenuation rate of the detected light signal

and the RNA copy number calculated by RT-qPCR in nasopharyngeal swabs from 58 PCR-confirmed COVID-19 patients and 30 PCR-negative swabs (Supplementary Table S5). The attenuation value of the detected light signal tended to increase proportionately with the amount of virus and showed a very strong correlation ($R^2 = 0.93$, P -value < 0.0001) (Supplementary Figure S3). A plateau of saturation was observed when the RNA copy number reached 10^6 copies, beyond which the attenuation rate was as high as 80%–90% regardless of further increase in the virus amount.

Currently, the US Food and Drug Administration has only approved the Ag-RDTs that use NP as a target region (Nguyen et al., 2020). This can cause the problem of cross reactivity due to the antigenic homology of NP conserved between other coronaviruses. This could be overcome by using N-terminally truncated NP (Δ N-NP), which is highly specific for SARS-CoV-2 and abolishes cross reactivity (Yamaoka et al., 2021a, b). Rapiim SARS-CoV-2-N uses two highly specific antibodies derived from Δ N-NP that exclusively detect SARS-CoV-2. Our study has shown Rapiim SARS-CoV-2-N to possess 100% specificity as all PCR-negative samples were flagged negative. Additionally, the epitope sequences of these two detector antibodies were compared with those of ~8000 SARS-CoV-2 strains isolated so far and were found to be 99.75% and 99.41% identical, respectively, suggesting that these antibodies can detect the mutational variants of SARS-CoV-2 (Yamaoka et al., 2021b).

COVID-19 is a biphasic illness with an initial viral phase followed by an inflammatory phase. Transmissibility of SARS-CoV-2 begins shortly after infection in the asymptomatic period, peaks around the time of symptoms onset, and rapidly decreases 7 days thereafter (Stanleyraj et al., 2021). This is reflected by parallel changes in the viral load in the respiratory tract of infected individuals over time, which reaches its highest level around the time of symptoms onset and declines linearly thereafter (Sethuraman et al., 2020). Most of the currently available Ag-RDTs are based on LFIA

platform, which is a rapid test method using antigen–antibody reaction as a detection principle and is based on an immunoassay performed by the unidirectional flow of sample over a test strip. However, it has the inherent drawback of lesser sensitivity. Lower sensitivity also indicates that the test may miss out detecting positive patients in the early stages of infection when used for screening. Among the various novel methods investigated to improve rapid tests, one is optical waveguide biosensors (Uematsu et al., 2016). We have developed Rapiim SARS-CoV-2-N utilizing this technology and have shown that it achieves a lower LOD compared to the conventional LFIAs. We have shown that even in swab specimens with a low amount of virus, i.e. over 10^4 copies, which give relatively lower cycle threshold (Ct < 30) of RT-qPCR, can almost always be tested positive by Rapiim SARS-CoV-2-N. Hence, it can be expected to reduce the number of false negatives while screening patients with low viral load in the early stages of infection. Rapiim SARS-CoV-2-N also abolishes errors of subjective interpretation such as ‘faint bands’ faced in LFIAs. Although we did not encounter the problem of false positivity due to sample viscosity in any of the 88 clinical samples tested, this should be evaluated on larger scale. Rapiim SARS-CoV-2-N is inexpensive and easy to use, although it requires a dedicated analyzer. Due to all these reasons, Rapiim SARS-CoV-2-N can supplement the molecular diagnosis in order to facilitate health-care workers to institute timely and appropriate therapeutic or preventive measures for COVID-19.

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