Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Note

Diagnostic test property of transcription-reverse transcription concerted reaction reagent TRCReady® SARS-CoV-2 i using nasopharyngeal swab samples

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ABSTRACT

TRCReady® SARS-CoV-2 i is a reagent for transcription-reverse transcription concerted reaction (TRC) to detect SARS-CoV-2 N2 gene, used with the automated rapid isothermal nucleic acid amplification test (NAAT) analyzer TRCReady®-80. Sensitivity and specificity of TRCReady® SARS-CoV-2 i was assessed by comparison with the results of real-time reverse transcription-polymerase chain reaction (RT-PCR) using nasopharyngeal swab samples. From November 2020 to March 2021, a total of 441 nasopharyngeal swabs were obtained and analyzed both with TRCReady® SARS-CoV-2 i and RT-PCR. Sensitivity and specificity of TRCReady® SARS-CoV-2 i were 94.6% (53/56) and 99.2% (382/385), respectively. Reaction time to positivity of TRCReady® SARS-CoV-2 i ranged from 1.166 to 9.805 (median: 2.887) min, and minimum detection sensitivity of TRCReady® SARS-CoV-2 i was 9 copies per test, with reaction time as 5.014 min. Detection of SARS-CoV-2 gene from nasopharyngeal swab sample using TRCReady® SARS-CoV-2 i shows comparative diagnostic test accuracy with RT-PCR, and can be used as a useful test to diagnose SARS-CoV-2 infection.

Coronavirus disease 2019 (COVID-19) is caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which has been a worldwide concern for more than 2 years. Nucleic acid amplification test (NAAT) to detect SARS-CoV-2 plays an important role for diagnosis, infection control and prevention of COVID-19, as well as for public health problems caused by COVID-19.

Transcription-reverse transcription concerted reaction (TRC) method is an isothermal amplification method of nucleic acid invented and reported in 1996 [1]. Since then, automated system from extraction of nucleic acid from samples to amplification using specific test kits has been commercially available as clinical laboratory test, for *Mycobacterium* [2,3], norovirus [4], *Chlamydia/Neisseria gonorrhoeae* and *Mycoplasma pneumoniae*.

TRCReady® SARS-CoV-2 (Tosoh Corporation, Kanagawa, Japan) is a reagent for TRC, which has been used to extract, amplify and detect RNA coding nucleocapsid protein (N2) of SARS-CoV-2, combined with the automated rapid isothermal NAAT analyzer TRCReady®-80 (Tosoh Corporation, Kanagawa, Japan) since the middle of 2020 in Japan. Especially, "TRCReady® SARS-CoV-2 i" (Tosoh Corporation, Kanagawa, Japan) is an updated version of TRCReady® SARS-CoV-2, which was improved to reduce the inhibition of amplification caused by saliva in samples and released in 2021. The aim of this study is to know the sensitivity and specificity of the updated reagent TRCReady® SARS-CoV-2 i, using real-time reverse transcription-polymerase chain reaction (RT-PCR) as a gold standard.

From November 2020 to March 2021, a total of 441 extracts from the nasopharyngeal swabs, which were collected from outpatients of St. Luke's International Hospital in Tokyo, Japan, were analyzed with rapid isothermal NAAT with the former reagent TRCReady® SARS-CoV-2 and the automated analyzer TRCReady®-80. Outpatients with at least one symptom compatible with COVID-19, such as malaise, shivering, fever, headache, eye symptoms, nasal symptoms, taste disturbance, smell

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disturbance, sore throat, cough, myalgia, nausea, vomiting and diarrhea, were tested in this study [5]. After initial tests were carried out, the extracts were stored at −80 °C without using any additional storage medium, after anonymization. The frozen extracts were thawed, and 15 μL of each extract was used for re-analysis using the new reagent TRCReady® SARS-CoV-2 i. Positive result of TRCReady® SARS-CoV-2 i was defined as when fluorescence intensity ratio reached 1.3 compared to the baseline, by amplification within 20 min. Positive results were determined and reaction time to positivity was also recorded by the analyzer automatically. The residual thawed extracts (approximately 5 μL each) were also analyzed by RT-PCR according to the reference method of the National Institute of Infectious Diseases in Japan (NIID) and the analyzer QuantStudio® 5 (Applied Biosystems, MA, USA) [6]. Threshold cycle (Ct) value of RT-PCR to define positive result was 40 [6]. To convert the Ct value to the number of viral copies, 100 copies, 1000 copies and 10,000 copies of AccuPlex™ SARS-CoV-2 Molecular Controls Kit-Full Genome (Seracare, MA, USA) were analyzed by RT-PCR method and calibration curve was drawn. Sensitivity and specificity with 95% confidence intervals of TRCReady® SARS-CoV-2 i was assessed by comparison with the results of RT-PCR, using R version 4.2.1. Reaction time to positivity in TRCReady® SARS-CoV-2 i and Ct values of RT-PCR were also evaluated for the samples with discrepancy of the two methods. The study protocol was approved by the ethics committee of St. Luke's International University, with approval No. 20-RK201.

Sensitivity and specificity of TRCReady® SARS-CoV-2 i were 94.6% (53/56, 95% confidence interval: 85.1–98.9%) and 99.2% (382/385, 95% confidence interval: 97.7–99.8%), respectively, using RT-PCR results as references. Fig. 1 shows the distribution of the results of TRCReady® SARS-CoV-2 i and RT-PCR. Ct values of RT-PCR shown in Fig. 1. Time to positivity of TRCReady® SARS-CoV-2 i showed concordance with Ct value of RT-PCR ($y = 8.84\ln(x) + 17.78$, $R^2 = 0.35$).

There were 53 samples positive for TRCReady® SARS-CoV-2 i, and reaction time to positivity ranged from 1.166 to 9.805 (median: 2.887) minutes. Minimum detectable sensitivity of TRCReady® SARS-CoV-2 i was 9 viral copies per test approximately, estimated from the Ct value of 37.0. Ct values of the three RT-PCR-positive/TRCReady® SARS-CoV-2 i-negative samples were 37.0, 38.0 and 38.2, with estimated viral copies as 11, 6 and 3, respectively. In contrast, three RT-PCR-negative/TRCReady® SARS-CoV-2 i-positive samples showed the reaction times to positivity as 3.581, 3.991 and 5.271 min, respectively.

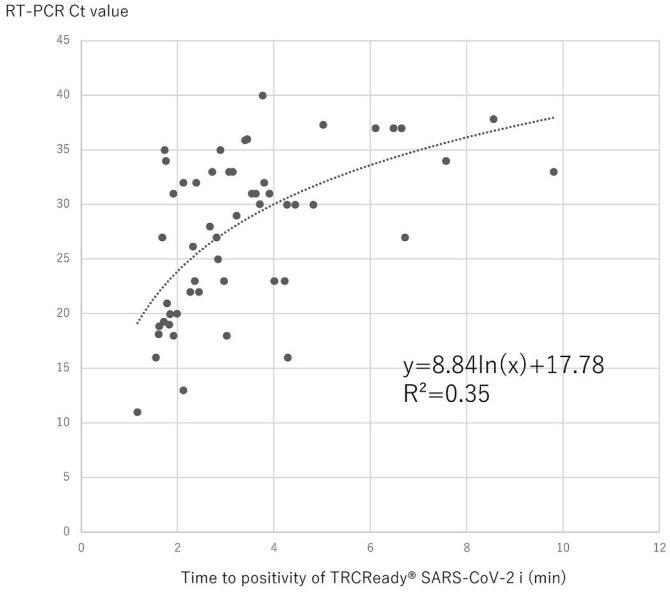

Fig. 1. Correlation of the time to positivity of TRCReady® SARS-CoV-2 i and RT-PCR. RT-PCR, real-time reverse transcription-polymerase chain reaction.

This is the first report of test diagnostic property of automated TRC method for SARS-CoV-2 gene using nasopharyngeal swab sample. Amplification of SARS-CoV-2 gene by TRC method takes 20 min, and total time from purification of nucleic acid to final result is approximately 40 min with specific automated equipment and reagent. Therefore, TRC method has its advantage as rapid NAAT. Generally, rapid RT-PCR tests were reported to have pooled sensitivity of 97% (95% CI: 94–99) with specificity 96% (95% CI: 94–98) [7]. One of the advantages of TRCReady® SARS-CoV-2 i with TRCReady®-80 is that extraction, isothermal amplification and detection of SARS-CoV-2 RNA is performed in one cartridge. Similar systems are available using RNA extraction step and isothermal amplification in one cartridge. ID NOW system (Abbott Laboratories, IL, USA) is using nicking enzyme amplification reaction method to amplify SARS-CoV-2 RNA and widely used as a point-of-care test, and was reported to have sensitivity and specificity as 98.5% and 97.5%, respectively, with nasopharyngeal swab at emergency room [8]. APTIMA and PANTHER system (Hologic, Inc. MA, USA), which can extract, amplify and detect SARS-CoV-2 RNA automatically by transcription-mediated amplification method, were reported to show sensitivity and specificity using nasopharyngeal swabs were 98.1% and 96.2%, respectively [9]. Therefore, diagnostic accuracy to detect SARS-CoV-2 gene by TRC method in this study were comparable to these rapid automated isothermal rapid NAAT tests. According to the recommendation from NIID, 50 copies/reaction of SARS-CoV-2 gene should be detected steadily as daily clinical laboratory test [6]. The results using clinical nasopharyngeal sample in this study also showed acceptable minimum limit of detection for SARS-CoV-2 gene. In contrast, TRC method continuously amplify RNA not only in samples originally but also amplified products during reaction time, using isothermal reaction, and there were three RT-PCR-negative/TRCReady® SARS-CoV-2 i-positive samples with usual reaction times to positivity, suggesting that reaction time to positivity could not reflect viral load simply in clinical samples.

Rapid antigen test with lateral flow immunoassay method is widely used conveniently in clinical setting. However, overall sensitivity and specificity were reported as 81% (95% CI: 72%–88%) and 99% (95% CI: 99%–100%), respectively, using NAAT as a reference standard, which led recommendation to prioritize using NAAT to diagnose SARS-CoV-2 infection [10]. Based on the result in this study, TRCReady® SARS-CoV-2 i also could be prioritized as a rapid NAAT than antigen testing.

There were several limitations in this study. This was a retrospective analysis using leftover of the extracts of nasopharyngeal swabs, and the samples were completely separated from the clinical information of patients or the results of former TRCReady® SARS-CoV-2 reagent. Therefore, patients' and samples' factors for discrepancy of both methods could not be analyzed, nor compare the updated reagent with the former reagent. In addition, detailed sequencing of the samples with discrepancy could not be performed due to small amount of extracts for additional sequencing, so the cause of discrepancy could not be understood correctly.

In conclusion, TRC method to detect SARS-CoV-2 gene from nasopharyngeal swab sample using TRCReady® SARS-CoV-2 i, could be a useful rapid NAAT, with comparative diagnostic test accuracy with RT-PCR and other previous reports of rapid NAAT.

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Authorship statement

All authors meet the ICMJE authorship criteria. YU was responsible for the organization and coordination of the study. MO was the chief investigator and responsible for the analysis. MO, MS, AY, YH, YS, YM, KU and SI carried out the sample analysis. MO, KU, SI, ST, YM, CF and YU developed the study design, analyzed data and drafted the manuscript. All authors contributed to the writing of the final manuscript.

Declaration of competing interest

KU, SI and ST are the employees of Tosoh Corporation. The reagent TRCReady® SARS-CoV-2 i and part of publication fee of this study was provided by Tosoh Corporation.

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