Brief Report

DOI: 10.5582/ddt.2022.01106

Examination of the utility of the COVID-19 detection kit, TRC Ready® SARS-CoV-2 i for nasopharyngeal swabs

Satoru Ishii[1,*], Moto Kimura[2], Tohru Miyoshi-Akiyama[3], Ataru Moriya[4], Masami Kurokawa[4], Erina Isaka[4], Junko Terada-Hirashima[1], Jin Takasaki[1], Shinyu Izumi[1], Masayuki Hojo[1], Haruhito Sugiyama[1]

[1] Department of Respiratory Medicine, National Center for Global Health and Medicine, Tokyo, Japan;
[2] Center for Clinical Sciences, National Center for Global Health and Medicine, Tokyo, Japan;
[3] Research Institute, National Center for Global Health and Medicine, Tokyo, Japan;
[4] Laboratory Testing Department, National Center for Global Health and Medicine, Tokyo, Japan.

SUMMARY The reverse transcription polymerase chain reaction (RT-PCR) offers high sensitivity, but has some drawbacks, such as the time required for the RNA extraction. Transcription reverse-transcription concerted reaction (TRC) Ready® SARS-CoV-2 i is easy to use and can be performed in about 40 minutes. TRC Ready® SARS-CoV-2 i and real-time one-step RT-PCR using the TaqMan probe tests of cryopreserved nasopharyngeal swab samples from patients diagnosed with COVID-19 were compared. The primary objective was to examine the positive and negative concordance rates. A total of 69 samples cryopreserved at -80° C were examined. Of the 37 frozen samples that were expected to be RT-PCR positive, 35 were positive by the RT-PCR method. TRC Ready® SARS-CoV-2 i detected 33 positive cases and 2 negative cases. One frozen sample that was expected to be RT-PCR positive was negative on both TRC Ready® SARS-CoV-2 i and RT-PCR. In addition, one frozen sample that was expected to be RT-PCR positive was positive by the RT-PCR method and negative by TRC Ready® SARS-CoV-2 i. Of the 32 frozen samples that were expected to be RT-PCR negative, both the RT-PCR method and TRC Ready® SARS-CoV-2 i yielded negative results for all 32 samples. Compared with RT-PCR, TRC Ready® SARS-CoV-2 i had a positive concordance rate of 94.3% and a negative concordance rate of 97.1%. TRC Ready® SARS-CoV-2 i can be utilized in a wide range of medical sites such as clinics and community hospitals due to its ease of operability, and is expected to be useful in infection control.

Keywords SARS-CoV-2, RT-PCR, TRC, Ct

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first reported in Wuhan City in the Hubei province of China in December 2019 and then spread globally. All genetic sequences of the virus were released on January 12, 2020 (*1*). The World Health Organization (WHO) declared it a pandemic on March 11, 2020 (*2*). The infection spread to Japan in February 2020, and a state of emergency was declared on April 7, 2020.

Data that can be used for COVID-19 diagnosis, such as severity markers (*3*) and diagnostic imaging (*4*) are increasing, but definitive diagnosis can be made by detecting pathogens, pathogen genes or pathogen antigens (*5*). Currently, COVID-19 testing uses a method of amplifying and detecting RNA sequences specific to the novel coronavirus by the reverse transcription polymerase chain reaction (RT-PCR) method. Although RT-PCR is characterized by high sensitivity, there are drawbacks, such as the requirement for manual performance of RNA extraction and the time-consuming nature of the measurements. Consequently, ways to reduce the burden on the laboratory technician and obtain results efficiently are needed.

The transcription reverse-transcription concerted reaction (TRC) method, which is a nucleic acid amplification test that amplifies and detects RNA at a constant temperature, can also be used (*6*). Using this method, the TRC Ready® system (Tosoh Bioscience, Tokyo, Japan) automates each process of the nucleic acid amplification test and has individually packaged reagents. The TRC Ready® system is also useful for detecting *Norovirus*, *Mycobacterium tuberculosis*

complex, *Mycobacterium avium*, *Mycobacterium intracellulare*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae* and *Chlamydia trachomatis*.

In the present study, the results from TRC Ready® SARS-CoV-2 i and of RT-PCR with cryopreserved nasopharyngeal swab samples of patients diagnosed with COVID-19 at our hospital were compared.

2. Materials and Methods

2.1. Study design and methods

The nasopharynxes of COVID-19 patients diagnosed at the National Center for Global Health and Medicine from October 2, 2020 to January 15, 2021 were wiped with FLOQSwabs (COPAN, Murrieta, CA, USA) and a general-purpose transport medium UTM (COPAN) was used. This study included 69 specimens that had been cryopreserved at -80°C.

The samples used in this study came from patients who participated in the prospective observational study of novel coronavirus infections (NCGM-G-003472-03) or the Filmarray Respiratory Panel 2.1 collection and storage of surplus specimens after examination (NCGM-G-004174-00) at the National Center for Global Health and Medicine. The subjects were those who consented to the secondary use of the stored specimens among those who participated in the study of collection and storage of surplus specimens after the examination (NCGM-G-004174-00). A retrospective study was conducted with the approval of the Ethics Committee (NCGM-G-004073-01).

2.2. Primary and secondary objectives

The primary objective was to examine the positive and negative concordance rates for TRC Ready® SARS-CoV-2 i compared with RT-PCR. The secondary objective was to compare the viral load of cryopreserved positive specimens with the cycle threshold (Ct). To confirm whether SARS-CoV-2 genes were detected in the samples for which TRC Ready® SARS-CoV-2 i and RT-PCR were not in concordance, residual liquid from nucleic acid purification was measured with real-time PCR, and sequence analysis was performed using an analysis primer for the obtained PCR products.

2.3. Inclusion criteria

Inclusion criteria for the study were age of at least 20 years, those who had a stored nasopharyngeal swab sample, and participants who consented to the "Prospective observational study of coronavirus infectious disease" (NCGM-G-003472-03).

2.4. Real-time one-step RT-PCR method using the TaqMan probe

The real-time one-step RT-PCR method using the TaqMan probe (QIAGEN, Tokyo, Japan) is the most commonly used method in Japan, and it involves a real-time reverse transcription-PCR method with hydrolysis probe using complementary strand DNA reverse transcribed from viral RNA as a template.

The QIAamp Viral RNA Mini Kit (QIAGEN) was used to isolate samples from viral RNA. RT-qPCR was performed using StepOne plus (Applied Biosystems, Tokyo Japan) according to the protocol of the National Institute of Infectious Disease, Japan (*7*).

The hydrolysis probe method uses a reporter dye (R) as a fluorescent dye at the 5' end and a 20-30 mer oligonucleotide labeled with a quencher (Q) at the 3' end. When the probe is intact, the reporter dye is quenched by the quencher and fluorescence resonance energy transfer, thus suppressing fluorescence. The probe then hybridizes to the virally derived cDNA that was reverse transcribed from viral RNA.

Subsequently, as the extension reaction by the primer proceeds, the probe is hydrolyzed by TaqDNA polymerase, so that the reporter dye is released from the probe and fluoresces. When probe hybridization and the PCR reaction are repeated for 40 cycles, the amount of free reporter dye increases in proportion to the amount of PCR product, so that the growth curve of the target gene sequence can be created in real time by monitoring fluorescence intensity. Those whose amplification curve is confirmed within 40 cycles are considered positive. Detection of SARS-CoV-2 genes from specimens and calculation of copy numbers were based on the real-time one-step RT-PCR method using the protocol of the National Institute Diseases on the N2 set.

2.5. TRC Ready® SARS-CoV-2 i

The TRC Ready® SARS-CoV-2 i is a nucleic acid amplification test based on the TRC method, which combines a DNA probe labeled with an intercalator fluorescent dye and a constant temperature RNA amplification method to amplify and detect RNA in one step (*6*). When the SARS-CoV-2 RNA promoter primer and SARS-CoV-2 RNA antisense primer included in this kit bind to SARS-CoV-2 RNA, the enzymes (reverse transcriptase, RNA polymerase) use the substrates (dNTP, NTP, ITP) used for transcription, reverse transcription, *etc*., and RNA is amplified and synthesized.

In parallel with this RNA amplification reaction, the fluorescently labeled SARS-CoV-2 RNA detection probe binds to the amplified and synthesized RNA, and the fluorescence intensity increases, so the fluorescence intensity in the reaction solution is measured over time. Thus, SARS-CoV-2 RNA in the sample is detected. TRCReady® SARS-CoV-2 i was used for detection. The TRCR® Purification Kit and TRCR® P-ASSIST were used for purification. The assay was performed on the Automated TRCR Real-time Monitor TRCReady®-80.

TRCReady® SARS-CoV-2 i cut-off value is positive when the fluorescence ratio exceeds 1.3 within 20 minutes from the start of the reaction.

2.6. Statistical analyses

SPSS version 24 (IBM Corporation, Armonk, NY, USA) was used for all statistical analyses. Spearman's rank correlation coefficient was used for the significance test comparing the two groups, with significance set at $p < 0.05$.

3. Results and Discussion

A total of 69 samples that were cryopreserved at -80 °C were examined. The median time from freezing the sample at -80°C to testing was 288 days (range 263-349). Of these samples, 37 were expected to be RT-PCR positive based on the RT-PCR test results at the time of collection, and 32 were expected to be negative (Figure 1). Of the 37 frozen samples that were expected to be RT-PCR positive, 35 were positive by the RT-PCR method. Using TRC Ready® SARS-CoV-2 i, there were 33 positive cases and 2 negative cases.

One frozen sample that was expected to be RT-PCR positive was negative on both TRC Ready® SARS-CoV-2 i and RT-PCR. In addition, one frozen sample that was expected to be RT-PCR positive was positive by the RT-PCR method and negative by TRC Ready® SARS-CoV-2 i.

For the 32 frozen samples that were expected to be RT-PCR negative, both the RT-PCR method and TRC Ready® SARS-CoV-2 i yielded negative results for all 32 samples (Table 1).

The positive concordance rate was 94.3% and the negative concordance rate was 97.1%. In the 35 patients who were positive by RT-PCR, median SARS-CoV-2 RNA viral load was 12,221 copies/mL (range 2-2,009,922), and median cycle threshold (Ct) was 27.3 (range 15.7-39.7). Examining the correlation between SARS-CoV-2 RNA viral load and Ct value, Spearman's rank correlation coefficient (r) was -0.591 and showed significance ($p = 0.0001$).

Ct values were high for the two samples that were positive by the RT-PCR method but negative by TRC Ready® SARS-CoV-2 i at 36.1 and 39.5 (Figure 2). Real-time PCR was performed for the nucleic acid purification products of the discordant samples. In a sequence analysis using analysis primers for the obtained PCR products, SARS-CoV-2 sequences were confirmed from all samples.

From the examination results, TRC Ready® SARS-CoV-2 i had a positive concordance rate of 94.3% and a negative concordance rate of 97.1% compared with the real-time one-step RT-PCR method using the TaqMan probe. The RT-PCR method offers high sensitivity and specificity and is widely used in Japan, but it is time-consuming and requires specific skills to perform

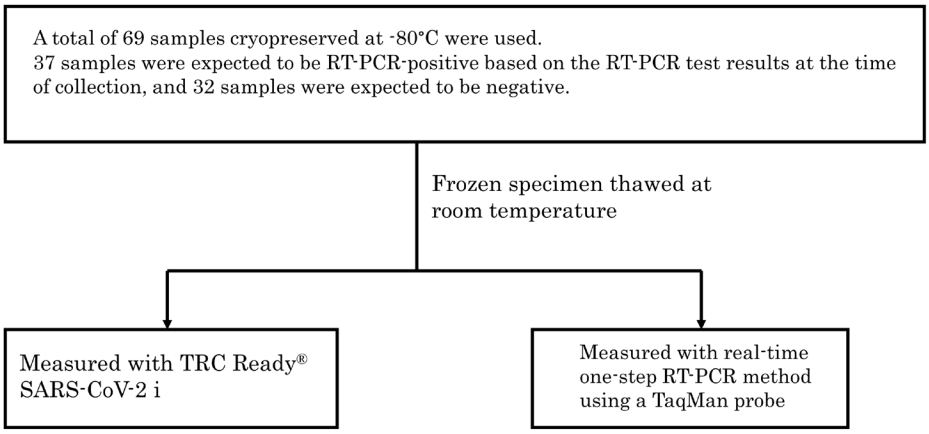

Figure 1. Study flowchart. A total of 69 nasopharyngeal swab samples that were cryopreserved at -80°C were examined. Of these 37 samples were expected to be RT-PCR positive based on RT-PCR test results at the time of collection and 32 samples were expected to be negative. Frozen samples were thawed to room temperature, then measured with the TRC Ready® SARS-CoV-2 i and real-time one-step RT-PCR method using a TaqMan probe.

Table 1. Comparison of results from TRC Ready® SARS-CoV-2 i and real-time one-step RT-PCR using the TaqMan probe for cryopreserved nasopharyngeal swab samples of patients diagnosed with COVID-19

		TRC Ready® SARS-CoV-2 i		Total
		Positive	Negative	
Real-time one-step RT-PCR method using the TaqMan probe	Positive	33	2	35
	Negative	1	33	34
Total		34	35	69

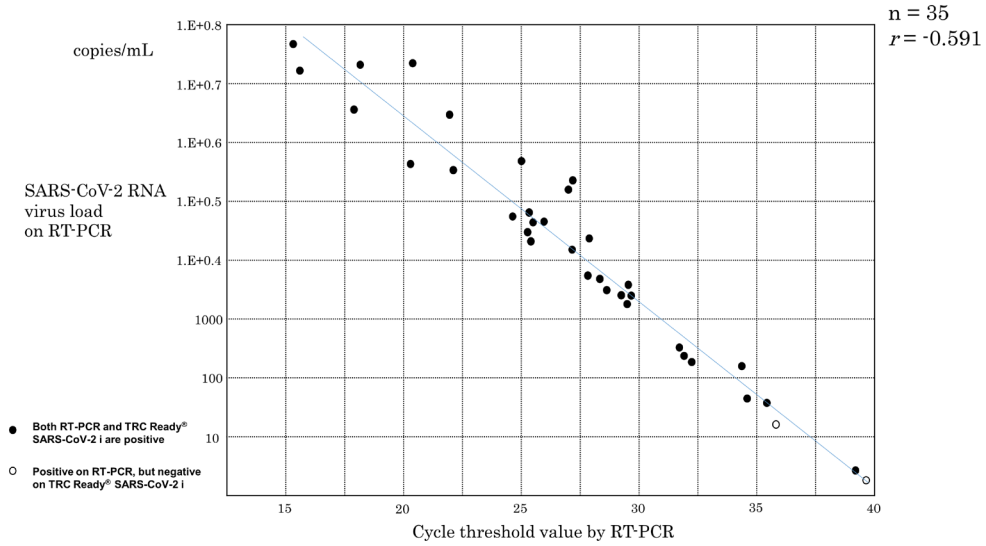

Figure 2. Correlation between SARS-CoV-2 RNA viral load and cycle threshold value in 35 RT-PCR positive patients. Examining the correlation between SARS-CoV-2 RNA viral load and Ct value, Spearman's rank correlation coefficient (r) was -0.591 (p = 0.0001). Ct values were high for the two samples that were positive by the RT-PCR method but negative by TRC Ready® SARS-CoV-2 i at 36.1 and 39.5.

appropriately.

On the other hand, TRC Ready® SARS-CoV-2 i provides the rapidity that is a feature of the nucleic acid amplification test method, and can be performed in about 40 minutes. In addition, six samples can be measured at the same time simultaneously, and the procedures are not difficult. The reaction reagents and starting solution of this kit are individually encapsulated for each measurement, so there is no need to prepare reagents at the time of measurement. Furthermore, since the reaction is completed in one closed tube, there is almost no risk of contamination. In addition, it is possible to perform measurement operations automatically using dedicated equipment.

With this kit, reaction inhibition originating in a sample can be detected by an internal control in the reagent, and false-negative results can be prevented. Real-time PCR was performed for the nucleic acid purification products of discordant samples, and in the sequence analysis, SARS-CoV-2 sequences were confirmed from all samples. Since there were no mutations in the regions amplified by RT-PCR or TRC Ready® SARS-CoV-2 i, the discordance is thought to have been caused by variation in measurements due to viral loads near the lower detection limit. Sethuraman *et al.* reported that high Ct levels make it difficult to isolate the virus responsible for an infectious disease from the sample, even if the genetic test is positive (*8*).

The TRC Ready® system is also useful for detecting *Norovirus*, *Mycobacterium tuberculosis* complex, *Mycobacterium avium*, *Mycobacterium intracellulare*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae* and *Chlamydia trachomatis* with a change of the reagents (*9,10*). Systems like TRC Ready®, which are easy to use and that can be used to detect various bacteria, can increase testing frequency and efficiency, thereby aiding in infection control in community hospitals.

4. Limitations

This was a retrospective study. Moreover, the number of positive and negative samples did not match because the samples were cryopreserved and usable.

5. Conclusion

Compared with the RT-PCR method, TRC Ready® SARS-CoV-2 i had a positive concordance rate of 94.3% and a negative concordance rate of 97.1%. TRC Ready® SARS-CoV-2 i can be used in a wide range of medical sites such as clinics and community hospitals due to its simple operability, and it is expected to be useful in infection control.

Acknowledgements

The authors would like to thank Mrs. Shimada for her excellent technical assistance. The authors would also like to thank the staff at the Department of Respirology for collecting the clinical samples.

Funding: This work was supported in part by Tosoh Bioscience (Tokyo, Japan). Tosoh Bioscience had no role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

Conflict of Interest: Satoru Ishii received research grants from Tosoh Bioscience. The other authors declare no conflicts of interest.

References

1. Wang C, Horby PW, Hayden FG, Gao GF. A novel coronavirus outbreak of global health concern. Lancet. 2020; 395:470-473.
2. World Health Organization. Statement on the third meeting of the international Health Regulations (2005) Emergency Committee regarding the outbreak of coronavirus disease (COVID-19). *https//www.who.int/news-room/detail/01-05-2020-statement-on-the-third-meeting-of-the-international-health-regulations-(2005)-emergency-committee-regarding-the-outbreak-of-coronavirus-disease-(covid-19)* (accessed 14 Jan 2021)
3. McElvaney OJ, McEvoy NL, McElvaney OF, *et al*. Characterization of the inflammatory response to severe COVID-19 illness. Am J Respir Crit Care Med. 2020; 202:812-821.
4. Ai T, Yang Z, Hou H, Zhan C, Chen C, Lv W, Tao Q, Sun Z, Xia L. Correlation of chest CT and RT-PCR testing for coronavirus disease 2019 (COVID-19) in China: A report of 1014 cases. Radiology 2020; 296:E32-40.
5. Sethuraman N, Jeremiah SS, Ryo A. Interpreting diagnostic test for SARS-CoV-2. JAMA. 2020; 323:2249-2251.
6. Ishiguro T, Saitoh J, Horie R, Hayashi T, Ishizuka T, Tsuchiya S, Yasukawa K, Kido T, Nakaguchi Y, Nishibuchi M, Ueda K. Intercalation activating fluorescence DNA probe and its application to homogeneous quantification of a target sequence by isothermal sequence amplification in a closed vessel. Anal Biochem. 2003; 314:77-86.
7. Shirato K, Nao N, Katano H, Takayama I, Saito S, Kato F, Katoh H, Sakata M, Nakatsu Y, Mori Y, Kageyama T, Matsuyama S, Takeda M. Development of genetic diagnostic methods for detection for novel coronavirus 2019 (nCoV-2019) in Japan. Jpn J Infect Dis. 2020; 73:304-307.
8. Wölfel R, Corman VM, Guggemos W, *et al.* Virological assessment of hospitalized patient with COVID-2019. Nature. 2020; 581:465-469.
9. Sato Y, Nirasawa S, Saeki M, Yakuwa Y, Ono M, Yanagihara N, Fujiya Y, Takahashi S. Evaluation of two commercial molecular diagnostic assays: The Xpert norovirus and the TRCReady NV. J Infect Chemother. 2021; 27:1115-1118.
10. Mazzarelli A, Cannas A, Venditti C, D'Arezzo S, De Giuli C, Truffa S, Butera O, Petroni F, Paglia MG, Vulcano A, Palmieri F, Nisii C, Di Caro A. Clinical evaluation of TRCReady M.TB for rapid automated detection of *M. tuberculosis* complex in respiratory sample. Int J Tuberc Lung Dis. 2019; 23:260-264.

Received November 24, 2022; Revised February 24, 2023; Accepted March 8, 2023.

**Address correspondence to:*
Satoru Ishii, Department of Respiratory Medicine, National Center for Global Health and Medicine, 1-21-1 Toyama Shinjuku-ku, Tokyo 162-8655, Japan.
E-mail: satishii@hosp.ncgm.go.jp

Released online in J-STAGE as advance publication March 22, 2023.