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Clinical usefulness of extraction-free PCR assay to detect SARS-CoV-2

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ABSTRACT

Due to the coronavirus disease 2019 pandemic, the demand for an easily accessible high-throughput screening test is increasing. We aimed to evaluate the usefulness of the extrac-tion-free polymerase chain reaction (PCR) as a screening test to detect severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). Real-time reverse transcription PCR was performed in 300 samples (260 SARS-CoV-2 positives and 40 negatives), using both the conventional nucleic acid extraction method (standard method) and the direct method without nucleic acid extraction (direct method). The overall agreement between the standard and direct methods was 86.8 % (kappa 0.60), and the sensitivity of the direct method compared to the standard method was 85.4 %. When the cycle threshold (Ct) value was less than 35, the sensitivity was approximately 90 %–98 %, and when Ct exceeded 35, it decreased to approximately 60 %–65 %. The extraction-free PCR could be useful as a screening test that processes many samples in a short time.

1. Introduction

The outbreak of coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) began in Wuhan, China, in December 2019 (Guan et al., 2020). Various clinical symptoms ranging from asymptomatic and mild to fatal progression often appear as respiratory symptoms but are usually associated with nonspecific symptoms such as diarrhea and headache. Therefore, it cannot be diagnosed only by symptoms; thus, real-time reverse transcription polymerase chain reaction (rRT-PCR) test to detect RNA of SARS-CoV-2 is a widely used diagnostic method (Tang et al., 2020).

The ongoing COVID-19 pandemic has put an exceptional strain on public health laboratories, hospital laboratories, and commercial laboratories as they attempt to keep up with demands for SARS-CoV-2 testing (Babiker et al., 2020). The current standard assay for diagnosis requires two steps: an RNA extraction from patient nasopharyngeal (NP) swab materials followed by one-step rRT-PCR. This standard procedure usually takes 3.5–4.0 h, considering the manual interventions. Recently, extraction-free PCR has been studied as an alternative that can reduce the time required for testing and solve the shortage of reagents or equipment by skipping the nucleic acid extraction process (Alcoba--Florez et al., 2020; Hasan et al., 2020; Lübke et al., 2020; Merindol et al., 2020). We aimed to evaluate the usefulness of the extraction-free PCR as a screening test to detect SARS-CoV-2.

2. Materials and methods

Nasopharyngeal swabs were collected in the universal viral transport medium (Becton, Dickinson and Company, Maryland, USA) from COVID-19 suspected patients. All samples were determined as positive and negative by nucleic acid extraction using the Real-Prep Viral DNA/RNA kit (BioSewoom Inc., Seoul, Korea) and rRT-PCR by Allplex 2019-nCoV assay (Seegene, Seoul, Korea). rRT-PCR was performed on each of the selected 300 (260 SARS-CoV-2 positives and 40 negatives) samples, using both the conventional nucleic acid extraction method (standard method) and the direct method without nucleic acid extraction (direct method) (Fig. 1).

2.1. Sample preparation for the extraction-free method (direct method)

A 15 μL specimen was heated at 98℃ for 6 min and then cooled at 4℃ for 5 min. For rRT-PCR testing, 5 μL of pretreated samples and 1 μL IC were added to the One-step RT-PCR Mastermix. One-step RT-PCR Mastermix and IC were included in the Allplex SARS-CoV-2 Assay kit (Seegene).

2.2. Nucleic acid extraction for the standard method

A 200 μL specimen was first spiked with 10 μL of IC, and then nucleic acids were extracted with the automated system of Real-Prep

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(BioSewoom) using the Real-Prep Viral DNA/RNA kit (BioSewoom) according to the methods described by the manufacturers. 5 μL of the extracted nucleic acid was added to the One-step RT-PCR Mastermix to prepare PCR.

2.3. SARS-CoV-2 rRT-PCR

SARS-CoV-2 rRT-PCR was performed by the Allplex SARS-CoV-2 Assay (Seegene) kit using the CFX96 Real-time PCR System (Bio-Rad Lab. Inc., Hercules, CA, USA). Allplex SARS-CoV-2 assay was designed to detect four target genes (E, RdRP, S, and N) in a single tube. Four target genes were detected in three channels (FAM, CalRed 610, and Quasar 670), and RdRP and S shared the same channel (CalRed 610). Thermal cycling was performed with 1 cycle of polymerase activation at 50℃ for 20 min and 95℃ for 15 min, followed by 45 amplification cycles each consisting of 95℃ (10 s), 60℃ (15 s) and 72℃ (10 s).

2.4. Statistical analysis

The overall agreement between the direct method and the standard method was evaluated using the Cohen's kappa coefficient. The paired *t*-test was used to compare the cycle threshold (Ct) value of the direct and standard methods. We determined the correlation between the direct and standard methods using Pearson's correlation coefficient. All statistical analyses were performed using SPSS software ver. 22.0 (IBM, Armonk, NY, USA).

3. Results

3.1. Test agreement rate of the direct method and standard method

When using the standard method, of the 260 positive samples, 258 were positive, 1 was presumptive positive, and 1 was negative. In the direct method, out of 260 positive samples, 212 were positive, 5 were presumptive positive, and 37 were negative. Forty negative samples showed negative results in both the standard and direct methods (Table 1). To calculate the test agreement and sensitivity, the presumptive positives were considered positive and five samples showing invalid results in the direct method were excluded. The overall agreement between the standard and direct methods was 86.8 % (kappa 0.60), and the sensitivity of the direct method compared to the standard method was 85.4 %.

The gene detection cycle threshold (Ct) value of the standard method was divided into three sections to determine whether there was a difference in the direct method's sensitivity according to the Ct value. When the Ct value was less than 35, the sensitivity was approximately 89 %–98 %, and when Ct exceeded 35, it decreased to approximately 60 %–65 % (Fig. 2).

3.2. Comparison of the cycle threshold (Ct) values of the direct method and standard method

To compare the Ct of the direct method and the standard method, we selected the results detected with both methods for each gene (Table 2). The Ct values of the E gene (N = 180), RdRP/S gene (N = 176), N gene (N = 200), and internal control (N = 295) in the direct method were all significantly high compared to the standard method (Table 3). When analyzing the Ct value correlation between the direct method and the standard method, the correlation coefficients of the E gene, RdRP/S gene, and N gene were 0.8872, 0.8729, and 0.8229, respectively. In internal control, there was no correlation between the standard method and the standard method (Fig. 3).

4. Discussion

Several microbial studies using molecular technology have been conducted omitting the nucleic acid extraction process, even before the COVID-19 pandemic (Herraez-Hernandez et al., 2013; Kang et al., 2014; Nishimura et al., 2010). Regarding the detection of the RNA virus, RT-PCR was performed without RNA extraction when detecting norovirus in human fecal samples or porcine reproductive and respiratory syndrome virus in pig serum. In those studies, researchers reported that it was possible to reduce time and cost or perform high-throughput tests by omitting the RNA extraction process (Herraez-Hernandez et al., 2013; Nishimura et al., 2010).

With the prolonged COVID-19 pandemic, there is a growing demand for easily accessible high-throughput screening. As part of that, several studies have been published that omit the nucleic acid extraction process from PCR testing (Alcoba-Florez et al., 2020; Hasan et al., 2020; Lübke et al., 2020; Merindol et al., 2020). In the present study, we evaluated the usefulness of extraction-free PCR with more positive samples (N = 260) compared with previous studies. Excluding the IC, the Ct values of the detected genes were highly correlated between the two methods. In the standard method, 10 μL of IC was added to the sample and extracted together, whereas, in the direct method, only 1 μL was added to the reaction tube. For this reason, it was thought that the direct method could be affected more by the technique of handling the specimen. As a result, the standard deviation (SD) of IC values in the direct method (SD 2.39) was more prominent than in the standard method (SD 1.39), and there was no correlation between the two methods of IC. Meanwhile, the overall agreement between the standard and direct methods was 86.8 % (kappa 0.60) and the sensitivity of the direct method compared to the

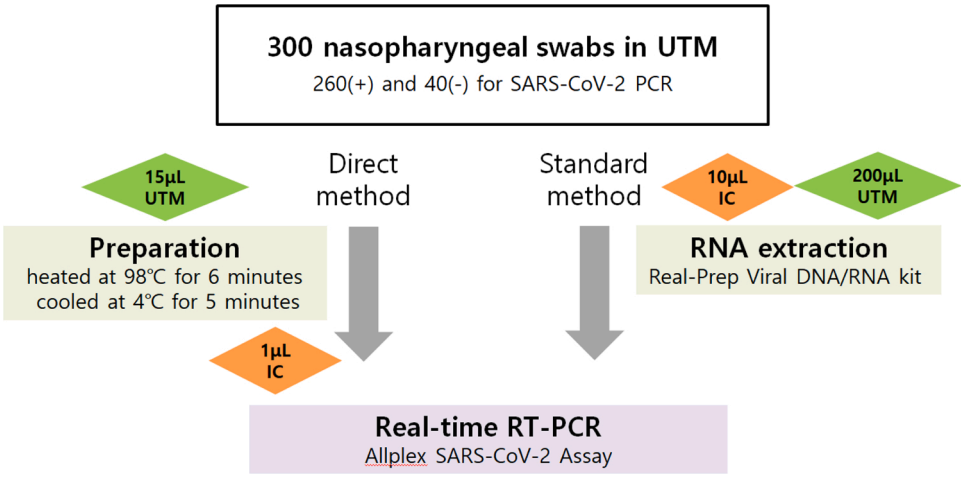

Fig. 1. Study design and sample selection.

Table 1
SARS-CoV-2 rRT PCT results of the standard and direct methods.

		Standard method				
		Positive	Presumptive positive*	Negative	Invalid	Total
Direct method	Positive	211	0	1	0	212
	Presumptive positive*	5	0	0	0	5
	Negative	37	1	40	0	78
	Invalid	5	0	0	0	5
	Total	258	1	41	0	300

* Considered positive when calculating agreement rate.

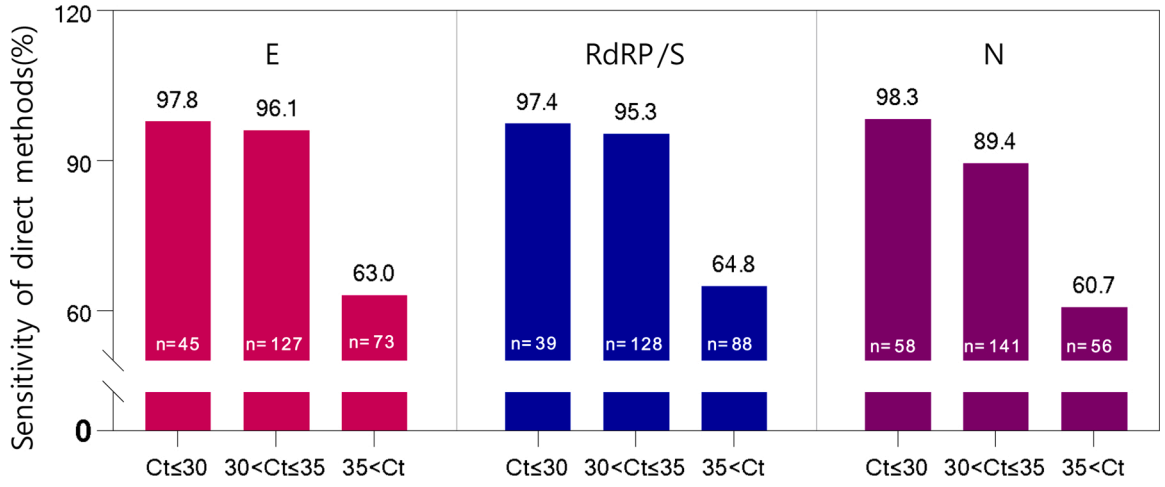

Fig. 2. The sensitivity of the direct method according to the cycle threshold (Ct) of E, RdRP/S, and N gene. The direct method's sensitivity was calculated by dividing each gene detection Ct value of the standard method into three sections.

Table 2
Detection results of each gene in the standard and direct methods.

		Standard method														
		Positive sample (N = 260)												Negative sample (N = 40)		
		E gene			RdRP/S gene			N gene			Internal control			Internal control		
		D	ND	Total	D	ND	Total	D	ND	Total	D	ND	Total	D	ND	Total
Direct method	D	180	3	183	176	4	180	200	2	202	255	0	255	40	0	40
	ND	70	7	77	70	10	80	53	5	58	5	0	5	0	0	0
	Total	250	10	260	246	14	260	253	7	260	260	0	260	40	0	40

D, detected; ND, not detected.

Table 3
Comparison of the cycle threshold (Ct) values of the direct and standard methods.

Gene	N	Cycle threshold (Ct) values				*P* value*
		Direct method		Standard method		
		Mean	SD	Mean	SD	
E	180	34.14	3.69	31.62	3.70	<0.01
RdRP/S	176	34.47	3.55	31.84	3.62	<0.01
N	200	33.79	3.69	31.27	3.41	<0.01
Internal control	295	25.13	2.10	24.02	1.32	<0.01

* *P*-values were determined with the paired *t*-test.

standard method was 85.4 %. The sensitivity differed according to the Ct value; when the Ct value was less than 35, the sensitivity was approximately 89 %–98 %. The viral load of patients withCOVID-19 generally reaches a peak within a few days before and after symptoms appear (Walsh et al., 2020). Since many of the positive samples included in this study were follow-up samples after the initial diagnosis, Ct values often exceeded 35. In other words, if only the samples for initial diagnosis were included, the sensitivity of the extraction-free PCR would also increase. Another limitation of this study is that the viral load of SARS-CoV-2 was not directly measured and was indirectly estimated by the Ct value.

We reported that 258 out of 260 positive samples were detected with the standard methods. Since have been used two different rRT-PCR kits, "Allplex 2019-nCoV" for samples selection and "Allplex SARS-CoV-2" for the comparison between workflows with and without nucleic acid extraction, this discrepancy could be due to a low amount of viral RNA in these samples (n = 2) and/or the differences between the two rRT-PCR kits (Blairon et al., 2021).

The advantage of extract-free PCR in our study is that the amount required for testing is small (15 μL). Besides, the nucleic acid extraction process can be omitted, eliminating the need for a nucleic acid extraction kit or equipment. If there is a problem with the supply of equipment or kits, the direct PCR without nucleic acid extraction could be a useful replacement test. Additionally, the number of samples when using automatic nucleic acid extraction equipment is limited. The extraction-free PCR does not have this limitation and can process a relatively larger

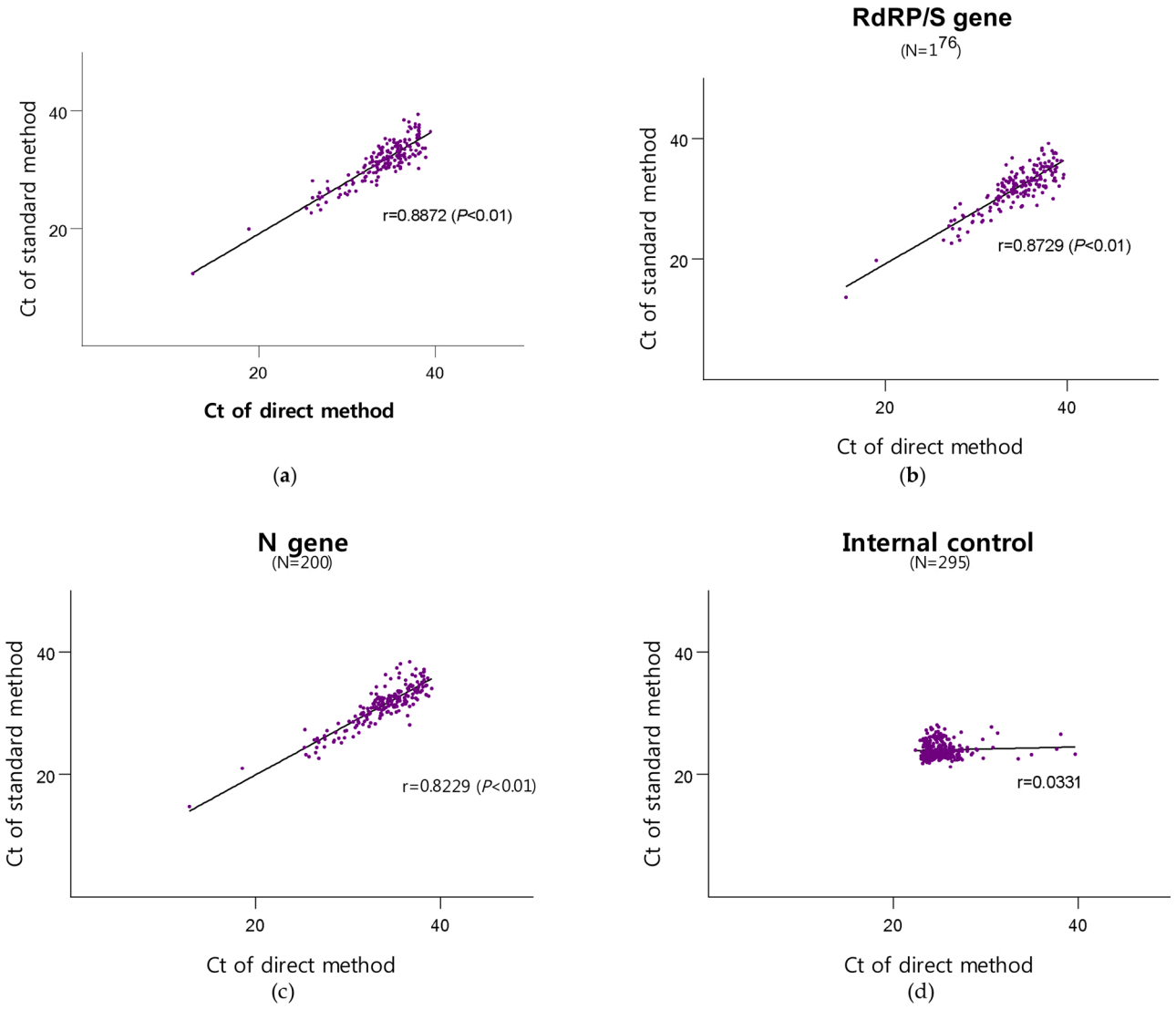

Fig. 3. Correlations of Ct values obtained by the direct versus standard method.

number of samples.

In conclusion, the extraction-free PCR was significantly correlated with standard methods using nucleic acid extraction. The sensitivity of the extraction-free PCR was excellent, except for the cases where the amount of nucleic acid was small. Therefore, the authors thought that the extraction-free PCR could be useful as a screening test that processes many samples in a short time.

Author contributions

Y.K.K. and S.H.C. conceived the study design. Y.K.K. analysed, collected the data and wrote the first draft of the manuscript. Y.K.K. and S.H.C. contributed in the analysis of data and preparation of the final version of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Institutional review board statement

The present study protocol was reviewed and approved by the Daegu Joint Institutional Review Board (approval No. 2020-09-005).

Informed consent statement

Since samples remaining after routine PCR tests were used, the Daegu Joint Institutional Review Board approved the study without the informed consent.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgment

Not applicable.

References

Alcoba-Florez, J., González-Montelongo, R., Íñigo-Campos, A., de Artola, D.G., Gil-Campesino, H., The Microbiology Technical Support Team et al, 2020. Fast SARS-

CoV-2 detection by RT-qPCR in preheated nasopharyngeal swab samples. Int. J. Infect. Dis. 97, 66–68.
Babiker, A., Myers, C.W., Hill, C.E., Guarner, J., 2020. SARS-CoV-2 testing. Am. J. Clin. Pathol. 153, 706–708.
Blairon, L., Piteüs, S., Beukinga, I., Tré-Hardy, M., 2021. Development and implementation of a RT-qPCR extraction-free protocol for the detection of SARS-CoV-2 and impact on the turn-around-time. J. Med. Virol. 93, 2538–2542.
Guan, W.J., Ni, Z.Y., Hu, Y., Liang, W.H., Ou, C.Q., He, J.X., Liu, L., Shan, H., Lei, C.L., Hui, D.S.C., Du, B., Li, L.J., Zeng, G., Yuen, K.Y., Chen, R.C., Tang, C.L., Wang, T., Chen, P.Y., Xiang, J., Li, S.Y., Wang, J.L., Liang, Z.J., Peng, Y.X., Wei, L., Liu, Y., Hu, Y.H., Peng, P., Wang, J.M., Liu, J.Y., Chen, Z., Li, G., Zheng, Z.J., Qiu, S.Q., Luo, J., Ye, C.J., Zhu, S.Y., Zhong, N.S., China Medical Treatment Expert Group for Covid-19, 2020. Clinical characteristics of coronavirus disease 2019 in China. N. Engl. J. Med. 382, 1708–1720.
Hasan, M.R., Mirza, F., Al-Hail, H., Sundararaju, S., Xaba, T., Iqbal, M., Alhussain, H., Yassine, H.M., Perez-Lopez, A., Tang, P., 2020. Detection of SARS-CoV-2 RNA by direct RTqPCR on nasopharyngeal specimens without extraction of viral RNA. PLoS One 15 e0236564.
Herraez-Hernandez, E., Alvarez-Perez, M., Navarro-Bustos, G., Esquivias, J., Alonso, S., Aneiros-Fernandez, J., Lacruz-Pelea, C., Sanchez-Aguera, M., Santamaria, J.S., de Antonio, J.C., Rodriguez-Peralto, J.L., 2013. HPV direct flow CHIP: a new human papillomavirus genotyping method based on direct PCR from crude-cell extracts. J. Virol. Methods 193, 9–17.
Kang, K., Yang, K., Zhong, J., Tian, Y., Zhang, L., Zhai, J., Zhang, L., Song, C., Gou, C.Y., Luo, J., Gou, D., 2014. A direct real-time polymerase chain reaction assay for rapid high-throughput detection of highly pathogenic North American porcine reproductive and respiratory syndrome virus in China without RNA purification. J. Anim. Sci. Biotechnol. 5, 45.
Lübke, N., Senff, T., Scherger, S., Hauka, S., Andrée, M., Adams, O., Timm, J., Walker, A., 2020. Extraction-free SARS-CoV-2 detection by rapid RT-qPCR universal for all primary respiratory materials. J. Clin. Virol. 130, 104579.
Merindol, N., Pépin, G., Marchand, C., Rheault, M., Peterson, C., Poirier, A., Houle, C., Germain, H., Danylo, A., 2020. SARS-CoV-2 detection by direct rRT-PCR without RNA extraction. J. Clin. Virol. 128, 104423.
Nishimura, N., Nakayama, H., Yoshizumi, S., Miyoshi, M., Tonoike, H., Shirasaki, Y., Kojima, K., Ishida, S., 2010. Detection of noroviruses in fecal specimens by direct RT-PCR without RNA purification. J. Virol. Methods 163, 282–286.
Tang, Y.W., Schmitz, J.E., Persing, D.H., Stratton, C.W., 2020. Laboratory diagnosis of COVID-19: current issues and challenges. J. Clin. Microbiol. 58, e00512–20.
Walsh, K.A., Jordan, K., Clyne, B., Rohde, D., Drummond, L., Byrne, P., Ahern, S., Carty, P.G., O'Brien, K.K., O'Murchu, E., O'Neill, M., Smith, S.M., Ryan, M., Harrington, P., 2020. SARS-CoV-2 detection, viral load and infectivity over the course of an infection. J. Infect. 81, 357–371.