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SHORT COMMUNICATION

Comparison of clinical diagnostic performance between commercial RRT-LAMP and RT-qPCR assays for SARS-CoV-2 detection

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The rapid and reliable detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) plays a key role in isolating infected patients and preventing further viral transmission. In this study, we evaluated the clinical diagnostic performances of a commercial real-time reverse transcription loop-mediated isothermal amplification (RRT-LAMP) assay (Isopollo® COVID-2 assay, M-monitor, Daegu, Korea) using eighty COVID-19 suspected clinical samples and compared these with the results of a commercial real-time reverse transcription polymerase chain reaction (RT-qPCR) assay (Allplex™ 2019-nCoV rRT-QPCR Assay, SeeGene, Seoul, Korea). The results of the RRT-LAMP assay targeting the *N* or *RdRp* gene of SARS-CoV-2 showed perfect agreement with the RT-qPCR assay results in terms of detection. Furthermore, the RRT-LAMP assay was completed in just within a 20-min reaction time, which is significantly faster than about the 2 h currently required for the RT-qPCR assay, thus enabling prompt decision making regarding the isolation of infected patients. The RRT-LAMP assay will be a valuable tool for rapid, sensitive, and specific detection of SARS-CoV-2 in human or unexpected animal clinical cases.

Key Words: SARS-CoV-2, Real-time RT-LAMP, Diagnostic performance

INTRODUCTION

Under the pandemic of coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), rapid and reliable diagnostic assays are essential for the timely isolation and treatment of infected patients and the prevention of further spread of the virus among individuals. Several real-time reverse transcription polymerase chain reaction (RT-qPCR) assays have been utilized in most diagnostic laboratories as the first-line standard diagnostic method for SARS-CoV-2 (Carter et al., 2020; Corman et al., 2020; Lu et al., 2020; van Kasteren et al., 2020). Furthermore, several reverse transcription loop-mediated isothermal amplification (RT-LAMP) assays have been developed to detect SARS-CoV-2 (Baek et al., 2020; Kitagawa et al., 2020; Lau et al., 2020; Rodel et al., 2020; Thomson et al., 2020; Yan et al., 2020). RT-LAMP assays that can amplify a target gene under a single reaction temperature using *Bst* DNA polymerase and four or six primers have comparable sensitivity and specificity to RT-qPCR assays for SARS-CoV-2 detection. In addition, RT-LAMP assays are less time consuming and can be easily adapted for use in relatively under-equipped laboratories. Therefore, RT-LAMP assays have been recognized as suitable alternatives to the RT-qPCR assay for detection of SARS-CoV-2. The Isopollo® COVID-2 assay (M-monitor, Daegu, Korea), hereafter referred to

as mRRT-LAMP assay, is a newly introduced commercial real-time RT-LAMP (RRT-LAMP) assay for the detection of SARS-CoV-2 that has been authorized for exportation in Korea. However, the diagnostic performance of the mRRT-LAMP assay has not yet been reported. Therefore, in this study, we comparatively evaluated the clinical diagnostic performances of the RRT-LAMP assay and a commercial RT-qPCR assay (Allplex™ 2019-nCoV rRT-QPCR Assay, SeeGene, Seoul, Korea), thereafter referred to as sgRT-qPCR, which has been certified and broadly used for SARS-CoV-2 detection in Korea, EU, and other countries (Carter et al., 2020; van Kasteren et al., 2020). Recently, SARS-CoV-2 infection has been reported in cats, dogs, tigers, lions, and minks suggesting animal-to-human and animal-to-animal transmission of the virus (Maddy et al., 2020). Therefore, it is essential to know the possibility of infection for other animal species and it is needed to have a diagnostic tool for unexpected animal clinical cases.

MATERIALS AND METHODS

Clinical samples and RNA extraction

Eighty nasopharyngeal swab specimens were used for clinical evaluation. These specimens were collected from patients with suspected COVID-19 hospitalized at Kyungpook National University Hospital (KNUH, Daegu, Korea) and placed in Virocult® viral transport media (Sigma). RNAs were extracted from the samples using a commercial RNA extraction kit (Real-Prep Viral DNA/RNA Kit, BioSewoom, Seoul, Korea) as per manufacturer's instruction. Extracted RNAs were stored at −80℃ until use. Sampling for this study was approved by the KNUH ethics committee (KNUH 2020-02-003-002).

sgRT-qPCR assay

The sgRT-qPCR assay (SeeGene) was performed according to the manufacturer's instructions. Briefly, RNA template (8 μL) was added to the master mix, and the sgRT-qPCR was performed with the flollowing reaction conditions: reverse transcription at 50℃ for 20 min, initial denaturation at 95℃ for 15 min, 45 cycles of denaturation at 94℃ for 15 s, and annealing and extension at 58℃ for 30 s using a real-time PCR system (CFX96™ Dx System, Bio-Rad, CA, USA). Ct values from Cal Red 610 (*RdRp* gene), Quasar 670 (*N* gene), and HEX (internal control) were acquired from each sample. Samples were considered positive when a signal was detected at Ct<40 for any gene, negative if the internal control was amplified but not the viral genes, and invalid when the internal control was not amplified.

mRRT-LAMP assay

The mRRT-LAMP assay (M-monitor) was performed according to the manufacturer's instructions. Briefly, RNA template (5 μL) was added to the master mix, and the mRRT-LAMP assay was performed for 40 cycles at intervals of 30 s at 58℃ isothermal condition using a real-time PCR system (CFX96™ Dx System, Bio-Rad). The time to positive (Tp) values from SYTO 9 intercalating fluorescent dye (*N* gene, *RdRp* gene, or internal control) were acquired from each sample. Samples were considered positive when a signal was detected at Ct<40 for any gene, negative if the internal control was amplified but not the viral genes, and invalid when the internal control was not amplified.

Comparison of clinical diagnostic performance using clinical samples

To comparatively evaluate the performance of the mRRT-LAMP and sgRT-qPCR assays for SARS-CoV-2 detection, each assay was performed with RNA templates extracted from 80 nasopharyngeal swabs described above. The concordance rate between both assays was calculated using the following formula: (number of consistent results by both methods/total numbers)× 100%. The Tp value of mRRT-LAMP or Ct value of sgRT-qPCR was converted to time elapsed to compare the re-

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action times. As the fluorescence signal of mRRT-LAMP was monitored every 30 s interval under isothermal conditions, the reaction time was calculated in minutes by dividing the Tp value by 2. In the case of sgRT-qPCR, as the reaction temperature changes per cycle, the reaction time required per cycle was calculated considering the ramping time (3.3℃/sec) determined for the real-time PCR system (Bio-Rad). Considering these parameters, the reaction time per cycle was calculated to be 67 s. In addition, the sgRT-qPCR assay also includes reverse transcription (20 min) and initial denaturation (15 min) prior to cycling. Accordingly, the total reaction time (time elapsed) for sgRT-qPCR was calculated by multiplying the Ct value of each sample by 67 sec and adding 35 min.

RESULTS AND DISCUSSION

The ongoing COVID-19 pandemic requires an accurate and efficient diagnostic method for the detection of SARS-CoV-2 in suspected patients and asymptomatic individuals. Most diagnostic assays for SARS-CoV-2 RNA detection rely on the RT-qPCR assays (Carter et al., 2020; Corman et al., 2020; Lu et al., 2020; van Kasteren et al., 2020), but RT-LAMP assays are promising alternatives (Baek et al., 2020; Kitagawa et al., 2020; Lau et al., 2020; Rodel et al., 2020; Thomson et al., 2020; Yan et al., 2020). In the present study, we evaluated the clinical diagnostic performance of a commercial mRRT-LAMP assay (M-monitor) and compared the results with those of a commercial sgRT-qPCR assay (SeeGen) for SARS-CoV-2 detection. We selected the sgRT-qPCR assay as the reference for comparative evaluation because it has been certified for Emergency Use Authorization by the United States Food and Drug Administration and other drug regulatory authorities (Carter et al., 2020) and has been proven to have comparable diagnostic performance as other commercial kits in a previous study (van Kasteren et al., 2020).

The results of the comparative clinical evaluation of the mRRT-LAMP and sgRT-qPCR assays are given in Table 1, 2. Internal control genes were successfully amplified by both mRRT-LAMP and sgRT-qPCR assays from all 80 clinical samples, indicating that the results of two assays could be interpreted as valid. In detecting *N* gene of SARS-CoV-2 from clinical samples, the

Table 1. Comparison of clinical performance between mRRT-LAMP and sgRT-qPCR for the detection of SARS-CoV-2

Target gene	sgRT-qPCR*	mRRT-LAMP		Total	Positive rate	Percent agreement
		Positive	Negative			
N gene	Positive	22	8	30	37.5%	90.0%
	Negative	0	50	50		
	Total	22	58	80		
	Positive rate		27.5%			
RdRp gene	Positive	15	4	19	23.8%	82.5%
	Negative	10	51	61		
	Total	25	55	80		
	Positive rate		31.3%			
N or *RdRp* gene	Positive	30	0	30	37.5%	100.0%
	Negative	0	50	50		
	Total	30	50	80		
	Positive rate		37.5%			

mRRT-LAMP, real-time reverse transcription loop-mediated isothermal amplification assay using Isopollo® COVID-2 assay (M-monitor, Daegu, Korea); sgRT-qPCR, real-time reverse transcription polymerase chain reaction assay using Allplex™ 2019-nCoV rRT-QPCR Assay (SeeGene, Seoul, Korea); SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
*The samples that are positive by the sgRT-qPCR were determined to be true positive in the hospital, because the assay has been certified and broadly used as standard diagnostic method for SARS-CoV-2 detection in Korea.

Table 2. Comparison of reaction times between mRRT-LAMP and sgRT-qPCR for the detection of SARS-CoV-2 genes from clinical samples

Methods	Target gene	Number of positive samples	Reaction time of each method (min)		
			Range	Mean	SD
sgRT-qPCR	*N*	30	58.3~78.8	73	4.4
	RdRp	19	64.5~75.1	71.9	2.9
mRRT-LAMP	*N*	22	7.5~16.3	11.6	2.3
	RdRp	25	8.0~15.8	11.7	2.2

mRRT-LAMP, real-time reverse transcription loop-mediated isothermal amplification assay using Isopollo® COVID-2 assay (M-monitor, Daegu, Korea); sgRT-qPCR, real-time reverse transcription polymerase chain reaction assay using Allplex™ 2019-nCoV rRT-QPCR Assay (SeeGene, Seoul, Korea); Range, the shortest detection time – the longest detection time; SD, standard deviation; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

positive ratio of mRRT-LAMP or sgRT-qPCR assay was 27.5% (22/80) or 37.5% (30/80), and the percentage of positive, negative, or overall agreement between the results of the mRRT-LAMP and the sgRT-qPCR assay was determined as 73.3% (22/30), 100.0% (50/50), or 90.0% (72/80), respectively. In detecting the *RdRp* gene of SARS-CoV-2 from clinical samples, the positive ratio of mRRT-LAMP or sgRT-qPCR assay was 31.3% (25/80) or 23.8% (19/80), and the percentage of positive, negative, or overall agreement between the results of the mRRT-LAMP and the sgRT-qPCR assay was determined as 78.9% (15/19), 83.6% (51/61), or 82.5% (66/80), respectively. For the detection of either the *N* or *RdRp* gene from each clinical sample, the positive ratio of mRRT-LAMP and sgRT-qPCR assay was the same as 37.5% (30/80), showing 100% of positive, negative, and overall agreement between both assays.

However, some discrepancies between the assay results were observed for individual *N* or *RdRp* gene detection. The reason for these discrepancies remains unclear but may be attributed to differences in sensitivity between the two assays, unknown inhibitors specific for each assay, or the degradation of individual target genes in clinical samples. However, when interpreting the assay results of either *N* or *RdRp* gene detection for each sample, both assays showed the same positive ratio of 37.5% and 100% percent agreement between the two assay results (Table 1). Several researchers have reported that false-negative or false-positive reactions can occur for various reasons, even with the best diagnostic method, causing serious problems for control and quarantine COVID-19 (Carter et al., 2020; Thomson et al., 2020). And also, considering the continuous genetic mutation of SARS-CoV-2 and the characteristics of the RT-LAMP method that requires the use of multiple primers, it cannot exclude the possibility that mutations in the gene sequence of the primer-binding site will occur, which will lower the diagnostic efficacy of the RT-LAMP assay. Therefore, it is necessary to update and improve the primers used in the RT-LAMP assay by continuously monitoring the virus for mutations to ensure accurate and sensitive diagnosis of SARS-CoV-2.

Inconsistencies of the results between different diagnostic methods or between target genes amplified with the same diagnostic method have been reported by researchers, which are similarly found in this study (Carter et al., 2020; Kitagawa et al., 2020; Rodel et al., 2020; Thomson et al., 2020; van Kasteren et al., 2020). Such inconsistencies of test results may inevitably occur in any diagnostic method, but a strategy to minimize such discrepancies is needed in order to minimize misdiagnosis caused by false-positive or false-negative reactions, Therefore, it is recommended to use a diagnostic method that can detect two or more targets simultaneously and it is more recommended to use another diagnostic method with a different operating mechanism as an alternative diagnostic method.

A comparison of reaction times between the mRRT-LAMP and sgRT-qPCR assays is presented in Table 2. The mean reaction time for *N* or *RdRp* gene-positive

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samples in the RT-qPCR assay was 73.0 (±4.4) min or 71.9 (±2.9) min, respectively. In contrast, the mean reaction time for *N* or *RdRp* gene-positive samples in the RRT-LAMP was 11.6 (±2.3) min or 11.7 (±2.2) min, respectively (Table 2). Therefore, the mean reaction times of the mRRT-LAMP assay was 61.4 min for *N* gene or 60.2 min for *RdRp* gene, which are shorter than those of the sgRT-qPCR assay, indicating that the developed mRRT-LAMP assay can be utilized as a faster diagnostic method than sgRT-qPCR for the detection of SARS-CoV-2 from clinical samples. The reason for the faster reaction speed of mRRT-LAMP is that it does not require RT and the initial denaturation steps, and because the reaction is performed under isothermal conditions, there is no time delay due to reaction temperature change.

In summary, the mRRT-LAMP assay allows rapid, sensitive, and specific detection of SARS-CoV-2 by targeting both *N* and *RdRp* genes of the virus and the assay will be a valuable diagnostic assay alternative to RT-qPCR for the detection of SARS-CoV-2 in human or unexpected animal clinical cases.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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