Evaluation of rapid diagnosis of novel coronavirus disease (COVID-19) using loop-mediated isothermal amplification

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ABSTRACT

With the rapid spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), there is an urgent need for more rapid and simple detection technologies at the forefront of medical care worldwide.

In this study, we evaluated the effectiveness of the Loopamp® 2019-SARSCoV-2 Detection Reagent Kit, which uses loop-mediated isothermal amplification (LAMP) technology. In this protocol, cDNA is synthesized from SARS-CoV-2 RNA using reverse transcriptase, followed by DNA amplification under isothermal conditions in one step. The RT-LAMP test kit amplified the targeted RNA of a SARS-CoV-2 isolate with a detection limit of 1.0×10^1 copies/μL, which was comparable to the detection sensitivity of quantitative reverse transcription PCR (RT-qPCR).

Comparison with the results of RT-qPCR for 76 nasopharyngeal swab samples from patients with suspected COVID-19 showed a sensitivity of 100 % and a specificity of 97.6 %. In the 24 RNA specimens derived from febrile Japanese patients with or without influenza A, no amplification was observed using RT-LAMP. RT-LAMP could be a simple and easy-to-use diagnostic tool for the detection of SARS-CoV-2.

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes coronavirus disease 2019 (COVID-19), has emerged as a serious threat to human health worldwide [1,2]. With the rapid increase in the number of patients, a reliable, rapid, and simple detection system for SARS-CoV-2 is needed that can be used in all medical institutions as quickly as possible [3].

Loop-mediated isothermal amplification (LAMP)-based analysis, which can be performed without a thermal cycler, is suitable for the diagnosis of infectious diseases as a point-of-care test in resource-limited settings [4,5]. In particular, the use of *bst* DNA polymerase with high strand displacement activity, to which reverse transcriptase (RT) activity has also been added, makes amplification of specific viral RNA possible in one step at a constant temperature.

This study examined the usefulness of a commercially available RT-LAMP-based diagnostics kit for COVID-19 (Loopamp® 2019-SARS-CoV-2 Detection Reagent Kit; http://loopamp.eiken.co.jp/), with the view

that if the approach proves feasible, it could support the rapid detection of SARS-CoV-2.

2. Materials and methods

2.1. Standard RNA of SARS-CoV-2

To evaluate the analytical sensitivity of the RT-LAMP method, we used purified and quantified total RNA of SARS-CoV-2, which was provided by the National Institute of Infectious Diseases, Japan, as a standard specimen for the molecular diagnosis of COVID-19. Analytical sensitivity was determined using 10-fold serially diluted standard RNA ranging from 1.0×10^3 copies/μL to 1.0 copy/μL and stored at -30 °C until required.

2.2. Clinical specimens

Seventy-six nasopharyngeal swab samples were examined. The

swabs were from patients with suspected COVID-19 who were admitted to Saitama Medical University Hospital, Japan, from February to March 2020. Viral RNA was extracted from the samples using a QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions, and RNA was eluted in 60 μL of the provided AVE buffer. Conventional quantitative reverse transcription PCR (RT-qPCR) for the specific amplification of the N gene of SARS-CoV-2 was performed using the previously reported TaqMan system with the following sets of primers and probe: 2.4 μM forward primer, 5′-AAA TTT TGG GGA CCA GGA AC-3′; 3.2 μM reverse primer, 5′-TGG CAG CTG TGT AGG TCA AC-3′; and 0.4 μM probe, 5′-FAM-ATG TCG CGC ATT GGC ATG GA-TAMRA-3′ [6]. Thermal cycling was carried out under the following conditions: reverse transcription at 50 °C for 30 min; initial denaturation at 95 °C for 15 min; and 40 cycles of denaturation at 94 °C for 15 s and annealing/extension at 60 °C for 60 s. This study was approved by Saitama Medical University Hospital Research Ethics Committee (Approval No. 19136).

2.3. Amplification of SARS-CoV-2 RNA with RT-LAMP

RT-LAMP for the specific detection of SARS-CoV-2 RNA was performed with a Loopamp® 2019-SARS-CoV-2 Detection Reagent Kit (Eiken Chemical, Tokyo, Japan) at 62.5 °C for 35 min according to the manufacturer's instructions. Positive amplification results were monitored by real-time measurement of turbidity with an LA-200 turbidimeter (Eiken Chemical). In this reaction, 10 μL RNA template was added to 15 μL of the provided master mix including a set of primers to make a final volume of 25 μL. The performance of the RT-LAMP kit was evaluated by comparing the results with those of conventional RT-qPCR.

2.4. Specificity of RT-LAMP

The specificity of the RT-LAMP reaction was evaluated using 12 RNA samples extracted from nasopharyngeal swabs taken from patients with influenza A at Saitama Medical University Hospital, Japan, from November to December 2019. In addition, 12 RNA samples negative for influenza A from febrile Japanese patients during the same period were also used in this study. During this sampling period, there were no cases of COVID-19 in Japan. The RT-LAMP reaction was performed using the same kit and conditions described above.

3. Results

3.1. Analytical sensitivity

A 10-fold serial dilution of SARS-CoV-2 RNA was amplified to determine the lower limit of detection with the RT-LAMP kit. Fig. 1 shows
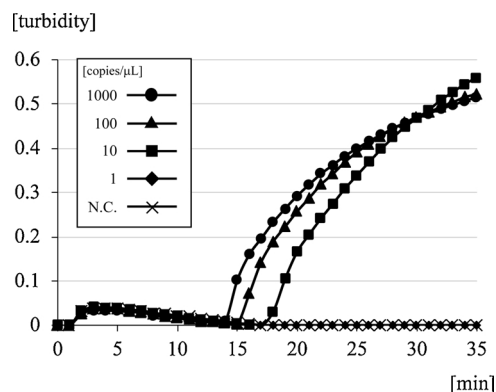

Fig. 1. Detection limit of the RT-LAMP method for SARS-CoV-2 with 10-fold serial dilutions of standard RNA.
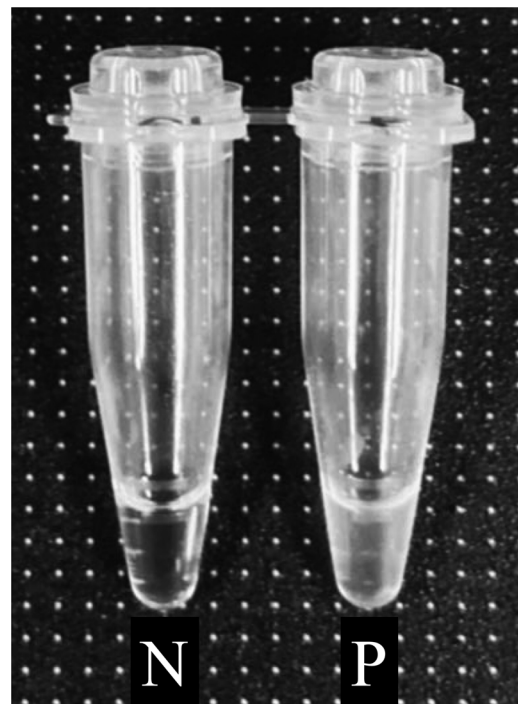

Fig. 2. Visual confirmation of turbidity under natural light after completion of the LAMP reaction.
Photographs of microtubes after the end of the RT-LAMP reaction under natural light. N, negative control; P, positive control.

the results for the detection of real-time turbidity with the LA-200 turbidimeter. The minimum amount of RNA detected was 1.0×10^1 copies/μL, which was achieved within 35 min in this procedure. After measurement, turbidity of the reaction solution could be observed visually under natural light (Fig. 2).

3.2. Utility of the RT-LAMP kit for clinical specimens

Based on the analysis of conventional RT-qPCR, 30 of the 76 patients were identified as positive for SARS-CoV-2; the median Ct value obtained was 30.85 (interquartile range, 25.31–36.08). Of the 76 patients who underwent conventional RT-qPCR, 32 were positive and 44 were negative by RT-LAMP. As shown in Table 1, the agreement between RT-qPCR and RT-LAMP was 97.4 % (74/76). Among them, 2 patients were found to be negative with RT-qPCR but positive with RT-LAMP. In the 24 RNA specimens derived from febrile Japanese patients with or without influenza A, no amplification was observed using RT-LAMP.

4. Discussion

RT-qPCR is a sensitive and specific nucleic acid amplification method that can be used to diagnose emerging viral infections, including COVID-19. However, it requires trained personnel, expensive equipment, and an extended period of time to generate test results.

Table 1
Correlation between the results of RT-qPCR and Loopamp 2019-nCoV.

		Loopamp 2019-nCoV		
		Positive	Negative	Total
RT-qPCR	Positive	30	0	30
	Negative	2	44	46
	Total	32	44	76

Conversely, RT-LAMP is extremely convenient to use (the isothermal reaction requires a simple heating device) and produces rapid results (within 30–60 min). In addition to these advantages, the amplification products generated by the RT-LAMP test kit can be detected by turbidity under natural light.

In this study, we found that the detection limit of the RT-LAMP test kit was 1.0×10^1 copies/μL within 35 min with real-time detection of its amplification products. Previous studies using the same molecular diagnostic strategy reported sensitivity ranging from 2.0×10^1 to 1.0×10^2 copies/reaction, indicating that this RT-LAMP test kit has extremely high sensitivity and is also valuable for diagnosis, in terms of not only its convenience but also its detection sensitivity [7,8]. It is highly specific because it uses a set of four primers that recognize at least six different sequences in SARS-CoV-2 RNA. In the present study, it was considered that there was no nonspecific amplification with the RT-LAMP test kit. The results for two samples, re-collected at more than 1 week after the initial SARS-CoV-2-positive result, were inconsistent with those for RT-qPCR (RT-LAMP was positive, whereas RT-qPCR was negative). This phenomenon may depend on the concentration gradient or the aspiration rate in the RNA extracts because the amount of sample used for RT-qPCR was 5 μL, while that used for RT-LAMP was 10 μL.

Our findings support the use of the Loopamp® 2019-SARS-CoV-2 Detection Reagent Kit for the early diagnosis of COVID-19 as a point-of-care test. The main limitations of our study are the small number of samples and the lack of validation of cross-reactivity with other respiratory pathogens.

Author contributions

TM designed the research; YK, YO, RK, KI, JS, and NT performed the research; MM, ST, and SM provided scientific guidance; YK and TM prepared the manuscript.

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Declaration of Competing Interest

All authors have no conflicts of interest to declare.

CRediT authorship contribution statement

Yutaro Kitagawa: Data curation, Formal analysis, Writing - original draft. **Yuta Orihara:** Data curation, Formal analysis. **Rieko Kawamura:** Data curation, Formal analysis. **Kazuo Imai:** Methodology, Project administration, Supervision, Writing - review & editing. **Jun Sakai:** Data curation. **Norihito Tarumoto:** Supervision, Validation, Writing - review & editing. **Masaru Matsuoka:** Supervision, Validation. **Shinichi Takeuchi:** Supervision, Validation. **Shigefumi Maesaki:** Supervision, Validation. **Takuya Maeda:** Conceptualization, Data curation, Formal analysis, Writing - review & editing.

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