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.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Evaluation of rapid diagnosis of novel coronavirus disease (COVID-19) using loop-mediated isothermal amplification

Yutaro Kitagawa, Yuta Orihara, Rieko Kawamura, Kazuo Imai, Jun Sakai, Norihito Tarumoto, Masaru Matsuoka, Shinichi Takeuchi, Shigefumi Maesaki, Takuya Maeda

PII: S1386-6532(20)30188-8
DOI: https://doi.org/10.1016/j.jcv.2020.104446
Reference: JCV 104446
To appear in: Journal of Clinical Virology

Received Date: 25 April 2020
Revised Date: 8 May 2020
Accepted Date: 14 May 2020

Please cite this article as: Kitagawa Y, Orihara Y, Kawamura R, Imai K, Sakai J, Tarumoto N, Matsuoka M, Takeuchi S, Maesaki S, Maeda T, Evaluation of rapid diagnosis of novel coronavirus disease (COVID-19) using loop-mediated isothermal amplification, Journal of Clinical Virology (2020), doi: https://doi.org/10.1016/j.jcv.2020.104446

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2020 Published by Elsevier.
Evaluation of rapid diagnosis of novel coronavirus disease (COVID-19) using loop-mediated isothermal amplification

Yutaro Kitagawa\textsuperscript{a}, Yuta Orihara\textsuperscript{a}, Rieko Kawamura\textsuperscript{a}, Kazuo Imai\textsuperscript{b}, Jun Sakai\textsuperscript{b}, Norihito Tarumoto\textsuperscript{b}, Masaru Matsuoka\textsuperscript{a}, Shinichi Takeuchi\textsuperscript{a}, Shigefumi Maesaki\textsuperscript{b}, Takuya Maeda\textsuperscript{a, c}\textsuperscript{*}

\textsuperscript{a}Department of Clinical Laboratory, Saitama Medical University Hospital, Saitama, Japan
\textsuperscript{b}Department of Infectious Disease and Infection Control, Saitama Medical University, Saitama, Japan
\textsuperscript{c}Department of Laboratory Medicine, Saitama Medical University, Saitama, Japan

\textbf{Running title:} Diagnosis of COVID-19 with LAMP

\textbf{Abstract Word Count:} 169
\textbf{Manuscript Word Count:} 1,099

*\textbf{Address for correspondence:}\
Takuya Maeda M.D., Ph.D.\
Saitama Medical University, 38 Morohongo, Moroyama-machi, Iruma-gun, Saitama 350-0495, Japan\
E-mail: t_maeda@saitama-med.ac.jp\
Phone: +81-49-276-1175, Fax.: +81-49-276-1175

\textbf{Highlights}

\begin{itemize}
  \item RT-LAMP test kits have good utility.
  \item Detection sensitivity of $1.0 \times 10^4$ copies/\mu L can be obtained within 35 min.
\end{itemize}
• RT-LAMP can be used as a point-of-care test because it is judged by turbidity under natural light.

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.

Materials and Methods

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 $1.0 \times 10^3$ copies/µL to 1.0 copy/µL and stored at -30 °C until required.
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).

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.

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.

Results

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. Figure 1 shows the results for the detection of real-time turbidity with the LA-200 turbidimeter. The minimum amount of RNA detected was $1.0 \times 10^{1}$ 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).

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 to 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.

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. 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 $1.0 \times 10^1$ 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 $2.0 \times 10^1$ to $1.0 \times 10^2$ 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.
CRediT author statement

Yutaro Kitagawa: Performed the research, Prepared the manuscript. Yuta Orihara: Performed the research, Prepared the manuscript. Rieko Kawamura: Performed the research, Prepared the manuscript. Kazuo Imai: Performed the research, Provided scientific guidance, Prepared the manuscript. Jun Sakai: Performed the research, Prepared the manuscript. Norihito Tarumoto: Performed the research, Provided scientific guidance. Masaru Matsuoka: Prepared the manuscript, Providing scientific guidance. Shinichi Takeuchi: Provided scientific guidance, Reviewed and Edited. Shigefumi Maesaki: Provided scientific guidance, Reviewed and Edited. Takuya Maeda: Designed the research, Prepared the manuscript, Reviewed and Edited.

Funding
This study did not receive funding support.

Conflict of interest
All authors have no conflicts of interest to declare.

Declaration of competing interest
The authors declare that they have no competing interests.

Acknowledgments
None.
References

[1] Huang. C, Wang. Y, Li. X, et al., Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China, Lancet. 395 (2020) 497-506.

[2] Li. Q, Guan. X, Wu. P, et al., Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. N Engl J Med, In press. doi: 10.1056/NEJMoA2001316.

[3] Wang. J, Zhou. M, Liu. F, Exploring the reasons for healthcare workers infected with novel coronavirus disease 2019 (COVID-19) in China. J Hosp Infect, In Press. doi: 10.1016/j.jhin.2020.03.002.

[4] Boehme. CC, Nabeta. P, Henastroza. G, et al., Operational feasibility of using loop mediated isothermal amplification (LAMP) for the diagnosis of pulmonary TB in microscopy centers of developing countries, J Clin Microbiol, 45 (2007) 1936-1940.

[5] Mitarai. S, Okumura. M, Toyota. E, et al., Evaluation of a simple loop mediated isothermal amplification test kit for the diagnosis of tuberculosis, Int J Tuberc Lung Dis, 15 (2011) 1211-1217.

[6] National Institute of Infectious Diseases, Japan. Manual for the Detection of Pathogen 2019-nCoV Ver.2.6. https://www.niid.go.jp/niid/ja/diseases/ka/corona-virus/2019-ncov/2484-idsc/9403-labo-manual.html.

[7] Park. GS, Ku. K, Baek. SH, et al., Development of Reverse Transcription Loop-mediated Isothermal Amplification (RT-LAMP) Assays Targeting SARS-CoV-2, J Mol Diagn, In Press. doi: 10.1016/j.jmoldx.2020.03.006.

[8] Yan. C, Cui. J, Huang. L, et al., Rapid and visual detection of 2019 novel coronavirus (SARS-CoV-2) by a reverse transcription loop-mediated isothermal amplification assay, Clin Microbiol Infect, In Press. doi: 10.1016/j.cmi.2020.04.001.
Figure Legends

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

![Graph showing turbidity over time for different dilutions.](image)

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

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