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Original Article

Evaluation of SARS-CoV-2 RNA quantification by RT-LAMP compared to RT-qPCR[1]

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ABSTRACT

Introduction: Coronavirus disease 2019 (COVID-19) is a global pandemic caused by a novel virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The viral load of SARS-CoV-2 is associated with mortality in COVID-19 patients. Measurement of viral load requires the use of reverse transcription quantitative PCR (RT-qPCR), which in turn requires advanced equipment and techniques. In this study, we aimed to evaluate the viral load measurement using reverse transcription loop-mediated isothermal amplification (RT-LAMP), which is a simpler procedure compared to RT-qPCR.

Materials and methods: RNA was extracted by using the QIAamp Viral RNA Mini Kit. The RT-LAMP assay was performed by using the Loopamp® 2019-SARS-CoV-2 detection reagent kit and 10-fold serial dilutions of known viral load RT-LAMP were used to measure Tt, which is the time until the turbidity exceeds the threshold. Based on the relationship between viral load and Tt, the linearity and detection sensitivity of the calibration curve were evaluated. In addition, 117 clinical specimens were measured, and RT-qPCR and RT-LAMP assay results were compared.

Results: The dilution linearity of the calibration curve was maintained at five orders of magnitude 1.0×10^6 to 1.0×10^1 copies/μL, and was confirmed to be detectable down to 1.0×10^0 copies/μL. The limit of quantification of RNA extracted from clinical specimens using RT-LAMP correlated well with that obtained using RT-qPCR ($r^2 = 0.930$).

Conclusion: The findings indicate that RT-LAMP is an effective method to determine the viral load of SARS-CoV-2.

1. Introduction

Coronavirus disease 2019 (COVID-19) is caused by a novel virus known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). COVID-19 was first reported in Wuhan, China in 2019 and has since been declared a global pandemic by the World Health Organization (WHO) due to its rapid spread worldwide [1]. Unlike other coronavirus infections such as severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS), the active infectious nature of SARS-CoV-2, and the fact that COVID-19 patients often exhibit mild symptoms or are asymptomatic have resulted in a pandemic [2,3]. Most mild and asymptomatic infections remain undiagnosed, and their number is estimated to be ten times the number of diagnosed cases [4]. Therefore, rapid and accurate diagnosis of infection is important in COVID-19 patients for infection control and treatment. An independent relationship between high viral load and mortality has also been reported; thus, it is important to accurately quantify viral load [5–7].

Currently, the most reliable and widely used test for the diagnosis of COVID-19 is the reverse transcription-quantitative PCR (RT-qPCR) test performed on upper respiratory tract specimens such as nasopharyngeal

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[1] Abbreviations: COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; RT-LAMP, reverse transcription loop-mediated isothermal amplification; RT-qPCR, reverse transcription quantitative PCR, MERS, Middle East respiratory syndrome, SARS, severe acute respiratory syndrome.

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swabs and saliva [8]. However, RT-qPCR has several limitations, including the need for skilled staff, sophisticated equipment for sample processing, sophisticated and expensive laboratory equipment for sample processing, and long reaction times. On the other hand, reverse transcription loop-mediated isothermal amplification (RT-LAMP) is a rapid, sensitive, and effective visual nucleic acid amplification method [9]. Its principle is based on the replacement of the DNA strand by *Bst* DNA polymerase and the formation of a stem-loop structure using four specific primers in six regions. The LAMP reaction takes place under isothermal conditions of 60–65 °C and does not require expensive and complex equipment such as a thermal cycler. RT-LAMP has been widely applied in the detection of RNA viruses such as influenza virus, SARS coronavirus, MERS coronavirus, and others [10–13]. In terms of the clinical performance of RT-LAMP and its correlation with RT-qPCR, the sensitivity of RT-LAMP for SARS-CoV-2 in upper and lower respiratory tract specimens has been reported to be comparable to that of RT-qPCR, although the number of clinical specimens used in these studies is small [14,15]. The quantification of DNA by the LAMP method using lambda DNA has been studied, but the quantification of RNA using clinical specimens has not been reported [16]. In this study, we aimed to evaluate the viral load measurement using RT-LAMP.

2. Materials and Methods

2.1. Clinical specimens

Among the nasopharyngeal swabs collected from COVID-19 patients at the Osaka Medical College Hospital between March to October 2020, we used 117 specimens that had been consented to by opting out. The process of virus inactivation was performed in a biosafety level 2 safety cabinet by trained staff who were wearing appropriate personal protective equipment.

2.2. RNA extraction

Viral RNA was extracted from the collected nasopharyngeal swabs by using the QIAamp Viral RNA Mini Kit (Qiagen) in combination with the automated QIAcube system (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The extracted RNA samples were stored at −80 °C and amplified by RT-qPCR and RT-LAMP.

2.3. RT-qPCR assay

RT-qPCR assays were performed according to the protocol developed by the National Institute of Infectious Diseases, which is nationally recommended in Japan for SARS-CoV-2 detection [17]. RT-qPCR assays were performed in a QuantStudio® 5 Real-time PCR System (Thermo Fisher, Massachusetts, USA) by using the QuantiTect Probe RT-PCR Kit (Qiagen) with 5 μL of template RNA and the following probe and set of primers: the nucleocapsid protein set no. 2 (N2), forward primer 5′-AAA TTT TGG GGA CCA GGA AC-3′, and reverse primer 5′-FAM-ATG TCG CGC ATT GGC ATG GA-TAMRA-3′(17). The thermal cycling conditions used are as follows: 50 °C for 30 min and 95 °C for 15 min, followed by 45 cycles of 95 °C for 15 s and 60 °C for 1 min. The total reaction time was 130 min. The number of cycles required for the fluorescence to reach the threshold was defined as the cycle threshold (Ct), if the threshold was not reached in 45 cycles, it was judged to be below undetermined.

2.4. RT-LAMP assay

The RT-LAMP assay was performed using the Loopamp® 2019-SARS-CoV-2 detection reagent kit (Eiken Chemical, Tokyo, Japan) according to the manufacturer's instructions. Fifteen microliters of primer mix2019-nCoVwas mixed with 10 μL of template RNA to make a total reaction volume of 25 μL, which was incubated at 62.5 °C for 35 min in LoopampEXIA® (Eiken Chemical), a real-time turbidity measuring device. The threshold time (Tt) was determined automatically by LoopampEXIA® and defined as the time taken for the turbidity to exceed the threshold value. If the turbidity did not exceed the threshold value after 35 min of measurement, it was judged to be negative.

Table 1
Reproducibility of Tt values obtained from RT-LAMP.

viral load (copies/μL)	Tt					CV (%)
	Run1	Run2	Run3	Run4	Average	
1.0×10^6	11:12	11:30	11:24	11:36	11:26	1.2%
1.0×10^5	12:36	12:42	12:48	13:00	12:46	1.0%
1.0×10^4	13:54	14:00	13:54	14:00	13:57	0.3%
1.0×10^3	15:12	15:18	15:06	15:06	15:11	0.5%
1.0×10^2	16:30	16:42	16:18	16:30	16:30	0.8%
1.0×10^1	18:00	17:48	18:12	18:06	18:02	0.7%
1.0×10^0	30:36	28:30	22:06	23:06	26:05	12.2%

*Tt: threshold time, CV: coefficient of variation.

2.5. Creating a calibration curve

Positive RNA controls were prepared in 10-fold serial dilutions from 1.0×10^6 to 1.0×10^1copies/μL of the vitro synthesized SARS-CoV-2 RNA, which was provided by the National Institute of Infectious Diseases, and measured to create a calibration curve by RT-qPCR(17). RNA was extracted from a pooled sample of 10 pooled clinical samples of nasopharyngeal swabs with high RNA viral load, and the RNA viral load was measured from the calibration curve obtained by RT-qPCR. The resulting RNA extracts were adjusted by Distilled deionized water (Nippon gene, Tokyo, Japan) with carrier RNA (QIAGEN) added to bring the viral load from 1.0×10^6 to 1.0×10^0 copies/μL, and measured by RT-qPCR and RT-LAMP.

From the results obtained, a calibration curve was prepared using the Ct for RT-qPCR and the Tt for RT-LAMP to evaluate the virus load in the clinical samples.

2.6. Statistical analysis

All statistical analyses were performed using JMP® 15 data analysis software (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Creation of a calibration curve using RT-LAMP

RNA extracts diluted 10-fold from 1.0×10^6 to 1.0×10^0copies/μL were measured by RT-LAMP assay and their Tt values were obtained (Table 1). We were able to detect up to 1.0×10^0 copies/μL in all quadruple measurements, and the linearity of the calibration curve was maintained from 1.0×10^6 to 1.0×10^1 copies/μL [Log (copies number of RNA) = 33.9229–0.0294409 * Tt, $r^2 = 0.995$]. The dilution linearity of the calibration curve was maintained at five orders of magnitude 1.0×10^6 to 1.0×10^1 copies/μL, and was confirmed to be detectable down to 1.0×10^0 copies/μL.

Table 2
Relationship between RT-LAMP and RT-qPCR results when measured in duplicated.

		RT-qPCR		Toal
		Positive	Undetermined	
RT-LAMP	Positive	104	7	111
	Negative	2	4	6
Total		106	11	117

*Each was measured in duplicate, and if any one of them was positive, it was considered positive.

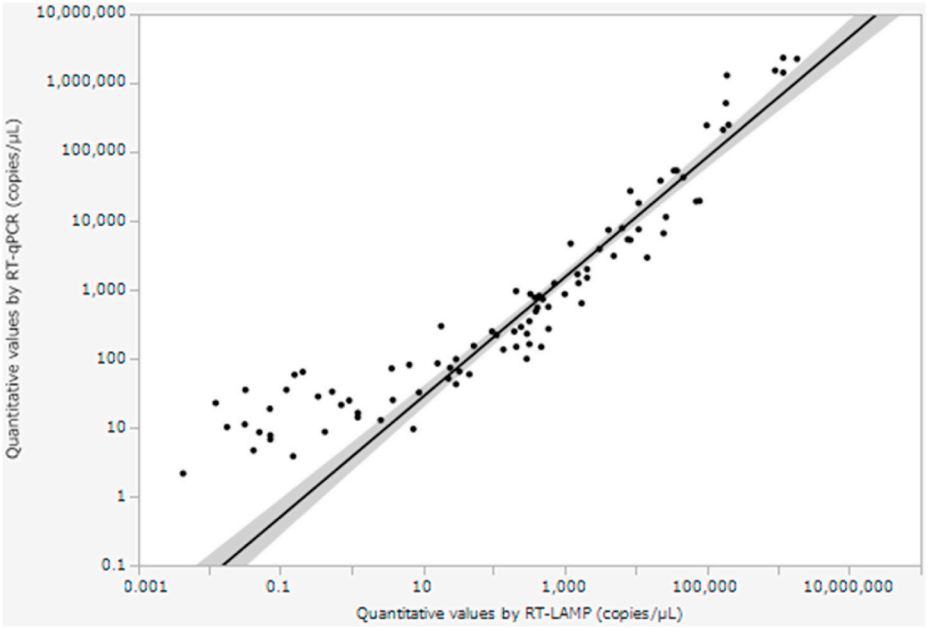

Fig. 1. Viral quantification by RT-LAMP and RT-qPCR per μL of RNA extract. RT-LAMP correlated well with RT-qPCR up to 1.0×10^1copies/μL.,$r^2 = 0.930$.

3.2. Correlation of RT-LAMP and RT-qPCR viral load in clinical specimens

Each of the 117 nasopharyngeal swabs was measured in duplicate and if any one of them was positive, it was considered positive. The result showed were positive in 90.6% (106/117) samples as detected by RT-qPCR and in 94.9% (111/117) samples as detected by RT-LAMP (Table 2). In addition, The mean viral load was also plotted for 96 cases that were positive for both methods in duplicate from 104 cases that were positive for both methods by RT-qPCR and RT-LAMP. RT-qPCR and RT-LAMP results showed good correlation, up to 1.0×10^1copies/μL. ($r^2 = 0.930$, Fig. 1).

4. Discussion

In this study, we investigated the measurement of viral load of SARS-CoV-2 by RT-LAMP. The dilution linearity of the calibration curve was maintained at five orders of magnitude 1.0×10^6 to 1.0×10^1 copies/μL, and was confirmed to be detectable down to 1.0×10^0 copies/μL. These results suggest that RT-LAMP can measure the amount of RNA viral load at unknown concentrations to some extent, similar to the RT-qPCR method commonly used in clinical laboratory as a quantitative method for SARS-CoV-2.

In addition, RT-LAMP was confirmed to be detectable down to 1.0×10^0 copies/μL, similar to what has been reported previously [14]. The percentage of positive results for RT-LAMP was higher than that for RT-qPCR in the duplicate measurement, but this may be due to the large number of RT-LAMP target regions (six) and the fact that the amount of RNA added in RT-LAMP was 10 μL, higher than that in RT-qPCR (5 μL).

For measurement of SARS-CoV-2 viral infectivity, culture tests are more useful than genetic tests [18,19]. However, compared to RT-qPCR, there are only a limited number of facilities that can perform viral cultures in hospital laboratories. A study examining the correlation between viral culture and RT-qPCR suggests that a Ct value less than 32 is indicative of a positive culture [20]. If we compare the same to the Tt value of RT-LAMP in this study, if the Tt value is shorter than 16 min, it is considered to be infectious. However, results need to be interpreted with caution as the Ct values vary depending on the measurement method [21].

This study has several limitations. Because this study used RNA extracted from clinical specimens to create a calibration curve, we did not assume that the RT-LAMP assay was performed directly from the samples. One factor that could affect the quantification is the possibility of false positives due to cloudy samples. For example, if samples, such as those of saliva, are tested without RNA extraction, it could affect the quantification. Secondly, we were unable to verify the differences between the amplification units. In this regard, we believe that its effect was small because the coefficient of variation of Tt was generally within 3% when the same RNA extract was measured in the three units in our laboratory (data not shown).

Translating qualitative tests into quantitative measures of viral load may help clinicians to risk-stratify patients and select the right treatment method. It may also have an impact on infection control based on infectivity.

5. Conclusions

RT-LAMP measures turbidity in real-time, suggesting that it can measure the amount of SARS-CoV-2 viral load somewhat close to RT-qPCR, and can detect the same as RT-qPCR.

Authorship statement

All authors meet the ICMJE authorship criteria. Kenta Minami was the chief investigator and responsible for the data analysis. Kenta Minami, Ryota Masutani, Youichi Suzuki, Toyofumi Nakanishi and Takashi Nakano developed the trial design. Contributors Meri Kubota, Naofumi Osaka and Akira Ukimura was responsible for the organization and coordination of the trial.

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Ethical approval

The Ethics Committee of Osaka Medical College (Osaka, Japan) approved the study design (Protocol number 2020-057).

Declaration of competing interest

The authors declare that they have no conflicts of interest.

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References

[1] Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395:497–506.
[2] Wei WE, Li Z, Chiew CJ, Yong SE, Toh MP, Lee VJ. Presymptomatic transmission of SARS-CoV-2 - Singapore, january 23-march 16, 2020. MMWR Morb Mortal Wkly Rep 2020;69:411–5.
[3] Hoxha A, Wyndham-Thomas C, Klamer S, Dubourg D, Vermeulen M, Hammami N, et al. Asymptomatic SARS-CoV-2 infection in Belgian long-term care facilities. Lancet Infect Dis 2021 Apr;21(4):e67. https://doi.org/10.1016/S1473-3099(20)30560-0. Epub 2020 Jul 3.
[4] Stringhini S, Wisniak A, Piumatti G, Azman AS, Lauer SA, Baysson H, et al. Seroprevalence of anti-SARS-CoV-2 IgG antibodies in Geneva, Switzerland (SEROCoV-POP): a population-based study. Lancet 2020;396:313–9.
[5] Pujadas E, Chaudhry F, McBride R, Richter F, Zhao S, Wajnberg A, et al. SARS-CoV-2 viral load predicts COVID-19 mortality. Lancet Respir Med 2020;8:e70.
[6] Bryan A, Fink SL, Gattuso MA, Pepper G, Chaudhary A, Wener MH, et al. SARS-CoV-2 viral load on admission is associated with 30-day mortality. Open Forum Infectious Diseases; 2020.
[7] Westblade LF, Brar G, Pinheiro LC, Paidoussis D, Rajan M, Martin P, et al. SARS-CoV-2 viral load predicts mortality in patients with and without cancer who are hospitalized with COVID-19. Canc Cell 2020;38. 661-71.e2.
[8] Sethuraman N, Jeremiah SS, Ryo A. Interpreting diagnostic tests for SARS-CoV-2. J Am Med Assoc 2020;323:2249–51.
[9] Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, et al. Loop-mediated isothermal amplification of DNA. Nucleic Acids Res 2000;28:E63.
[10] Hong TC, Mai QL, Cuong DV, Parida M, Minekawa H, Notomi T, et al. Development and evaluation of a novel loop-mediated isothermal amplification method for rapid detection of severe acute respiratory syndrome coronavirus. J Clin Microbiol 2004;42:1956–61.
[11] Bhadra S, Jiang YS, Kumar MR, Johnson RF, Hensley LE, Ellington AD. Real-time sequence-validated loop-mediated isothermal amplification assays for detection of Middle East respiratory syndrome coronavirus (MERS-CoV). PloS One 2015;10:e0123126.
[12] Huang P, Wang H, Cao Z, Jin H, Chi H, Zhao J, et al. A rapid and specific assay for the detection of MERS-CoV. Front Microbiol 2018;9:1101.
[13] Parida M, Horioke K, Ishida H, Dash PK, Saxena P, Jana AM, et al. Rapid detection and differentiation of dengue virus serotypes by a real-time reverse transcription-loop-mediated isothermal amplification assay. J Clin Microbiol 2005;43:2895–903.
[14] Kitagawa Y, Orihara Y, Kawamura R, Imai K, Sakai J, Tarumoto N, et al. Evaluation of rapid diagnosis of novel coronavirus disease (COVID-19) using loop-mediated isothermal amplification. J Clin Virol 2020;129:104446.
[15] Yan C, Cui J, Huang L, Du B, Chen L, Xue G, 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 2020;26:773–9.
[16] Mori Y, Kitao M, Tomita N, Notomi T. Real-time turbidimetry of LAMP reaction for quantifying template DNA. J Biochem Biophys Methods 2004;59:145–57.
[17] Shirato K, Nao N, Katano H, Takayama I, Saito S, Kato F, et al. Development of genetic diagnostic methods for detection for novel coronavirus 2019(nCoV-2019) in Japan. Jpn J Infect Dis 2020;73:304–7.
[18] Rhee C, Kanjilal S, Baker M, Klompas M. Duration of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infectivity: when is it safe to discontinue isolation? Clin Infect Dis 2021 Apr 26;72(8):1467–74. https://doi.org/10.1093/cid/ciaa1249.
[19] Arons MM, Hatfield KM, Reddy SC, Kimball A, James A, Jacobs JR, et al. Presymptomatic SARS-CoV-2 infections and transmission in a skilled nursing facility. N Engl J Med 2020;382:2081–90.
[20] Basile K, McPhie K, Carter I, Alderson S, Rahman H, Donovan L, et al. Cell-based culture of SARS-CoV-2 informs infectivity and safe de-isolation assessments during COVID-19. Clin Infect Dis 2020 Oct 24:ciaa1579. https://doi.org/10.1093/cid/ciaa1579. Online ahead of print.
[21] Binnicker MJ. Challenges and controversies to testing for COVID-19. J Clin Microbiol 2020;58. e01695-20.