Original Article

JHSMR Journal of Health Science and Medical Research

Comparing the Effectiveness of Colorimetric Reverse Transcription–Loop–Mediated Isothermal Amplification (SCoV–2 Detection Kit L1) with that of Reverse Transcription–Quantitative Polymerase Chain Reaction in SARS–CoV–2 Detection

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Received 3 September 2024 ● Revised 2 January 2025 ● Accepted 27 January 2025 ● Published online 15 May 2025

Abstract:

Objective: To determine the diagnostic sensitivity and specificity of reverse transcription–loop–mediated isothermal amplification (RT–LAMP) compared to those of reverse transcription–quantitative polymerase chain reaction (RT–qPCR) for coronavirus disease 2019 (COVID–19).

Material and Methods: A total of 382 nasopharyngeal swab samples obtained from 154 patients with COVID–19 were tested using RT–LAMP and RT–qPCR. The sensitivities and specificities of RT–LAMP were compared with those of RT–qPCR and analysed as a function of time from onset.

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J Health Sci Med Res
doi: 10.31584/jhsmr.20251208
www.jhsmr.org

Results: Up to the third day after onset, the RT-LAMP SARS-CoV-2 positivity was 68.33%, and the sensitivity and specificity compared to those of RT-qPCR were 100.0%. However, on the third day after onset, the RT-LAMP SARS-CoV-2 positivity decreased to less than 50%. The limit of detection for the RT-LAMP assay was $\log_{10}$ SARS-CoV-2 RNA 2.2 copies/reaction. RT-LAMP had the same diagnostic accuracy as RT-qPCR until day 9 after symptom onset.
Conclusion: The findings suggest that RT-LAMP can be used as an alternative to RT-qPCR as a diagnostic tool for detecting COVID-19 during the acute symptomatic phase of COVID-19.

Keywords: COVID-19, reverse transcription-loop-mediated isothermal amplification, reverse transcription-quantitative polymerase chain reaction, SARS-CoV-2

Introduction

The World Health Organization (WHO) declared severe acute respiratory syndrome coronavirus (SARS-CoV)-2 a global public health emergency on January 30, 2020[1]. Coronavirus disease 2019 (COVID-19), which originated in the Wuhan Province, China, resulted in travel restrictions, bans on public gatherings, and a negative impact on the global economy[2,3]. COVID-19 is marked by a high rate of illness but relatively low mortality, posing a significant risk, especially to elderly individuals with weakened immune systems and those with underlying health conditions. Current data indicates that the virus has an estimated case fatality rate of approximately 1%[4]. This rate is several times higher than that of typical seasonal flu and falls between the severity of the 1957 influenza pandemic (0.6%) and the 1918 pandemic (2%)[4]. However, it is less severe than SARS, which had a case fatality rate of 9.5%, and Middle East Respiratory Syndrome (MERS), which was at 34.4%[5]. Additionally, an average individual infected with COVID-19 spreads the virus at a rapid, exponential rate. Strong evidence suggests that the virus can be transmitted by individuals who show no symptoms or only mild symptoms[6], complicating efforts to control its spread compared to other coronaviruses like SARS-CoV and MERS-CoV. COVID-19 has resulted in roughly 10 times more cases than SARS-CoV in just one-fourth of the time[7]. Unlike MERS-CoV and SARS-CoV (which likely had camels and civet cats as intermediate hosts, respectively), the source of COVID-19 remains uncertain. While bats are suspected to be the reservoir hosts, recent studies suggest that intermediate carriers might include snakes or pangolins, according to WHO and other sources[8-11].

The median incubation period for COVID-19 is 4 days, ranging from 0 to 24 days, with the longest reported incubation period being 24 days[12]. Notably, some individuals infected with COVID-19 do not show obvious clinical symptoms[13,14]. The extended incubation period and the potential for asymptomatic infections suggest a high risk of community transmission of SARS-CoV-2[15,16]. To mitigate this risk, there is a need for rapid point-of-care tests to identify the virus in suspected cases at community clinics and hospitals, and possibly even through house-to-house testing. Currently, samples from suspected cases at community clinics and hospitals are often initially screened using Antigen Test Kits (ATKs), while reverse transcription-quantitative polymerase chain reaction (RT-qPCR), which requires more expensive equipment and specialised technicians, serves as the reference standard for confirmatory testing. The turnaround time for results can be up to 72 hours. Such delays can lead to anxiety

and may contribute to further virus spread, as there is no guarantee that individuals will self-isolate after undergoing standard real-time RT-PCR testing at a centralised lab[17].

Point-of-care (POC) testing for COVID-19 can alleviate anxiety, minimise lengthy turnaround times, and help reduce the virus's spread. A device for point-of-care testing that is quick, reliable, cost-effective, and can be used on-site or in the field without needing trained personnel[18] is essential and urgently needed for the detection of SARS-CoV-2. Testing to reduce the spread of an outbreak, such as COVID-19, substantially controls infectious diseases[19-21].

There is a strong demand for new methods to detect COVID-19, and one promising approach is loop-mediated isothermal amplification (LAMP). Unlike traditional PCR tests, LAMP amplifies nucleic acids at a constant temperature, eliminating the need for specialised equipment like a thermal cycler. This distinctive method of nucleic acid amplification allows LAMP-based assays for viral RNA/DNA to be faster, simpler, and more cost-effective for diagnosing the virus than RT-qPCR assays. The LAMP method offers other several advantages, such as its broad tolerance to pH and temperature variations, the capability to use unprocessed samples, and the flexibility in readout methods. Despite these benefits, it maintains a specificity and sensitivity comparable to that of PCR tests. This study aimed to compare the reverse transcription-LAMP (RT-LAMP) assay specificity and sensitivity for SARS-CoV-2 detection using the WHO-recommended RT-qPCR assay.

Material and Methods

Clinical specimens

The study was approved by the Human Research Ethics Committee of the Faculty of Medicine, Prince of Songkla University (protocol code REC 64-203-14-1). The inclusion criteria for the study were (1) patients aged ≥18 years, (2) those admitted to the hospital between 1 October 2020 and 31 March 2022, and (3) diagnosed with either community-acquired respiratory tract infection or healthcare-associated respiratory tract infection. Exclusion criteria were (1) records with less than 50% data completeness, (2) an initial diagnosis of hospital-acquired or ventilator-associated respiratory tract infection, and (3) co-infection of SARS-CoV-2 with other pathogens. All 382 nasopharyngeal swab samples were retrieved from patients at Songklanagarind Hospital, Songkhla, Thailand, between April 12 and July 2, 2021. Severity was classified according to the National Institutes of Health COVID-19 Treatment Guidelines (https://www.covid19 treatment guidelines.nih.gov). Swab samples were collected using a flocked sterile plastic swab applicator and placed in a 3 mL BD Universal Viral Transport Medium (Becton Dickinson and Company, Franklin Lakes, NJ, USA).

RNA extraction

The total RNA of SARS-CoV-2 was extracted using the magLEAD automated system (Precision System Science Co., Ltd). The concentration and purity of extracted RNA were assessed using a NanoDrop spectrophotometer, and the integrity of extracted RNA was observed using agarose gel electrophoresis.

RT-qPCR method

The RT-qPCR was used as a reference method to detect the ORF1ab and N genes, which exhibit substantial conservation across SARS-CoV-2. The primer/probe sets for sequencing the ORF1ab gene and N gene are designed to encompass the Alpha, Beta, Delta, Gamma, and Omicron variants of concern currently recognized by WHO. The RT-qPCR procedure was performed as previously described[22].

As RT-qPCR was used as the standard technique, we employed the SCoV-2 Detection Kit L1, which contains 6 primers targeting the spike (*S*) and nucleocapsid (*N*)

genes of SARS-CoV-2. The kit includes an external positive control composed of SARS-CoV-2 DNA, *S* and *N* genes prepared from recombinant DNA, and a negative control using RNase/DNase-free distilled water. The procedure utilised a reverse transcriptase enzyme for cDNA synthesis and *Bst* DNA polymerase for DNA amplification. Further details about the equipment used in the RT-qPCR process can be provided upon request.

RT-LAMP primer design

Each primer set contained 6 specific lines for detecting the 2019 novel coronavirus (Supplementary Table 1). It was designed from the N and S regions of the complete genome of SARS-CoV-2 isolate Wuhan-Hu-1 (accession no. NC_045512) and the human RNase P gene, which is an internal gene, using Primer Explorer version 4 (http://primerexplorer.jp/elamp4.0.0/index.html) and the NEB LAMP Primer Design Tool (https://lamp.neb.com). The synthetic primer sets were ordered from Macrogen (Seoul, South Korea). This LAMP assay, using these primer sets, can detect all SARS-CoV-2 types but cannot differentiate specific mutations or variants.

RT-LAMP reaction

The SCoV-2 Detection Kit L1, an in-house COVID-19 test kit, contains 6 primers specific to *S* and *N* genes of the virus. The procedure began with the conversion of RNA to cDNA by reverse transcriptase. Then, the *Bst* DNA polymerase enzyme that was stable and well-functioning at 60–65 °C was used. The amount of DNA was increased by strand displacement. Increasing the amount of DNA increases the acidity of the solution. Thus, the pH level changed from 8.2 to 6.0, causing Phenol Red, which served as an indicator, to change from red to yellow.

Standard control of the inspection process was done using the SCoV-2 Detection Kit L1, consisting of external positive and negative controls. The positive control consisted of SARS-CoV-2 DNA, *S*, and *N* genes prepared from the recombinant DNA pUC57_21,563-25,384, pcDNA3.1(+) 28,274-29,553, and specific primers. The negative control was prepared using RNase/DNase-free distilled water.

Positive plasmid DNA samples that contained the SARS-CoV-2 genes Supplementary Table 2 and RNA from positive and negative 2019 coronavirus-infected samples were tested with the RT-LAMP using a total of 25 µL LAMP reaction. LAMP was conducted at 65 °C for 60 minutes in a water bath. The proportion of the method components and RNA solution extracted from the samples used for the detection was as follows: outer primer F3 and outer primer B3 with 0.2-µM concentration, FIP inner primer, BIP inner primer at 1.6 µM, loop primers, LoopF, and Loop B, at 0.4 µM, WarmStart® Colorimetric LAMP 2X Master Mix (NEB, UK) solution that contained 4 types of bases (dNTPs), *Bst* DNA polymerase enzyme, and phenol red solution. RNA samples (1 µL) were added. Distilled water was later added to adjust the volume to 25 µL. After 30–60 minutes of incubation, the results were immediately observed with the naked eye. If the samples had increased amounts of DNA, the colour of the LAMP changed to yellow after the reaction. If the samples tested negative, the LAMP mixture remained pink after the reaction.

Statistical analysis

Two-by-two tables were created, and the analytical performance metrics, including sensitivity and specificity of RT-LAMP, were calculated with 95% confidence intervals (CIs) and compared to those of RT-qPCR. Pearson's chi-square test was used to calculate p-values for comparing the positivity rates between RT-LAMP and RT-qPCR in each group. The Kruskal-Wallis test was applied to analyse the genomic copy numbers across groups. Additionally, the Wilcoxon rank-sum test was utilised to compare genomic copy numbers for positive and negative RT-LAMP test results. Statistical significance was set at p-value<0.05. All

statistical analyses were performed using the R program 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria, 2018).

The equations used to calculate sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy are below:

Sensitivity = (TP/ (TP + FN))

Specificity = (TN / (TN + FP))

Positive predictive value (PPV) = (TP / (TP + FP))

Negative predictive value (NPV) = (TN / (TN + FN))

Diagnostic accuracy = ((TP + TN) / (TN + FP + FN + TN)

TP, TN, FP, and FN represent true positive, true negative, false positive, and false negative, respectively.

Results

Patient characteristics

Table 1 presents the characteristics of the studied patients. A total of 382 pairs of nasopharyngeal and throat swabs were obtained from the patients. Two hundred and twenty-eight (59.69%) samples were RT-qPCR negative and 154 (40.31%) were RT-qPCR positive. One hundred and ninety-six (51.3%) patients were male. The median (interquartile range; IQR) age was 46.0 (30.2–61.0) years and the median (IQR) onset of symptoms was 2 (1–4) days.

Among 154 patients diagnosed with COVID-19 via nasopharyngeal and throat swab RT-qPCR, the median (IQR) age was 46.0 (31.0–59.0) years. Thirty-four (26%) individuals presented with a runny nose and cough, and the IQR onset of symptoms occurred before 2 (1–4) days. Common symptoms at presentation were cough and runny nose (34, 26%), sore throat (31, 26%), and dyspnoea (27, 20.6%). The comorbidities were diabetes, hypertension, dyslipidemia (26; 59.1%), and cancer (5; 11.4%). Most patients had mild symptoms (124; 80.5%), length of hospital stay (IQR) was 11 (10–15) days. Sixty-four (41.6%) received antiviral medication (Supplementary Table 3).

When compared to 228 individuals with negative RT-qPCR nasopharyngeal and throat swab results, symptoms at presentation and fever were statistically significant. Diabetes, hypertension, dyslipidaemia, and cancer were significantly associated with comorbidities. The other characteristics were not significantly different.

RNA quantification by RT-qPCR

Figure 1 displays the RNA copy numbers of SARS-CoV-2, which were measured by RT-qPCR for each group, with the median number of $\log_{10}$ SARS-CoV-2 RNA (copies/reaction) in each group. Group A consists of samples collected on the 1st to 3rd days after symptom onset, Group B on the 4th to 6th days, Group C on the 7th to 9th days, and Group D on the 10th day or later. The median RNA copy number of all positive samples was 6.7 copies/reaction (IQR, 2.3–7.4). Compared to other groups, the RNA copy numbers of positive samples in Group A were significantly higher, with a median of 6.9 copies per reaction (IQR, 6.2–7.5). Group B numbers were significantly higher than those in Group C, which were significantly higher than in Group D. The median RNA copy numbers of the samples in groups B to D were 6.4 (IQR, 5.6–7.4), 5.3 (IQR, 4.2–6.4), and 2.2 copies/reaction (IQR, 1.7–2.9), respectively.

Comparison of RT-LAMP with RT-qPCR

Table 2 demonstrates the sensitivity and specificity of RT-LAMP and RT-qPCR. In all RT-LAMP assays, the colour of the reaction tube changed from red to yellow (indicating a positive result for SARS-CoV-2 RNA), which was assessed visually (Figure 2). The positivity rate for RT-qPCR was 40.31% (154/382), whereas for RT-LAMP it was 41.36% 166 (158/382). The sensitivity, specificity, and accuracy of RT-LAMP were 97.4% (95% CI, 93.5–99.3%), 96.5% (95% CI, 93.2–98.5%), and 96.9% (95% CI, 94.6–98.4%), respectively. The positivity rates were as follows: Group A had 35.49% with RT-LAMP and 33.10%

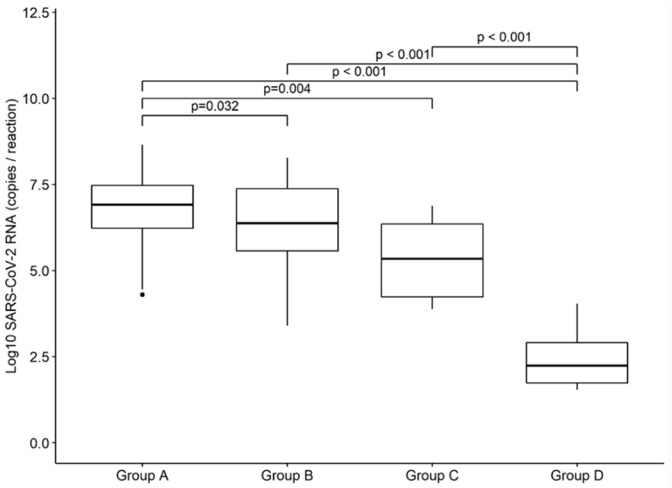
with RT-qPCR; Group B had 68.33% with RT-LAMP and 66.67% with RT-qPCR; Group C had 43.7% with both RT-LAMP and RT-qPCR; and Group D had 46.15% with RT-LAMP and 76.92% with RT-qPCR (Figure 3). There were significant differences in the positivity rates between RT-LAMP and RT-qPCR across the groups. Notably, RT-LAMP's positivity rate was significantly lower than that of RT-qPCR in Group D. The sensitivity of RT-LAMP was 100% compared to RT-qPCR in groups A–C, while its specificity was 100% in groups C and D. Additionally, the positive predictive value was 100% in groups C and D, and the accuracy was 100% only in Group C. Only 4, 7, and a single sample yielded false-negative results with RT-LAMP in groups D, A, and B, respectively.

Of the 382 samples tested using RT-LAMP and RT-qPCR, 154 were RT-qPCR-positive and contained $\log_{10}$ 1.371–8.477 SARS-CoV-2 RNA copies/reaction (Figure 4). No samples contained 1.0×10^{1} to 1.0×10^{2} copies/reaction. The number of samples with SARS-CoV-2 RNA copy numbers of 1.0×10^{2}, 1.0×10^{3}, 1.0×10^{4}, and $\geq1.0\times10^{4}$ copies/reaction were 3, 4, 7, and 140, respectively. Figure 5 exhibits the RT-LAMP positivity rates for each specific RNA copy number of SARS-CoV-2. RT-LAMP detected all samples (100%) with more than 1.0×10^{3} copies/reaction of SARS-CoV-2 RNA. In contrast, for samples with RNA copy numbers in the range of 1.0×10^{2} to 1.0×10^{3} copies/reaction, the RT-LAMP positivity rate was 75%.

Table 1 Characteristics of patients diagnosed with COVID-19 by RT-qPCR from nasopharyngeal and throat swabs

Variable	Overall (n=382)	Negative (n=228)	Positive (n=154)	p-value
Age (years), median (IQR)	46.0 (30.2–61.0)	46.0 (30.0–63.0)	46.0 (31.0–59.0)	0.636
Sex				0.757
Female, n (%)	186 (48.7)	113 (49.6)	73 (47.4)	
Male, n (%)	196 (51.3)	115 (50.4)	81 (52.6)	
Onset of symptoms before test, median (IQR)	2 (1–4)	2 (1–3)	2 (1–4)	0.094
Symptom at presentation				<0.001
Cough, n (%)	61 (21.3)	27 (17.4)	34 (26.0)	
Diarrhea, n (%)	21 (7.3)	16 (10.3)	5 (3.8)	
Dyspnoea, n (%)	59 (20.6)	32 (20.6)	27 (20.6)	
Runny nose, n (%)	56 (19.6)	22 (14.2)	34 (26.0)	
Sore throat, n (%)	72 (25.2)	41 (26.5)	31 (23.7)	
Vomiting, n (%)	17 (5.9)	17 (11.0)	0 (0)	
Fever (BT >37.5)				<0.001
No, n (%)	296 (77.5)	191 (83.8)	105 (68.2)	
Yes, n (%)	86 (22.5)	37 (16.2)	49 (31.8)	
Comorbidity				
Asthma/Obstructive pulmonary disease, n (%)	6 (4.8)	2 (2.4)	4 (9.1)	0.182
Cancer e.g., liver cancer, DLBCL, lung cancer, n (%)	39 (31.0)	34 (41.5)	5 (11.4)	0.001
ESRD, n (%)	6 (4.8)	3 (3.7)	3 (6.8)	0.420
HIV, n (%)	3 (2.4)	3 (3.7)	0 (0)	0.551
Diabetes, hypertension, or dyslipidemia, n (%)	38 (30.2)	12 (14.6)	26 (59.1)	<0.001
Ischemic heart disease, n (%)	18 (14.3)	14 (17.1)	4 (9.1)	0.340
Cerebrovascular disease, n (%)	11 (8.7)	11 (13.4)	0 (0)	0.008

IQR=interquartile range, HCC=hepatocellular carcinoma, DLBCL=diffuse large B cell lymphoma, ESRD=end-stage renal disease, HIV=human immunodeficiency virus, COVID-19=coronavirus disease 2019, RT-qPCR=reverse transcription-quantitative polymerase chain reaction



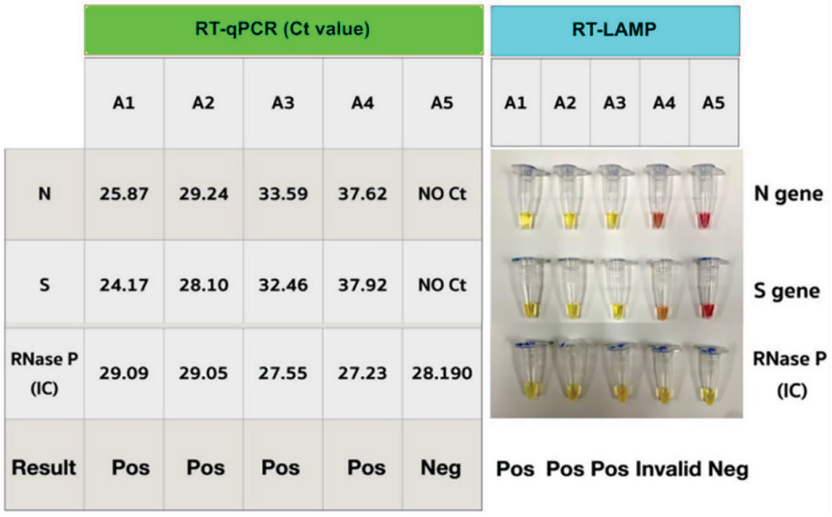
SARS-CoV-2=severe acute respiratory syndrome-coronavirus-2, RT-qPCR=reverse transcription-quantitative polymerase chain reaction

Figure 1 RNA copy numbers of SARS-CoV-2 determined by RT-qPCR in groups A–D. Groups A–D represent the samples collected on the 1st to 3rd days, the 4th to 6th days, the 7th and 9th days, and the 10th or more days after symptom onset, respectively. The box represents the 25th and 75th percentiles, with the thin line within the box indicating the median

Table 2 Sensitivity and specificity of RT-LAMP and RT-qPCR for detecting SARS-CoV-2

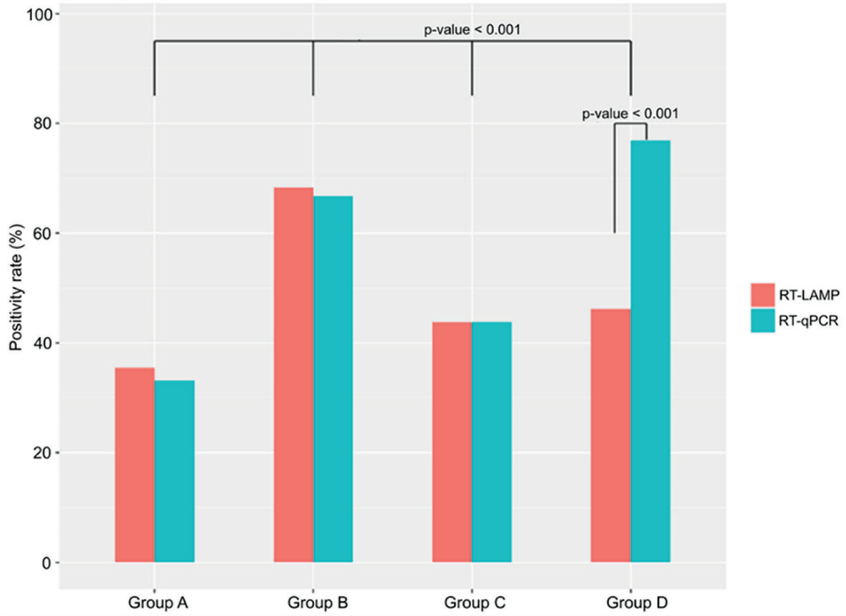
LAMP result	No. of samples with RT-qPCR result			Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Accuracy (95% CI)
	Positive	Negative	Total					
Overall								
Positive	150	8	158	97.4 (93.5, 99.3)	96.5 (93.2, 98.5)	94.9 (90.3, 97.8)	98.2 (95.5, 99.5)	96.9 (94.6, 98.4)
Negative	4	220	224					
Total	154	228	382					
Group A								
Positive	97	7	104	100.0 (96.3, 100.0)	96.4 (92.8, 98.6)	93.3 (86.6, 97.3)	100.0 (98.1, 100.0)	97.6 (95.1, 99.0)
Negative	0	189	189					
Total	97	196	293					
Group B								
Positive	40	1	41	100.0 (91.2, 100.0)	95.0 (75.1, 99.9)	97.6 (87.1, 99.9)	100.0 (82.4, 100.0)	98.3 (91.1, 100.0)
Negative	0	19	19					
Total	40	20	60					
Group C								
Positive	7	0	7	100.0 (59.0, 100.0)	100.0 (66.4, 100.0)	100.0 (59.0, 100.0)	100.0 (66.4, 100.0)	100.0 (79.4, 100.0)
Negative	0	9	9					
Total	7	9	16					
Group D								
Positive	6	0	6	60.0 (26.2, 87.8)	100.0 (29.2, 100.0)	100.0 (54.1, 100.0)	42.9 (9.9, 81.6)	69.2 (38.6, 90.9)
Negative	4	3	7					
Total	10	3	13					

Groups A–D represent the samples collected on the 1st to 3rd days, the 4th to 6th days, the 7th and 9th days, and the 10th or more days after symptom onset, respectively. PPV=positive predictive value, NPV=negative predictive value, RT-LAMP=reverse transcription-loop-mediated isothermal amplification, RT-qPCR=reverse transcription-quantitative polymerase chain reaction, SARS-CoV-2=severe acute respiratory syndrome-coronavirus-2



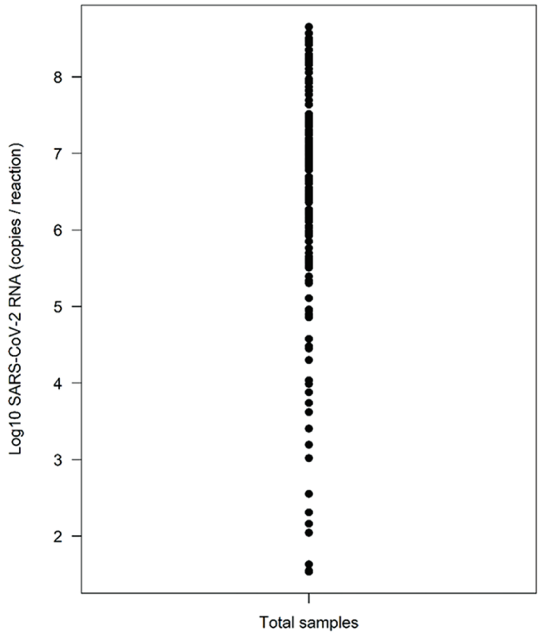
IC=internal control, Pos=positive, Neg=negative, RT-LAMP=reverse transcription-loop-mediated isothermal amplification, RT-qPCR=reverse transcription-quantitative polymerase chain reaction, Yellow denotes a positive reaction (+), While pink denotes a negative reaction (–)

Figure 2 Comparison of RT-LAMP reaction results and RT-qPCR Ct values



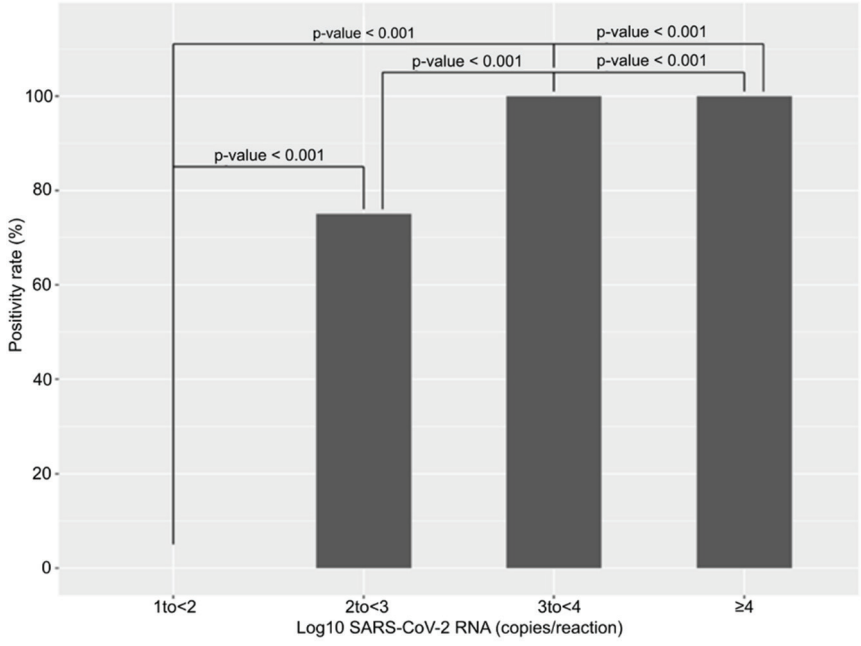
RT-LAMP=reverse transcription-loop-mediated isothermal amplification, RT-qPCR=reverse transcription-quantitative polymerase chain reaction, COVID-19=coronavirus disease 2019, Groups A–D represent the samples collected on the 1st to 3rd days, the 4th to 6th days, the 7th and 9th days, and the 10th or more days after symptom onset, respectively

Figure 3 Positivity rates of RT-LAMP and RT-qPCR in each sample group from patients with a confirmed diagnosis of COVID-19



SARS-CoV-2=severe acute respiratory syndrome-coronavirus-2, RT-qPCR=reverse transcription-quantitative polymerase chain reaction

Figure 4 SARS-CoV-2 viral load in positive samples tested with RT-qPCR

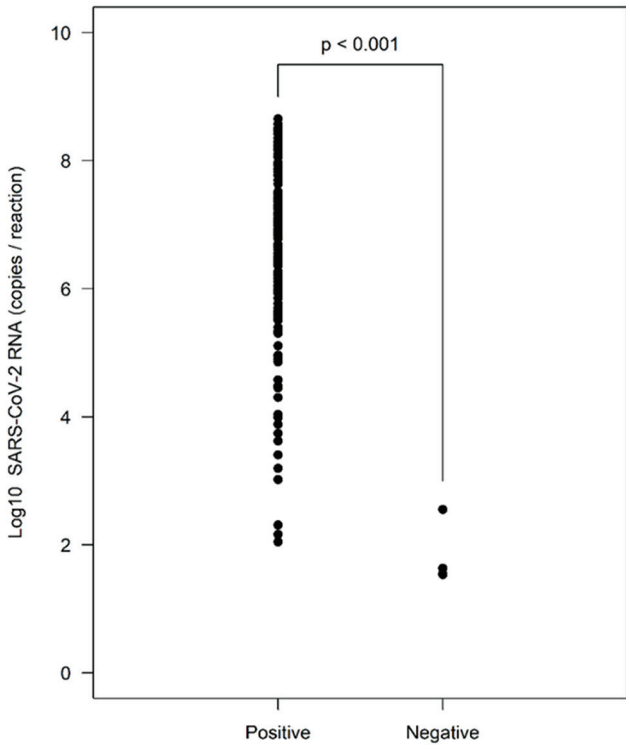


RT-LAMP=reverse transcription-loop-mediated isothermal amplification, SARS-CoV-2 RNA=severe acute respiratory syndrome coronavirus-2 ribonucleic acid, RT-qPCR=reverse transcription-quantitative polymerase chain reaction

Figure 5 Positivity rate of RT-LAMP for each genomic copy number of SARS-CoV-2 RNA as determined by RT-qPCR

Limit of detection for RT-LAMP

Figure 6 shows the difference in RNA copy numbers of SARS-CoV-2, as determined by RT-qPCR, between positive and negative RT-LAMP test results. The genomic copy numbers for positive RT-LAMP test results were significantly higher than those for negative RT-LAMP test results, with a median of $\log_{10}$ SARS-CoV-2 RNA 6.7 copies/reaction (IQR, 5.7–7.4) and 1.59 copies/reaction (IQR, 1.54–2.09), respectively. The limit of detection (LOD) for RT-LAMP, as established using 154 RT-qPCR-positive samples, was 2.2 $\log_{10}$ SARS-CoV-2 RNA copies/reaction.



SARS-CoV-2=severe acute respiratory syndrome-coronavirus-2, RT-qPCR=reverse transcription-quantitative polymerase chain reaction, RT-LAMP=reverse transcription-loop-mediated isothermal amplification

Figure 6 SARS-CoV-2 viral load in positive samples tested with RT-qPCR, comparing positive and negative RT-LAMP test results

Discussion

In this study, we examined the sensitivity and specificity of RT-LAMP in comparison to RT-qPCR, based on the duration since the onset of COVID-19 symptoms. Up to the 9th day after symptoms appeared, RT-LAMP demonstrated 100% sensitivity and specificity, indicating that it had the same accuracy of diagnosis as RT-qPCR during the acute phase of the infection. A previous study has also reported that RT-LAMP has high sensitivity compared to RT-qPCR for identifying SARS-CoV-2 in clinical respiratory samples[23]. Chow et al. found that out of 223 respiratory samples confirmed to be SARS-CoV-2 positive by RT-qPCR, 212 samples tested positive using COVID-19 RT-LAMP after 60 minutes, and 219 samples after 90 minutes, resulting in sensitivities of 95.07% and 98.21%, respectively[24]. Inaba et al. reported that the RT-LAMP sensitivity was 56.6% (95% CI, 43.3–69.0%), while its specificity was 98.4% (95% CI, 91.3–100.0%)[25]. Therefore, the SCoV-2 Detection Kit L1 had sensitivity and specificity on the ninth day after the onset of symptoms, which was higher than that reported in a previous study. However, it was uncertain how the diagnostic performance of RT-LAMP would vary over time from the onset of symptoms in real-life clinical settings, as most prior studies did not account for the stage of infection of the patients from whom the samples were obtained. This study showed that the RT-LAMP method had a positivity rate of 66.67%, which was similar to that of RT-qPCR up to the 10th day after symptom onset in patients with PCR-confirmed COVID-19. This included the sensitivity of RT-LAMP at 97.4% (with 95% CI, 93.5–99.3%), specificity at 96.5% (95% CI, 93.2–98.5%), positive predictive value at 94.9% (with 95% CI, 90.3–97.8%), negative predictive value at 98.2% (with 95% CI, 95.5–99.5%), and accuracy at 96.9% with 95% CI, 94.6–98.4%). In contrast, the positivity rates of RT-LAMP and RT-qPCR in the group with symptom onset before

the third day were lower than those of the other groups, due to having the highest negative SARS-CoV-2 sample collection. Inaba et al. also investigated the sensitivity and specificity of RT-LAMP in comparison to RT-qPCR throughout the course of COVID-19 and reported a high positivity rate of 92.8%, which is similar to that of RT-qPCR, up to the ninth day after symptom onset in patients with PCR-confirmed COVID-19[25]. However, beyond the 10th day of symptom onset, the sensitivity and positivity rate of the RT-LAMP assay decreased.

Up to the 9th day after symptom onset, the genomic copy numbers of SARS-CoV-2 RNA were generally higher compared to those observed after the 10th day. Furthermore, the RNA copy numbers of SARS-CoV-2 in negative RT-LAMP test results were significantly lower than those in positive RT-LAMP test results. Inaba et al. also found that samples with SARS-CoV-2 RNA copy numbers exceeding 1×10^2 copies/reaction had a 91.7% positivity rate with RT-LAMP. In contrast, RT-LAMP showed a much lower positivity rate of just 21.9%[24] for samples with fewer than 1.0×10^1 SARS-CoV-2 RNA copies/reaction. Consequently, the reduced RNA copy numbers of SARS-CoV-2 observed after the 10th day of symptom onset likely contributed to the decreased performance of RT-LAMP during the later stages of the illness. While RT-LAMP is an effective diagnostic tool for COVID-19 and can be used as an alternative to RT-qPCR during the acute symptomatic phase, it is less suitable for patients presenting later in the course of the illness or for confirming the clearance of SARS-CoV-2 in individuals who have previously tested positive.

We assessed the diagnostic accuracy of RT-LAMP in comparison to RT-qPCR at 3-day intervals. The decision to use 3-day intervals was based on 2 conditions. Firstly, the median interval between symptom onset and the day of the first PCR test for the 382 COVID-19 patients whose samples were analysed in this study was 2 days, and the latest group symptom onset was more than 10 days[26]. Secondly, Mallett et al. found that the positivity rate of RT-qPCR significantly decreased 10 days after symptom onset; however, the effect on RT-LAMP's positivity rate after 10 days remains unclear[26]. Therefore, we initially compared the diagnostic sensitivity and specificity of RT-LAMP and RT-qPCR during the first 10 days after symptom onset and then evaluated the diagnostic accuracy of both methods within the first 10 days and beyond that period.

The strengths of the present study are numerous sample collections compared with previous studies, as well as several LODs of RT-LAMP using the SCoV-2 Detection Kit. L1 had a $\log_{10}$ SARS-CoV-2 RNA of 2.2 copies/reaction, with an RT-LAMP positivity rate of 46.15%. Two previous studies have reported different LODs. Park et al. indicated that the detection limit of RT-LAMP was between 1 and 2 $\log_{10}$ SARS-CoV-2 RNA copies/reaction. This suggests that the RT-LAMP test kit provided high sensitivity, even when the RNA copy numbers were $\log_{10}$ SARS-CoV-2 RNA of 2 copies/reaction or lower; however, the positivity rate of RT-LAMP was low, potentially leading to false-negative results using this method[27]. Additionally, Inaba et al. reported a detection limit for RT-LAMP using the Loopamp SARS-CoV-2 Detection Kit of $\log_{10}$ SARS-CoV-2 RNA 6.7 copies/reaction, with an RT-LAMP positivity rate of 29.0%[24]. Therefore, SCoV-2 Detection Kit L1 also had a lower limit for detecting SARS-CoV-2 RNA than in previous studies.

As POC testing for COVID-19, rapid ATKs and RT-LAMP are both key POC methods. ATKs are favored for their speed, simplicity, and affordability, delivering results within 15–30 minutes with minimal training. However, they have lower sensitivity compared to nucleic acid-based methods, leading to possible false negatives at low viral loads, necessitating follow-up RT-qPCR testing. In contrast, RT-LAMP provides higher sensitivity similar to RT-qPCR

and produces results within 30–60 minutes with portable equipment, making it a promising POC option. Nonetheless, RT-LAMP is more costly than ATKs and requires more technical expertise and some laboratory infrastructure, which can limit its accessibility in resource-limited settings.

This study had several limitations. Firstly, the findings were based on data from a single centre, and the sample size was relatively small, including sample collection at the onset of symptoms for more than 10 days and sample collection with $\log_{10}$ SARS-CoV-2 RNA <2 copies/reaction. Studies involving multiple centres and a larger number of patients with a wider range of conditions are required in order to comprehensively assess the clinical utility of RT-LAMP for SARS-CoV-2. Secondly, we did not assess the quantity and quality of RNA extracted from the samples. Thirdly, RT-LAMP and RT-qPCR assays were conducted in triplicate for each sample. In real-world clinical settings, testing multiple samples simultaneously is crucial, and RNA quantity and quality are often not analysed, with RT-LAMP and RT-qPCR typically performed on individual samples. Our aim in this study was to compare the diagnostic accuracy of RT-LAMP and RT-qPCR in practical clinical scenarios. Consequently, we did not evaluate RNA quantity and quality and performed the assays in triplicate for each sample in order to reflect standard clinical practices. Thus, the accuracy of our results might be somewhat lower than what could be achieved in a more controlled experimental environment. Finally, we did not evaluate a cross-reaction of the RT-LAMP test for COVID-19 infections with the other respiratory viral infections.

Conclusion

RT-LAMP demonstrated a sensitivity compared to that of RT-qPCR for detecting COVID-19 during the acute phase of the illness. It can serve as a viable alternative diagnostic tool in hospitals and clinics where on-site RT-qPCR testing is not feasible. Future studies should address its performance across different viral loads and disease stages, evaluate the long-term stability of reagents, and focus on integrating RT-LAMP with existing diagnostics while ensuring it is robust against cross-reactivity with other pathogens.

Acknowledgement

We would like to acknowledge Miss Piyarat Nikomrat and Miss Suratsawadee Kimtan for their efforts in managing the ethics submission process.

Funding sources

This study was supported by the National Science, Research, and Innovation Fund (NSRF) under Grant No. RDO6405059f, and the Prince of Songkla University under Grant No. RDO6405059M.

Conflict of interest

The authors declare no conflicts of interest.

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Supplementary Table 1 Nucleotide sequences of primers for RT-LAMP detecting SARS-CoV-2

Primer	Nucleotide (5′-3′)	Target gene
F3_N4	TGTCTGGTAAAGGCCAACAA	*N*
B3_N4	GCAATTTGCGGCCAATGT	
FIP_N4	GCAGTACGTTTTTGCCGAGGCTTTTTTTCAACAAGGCCAAACTGTCAC	
BIP_N4	AACACAAGCTTTCGGCAGACGTTTTTTTTGATTAGTTCCTGGTCCCCA	
LF_N4	GCCTCAGCAGCAGATTTCTTA	
LB_N4	GGTCCAGAACAAACCCAAGG	
F3_S2	GTCTCTGGGACCAATGGT	*S*
B3_S2	AAACACCCAAAAATGGATCA	
FIP_S2	TTAGACTTCTCAGTGGAAGCAAAATTTTTTTCTAAGAGGTTTGATAACCCTGTC	
BIP_S2	ACTACTTTAGATTCGAAGACCCAGTTTTTTTGACTTTAATAACAACATTAGTAGCG	
LF_S2	CACCATCATTAAATGGTAG	
LB_S2	CCCTACTTATTGTTAAT	
F3_RnaseP2	TTGATGAGCTGGAGCCA	Rnase P
B3_RnaseP2	CACCCTCAATGCAGAGTC	
LF_RnaseP2	ATGTGGATGGCTGAGTTGTT	
LB_RnaseP2	CATGCTGAGTACTGGACCTC	
FIP_RnaseP2	GTGTGACCCTGAAGACTCGGTTTTAGCCACTG ACTCGGATC	
BIP_RnaseP2	CCTCCGTGATATGGCTCTTCGTTTTTTTCTTACA TGGCTCTGGTC	

RT-LAMP=reverse transcription-loop-mediated isothermal amplification, SARS-CoV-2=severe acute respiratory syndrome-coronavirus-2, *N*=nucleocapsid, *S*=spike

Supplementary Table 2 Demonstration of positive plasmid DNA containing SARS-CoV-2 gene

Gene names	Plasmid names	Sources
S	pUC57_21,563-25,384	pUC57-2019-nCoV-S (original) Lot No. MC_0101080/PB40842 (GenScript Biotech, China)
N	pcDNA3.1(+) 28,274-29,553	pcDNA3.1(+)-N-eGFP-N Protein Lot No. MC_0101137/PB40991 (GenScript Biotech, China)

DNA=deoxyribonucleic acid, SARS-CoV-2=severe acute respiratory syndrome-coronavirus-2, *S*=spike, *N*=nucleocapsid

Supplementary Table 3 Other characteristics of patients diagnosed with COVID-19 by RT-qPCR from nasopharyngeal and throat swabs

Variable	N (%), N (154)
Median Age, years (IQR)	46.0 (31.0–59.0)
Median interval between symptom onset to the day of the first PCR test, days (IQR)	2 (1–4)
Sex	
Male	81 (52.6)
Female	73 (47.4)
Comorbidities	
Asthma/Obstructive pulmonary disease, n (%)	4 (9.1)
Cancer e.g., HCC, DLBLC, lung cancer, n (%)	5 (11.4)
ESRD, n (%)	3 (6.8)
Diabetics, hypertension, or dyslipidemia, n (%)	26 (59.1)
Ischemic heart disease, n (%)	4 (9.1)
Severity	
Mild	124 (80.5)
Moderate	13 (8.4)
Severe	17 (1.1)
Length of hospital stay, median (IQR)	11 (10–15)
Antiviral drug	
Favipiravir	30 (19.5)
Lopinavir/Ritonavir, Azithromycin, Hydroxychloroquine	8 (5.2)
Remdesivir	26 (16.9)
No	90 (58.4)

COVID-19=coronavirus disease 2019, RT-qPCR=reverse transcription-quantitative polymerase chain reaction, IQR=interquartile range, PCR=polymerase chain reaction, HCC=hepatocellular carcinoma, DLBLC=diffuse large B cell lymphoma