

Rapid Detection of COVID-19 by RT-LAMP PCR Technique and its Comparison with Real-Time RT-PCR Method

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ABSTRACT

Background and Aim: Rapid antigen and antibody, serological tests, and RT - PCR-based molecular methods are widely used to detect microorganisms worldwide. This study aimed to detect the covid-19 using Isothermal nucleic acid amplification techniques.

Materials and Methods: In this study, we collected 200 samples of nasopharynx and oropharynx from Jamaran Heart Hospital in Tehran. Covid -19 was examined by LAMP-RT PCR and Real-Time RT- PCR techniques. The nasopharynx and oropharynx nucleic acids were extracted both by pishtaz RNA extraction kit. LAMP-RT-PCR was performed on direct samples, while Real-Time RT-PCR samples were tested only using RNA extraction samples. The probe-primer mixture of this kit was designed using a dual-target gene method that simultaneously targets the genomic sequences of the spike region and N nucleocapsid. Fluorescence was measured using Real-Time RT-PCR and LAMP-RT PCR.

Results: Clinical specimens (56%) were positive using Real-Time RT-PCR technique, with mean Ct values less than 30. Clinical samples (52%) were positive for Covid-19 in the RT-LAMP technique of RNA extraction samples. RT-LAMP technique indicated a sensitivity of 92.8%. Also in the RT-LAMP method without RNA extraction, the sensitivity of the technique was 85.7%.

Conclusion: The LAMP-RT PCR technique is emerging as an appropriate alternative to the Real-Time RT-PCR method. This technique has basic advantages, such as constant temperature amplification, thermal cycle elimination, faster results, and potentially greater detection capacity. Therefore, the RT-LAMP-PCR can be used as a quick and cost-effective technique that can be employed in all areas.

Keywords: Rapid, Detection, SARS-CoV-2, RT-LAMP-PCR, Real-time RT-PCR

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1. Introduction

Covid-19 was first discovered in Wuhan in December 2019. It has since spread to more countries around the world and was recognized by the World Health Organization on March 11, 2020, as a pandemic (1).

In 2021, the existence of this disease was confirmed worldwide, surpassing those outside China on March 16, 2020 (2).

Covid-19 belongs to a family of single-strand RNA viruses called *Coronaviridae*, which affects mammals, birds, and reptiles.

In humans, it can cause mild infections such as the common cold with a rate of 10-30% of upper respiratory system infections in adults. Coronaviruses can cause respiratory tract, Gastrointestinal, and neurological diseases. It should be noted that the

recovery period of this disease varies from person to person, depending on their physical and mental condition. But generally, recovery may take about two weeks (3).

As the *coronavirus* infects the respiratory system, common symptoms include fever, myalgia, headache, and dry cough. *SARS-COV2* can lead to short fever, fatigue, headache and body pain, cough, runny nose, sore throat, wheezing and chest heaviness, and in some cases, diarrhea, kidney failure, and death (4).

Diagnosis of COVID-19 is based on common symptoms such as fever, cough, decreased sense of smell, and taste, and body pain (5, 6).

Initially, laboratory methods are used to diagnose this disease, including the RT-PCR test performed on nasopharynx and oropharynx samples (7). Serological tests are another available method for diagnosing and confirming coronavirus infection (8).

There are many microbiological, immunological, and molecular methods for detecting Covid-19, each with its advantages and disadvantages. The disadvantages of these methods are the need for special equipment, high cost, time consumption, and false positive or negative results. Among these, RT-PCR is the most accurate method with the possibility of a false-positive result. The LAMP-RT-PCR method is one of the effective techniques for identifying microorganisms with appropriate sensitivity and specificity (9).

This method is simple, highly specific, fast, and cost-effective. Some viruses that are detected by the LAMP-RT-PCR method contain human papillomavirus, cytomegalovirus, human herpesvirus (HSV-6), influenza virus, human immunodeficiency virus (HIV),

respiratory syndrome virus (SARS) without nucleic acid extraction (10-13).

This study aimed to detect Covid-19 in oropharyngeal and nasopharyngeal specimens using LAMP-RT-PCR and compare the results simultaneously with Real-Time RT-PCR. One of the innovations of this research is that the primers designed by Spike and N regions used in this research are new regions. Another advantage is the consumption of less enzyme concentration (320U) up to 0.2 μ L, while the usual concentrations used are 1 μ L. Another advantage of this research is quick and correct identification without requiring nucleic acid extraction, and expensive devices are not required.

2. Materials and Methods

2.1. Sample collection

A total of 200 clinical specimens were collected from the oropharynx and nasopharynx of patients at Jamaran Heart Hospital in Tehran in 2021.

2.2. Extraction of mRNA

The mRNA extraction was performed using Pishtaz Teb Kit for real-time RT-PCR reaction.

2.3. Direct Sample

Direct nasopharyngeal and oropharyngeal samples were used without mRNA extraction to identify Covid-19 by LAMP-RT-PCR.

2.4. Primer Design

Suitable primers for Spike and N genes were designed using Explorer v5 software, (Figs 1,2).

PrimerExplorer V5
Software

1. Push "Primer Information" button to download Primer Information format file for loop primer designing.
2. Push "Save" button to download the primer information in the screen display layout.

DesignId 201108050724

Primer Information

Save

1	ID:1			dimer(minimum)dG=-2.34				
label	5'pos	3'pos	len	Tm	5'dG	3'dG	GCrate	Sequence
F3	15	36	22	56.86	-3.83	-4.59	0.36	TGTTTTATTGCCACTAGTCTCT
B3	189	209	21	56.77	-4.17	-4.67	0.38	ACATGTATAGCATGGAACCAA
FIP			48					ACCACGTGTGAAAGAATTAGTGTAT-GTCAGTGTGTTAATCTTACAACC
BIP			48					CCTGACAAAGTTTTTCAGATCCTCA-GTAACATTGGAAAAGAAAGGTAAG
F2	38	60	23	57.00	-4.90	-5.16	0.39	GTCAGTGTGTTAATCTTACAACC
F1c	81	105	25	60.50	-6.33	-3.78	0.36	ACCACGTGTGAAAGAATTAGTGTAT
B2	165	188	24	55.90	-3.90	-4.18	0.33	GTAACATTGGAAAAGAAAGGTAAG
B1c	115	138	24	60.68	-5.35	-5.11	0.42	CCTGACAAAGTTTTTCAGATCCTCA

Figure 1. LAMP primer sequence designed for Spike gene

PrimerExplorer V5

Software

1. Push "Primer Information" button to download Primer Information format file for loop primer designing.
2. Push "Save" button to download the primer information in the screen display layout.

DesignId 201108045726

Primer Information		Save	
1	ID:7	dimer(minimum)dG=-2.36	
label	5'pos	3'pos	len Tm 5'dG 3'dG GCrate Sequence
F3	252	270	19 59.21 -5.33 -5.32 0.53 TGGCTACTACCGAAGAGCT
B3	449	468	20 59.64 -6.65 -4.74 0.45 TGCAGCATTGTTAGCAGGAT
FIP		41	TCTGGCCCAGTTCCTAGGTAGT-GACGAATTCGTGGTGGTGA
BIP		40	AGACGGCATCATATGGGTTGCA-GCGGGTGCCAATGTGATC
F2	275	293	19 59.26 -5.20 -5.42 0.53 GACGAATTCGTGGTGGTGA
F1c	332	353	22 64.16 -6.10 -4.57 0.55 TCTGGCCCAGTTCCTAGGTAGT
B2	430	447	18 60.66 -7.52 -4.41 0.61 GCGGGTGCCAATGTGATC
B1c	381	402	22 64.29 -6.02 -5.57 0.50 AGACGGCATCATATGGGTTGCA

Figure 2. Lamp primer sequence designed for N gene

Table 1. Reaction components for PCR LAMPs

Component	25 µl Reaction	Final Concentration
10X Isothermal Amplification Buffer	2.5 µl	1X (contains 2 mM MgSO ₄)
MgSO₄ (100 mM)	1.5 µl	6 mM (8 mM total)
dNTP Mix (10 mM)	3.5 µl	1.4 mM each
FIP/BIP Primers (25X)	1 µl	1.6 µM
F3/B3 Primers (25X)	1 µl	0.2 µM
B2/F 2 (25X) B1c/F1c(25X)	1 µl	0.4 µM
Bst 2.0 DNA Polymerase (8,000 U/ml)	0.2µl	320 U/ml
DNA Sample	variable	> 10 copies or more
Nuclease-free Water	to 25 µl	
Total Reaction Volume		25 µl

2.5. Preparation of Optimal LAMP Reaction Mixture

In the LAMP reaction, for each clinical sample, a total volume of 25 microliters containing primers B3 and F 3 was prepared based on [table 1](#).

2.6. Real-Time RT-PCR

The reaction protocol was carried out according to Pishtaz Teb PCR-RT one-Step. The mixture of probe and primer of this kit is designed using a gene target-

ual method, which sequences conserved genomes of RdRp region and N protein nucleocapsid simultaneously. The aim is to amplify this using the PCR reaction solution provided in the kit. The pattern can be qualitatively analyzed, and fluorescence signals can be measured using Real-Time PCR device. Additionally, this diagnostic kit includes a solution containing a probe and an internal control primer (RNase P) to increase the accuracy of the sampling process, and extraction is performed to avoid false negative results ([Table 2,3](#)).

Table 2. Preparation of the reaction mixture

Materials required for each test	Materials required for each test(1x)	
Template RNA	5 μ L	5 μ L
Enzyme mixture	9 μ L	9 μ L
Primer-Probe RdRp/N/ICON (FAM-HEX/ROX)	1 μ L	1 μ L
DW (RNase Free)	5 μ L	-
Final Volume	15 μ L	10 μ L

Table 3. Q-PCR routine program

Stage	Temperature	Time	Cycle
Reverse Transcription	50 °C	20 min	1
CDNA Initial Denaturation	95 °C	3 min	1
Denaturation	94 °C	10 sec	45
Annealing, Extension, and Fluorescence measurement	55 °C	40 sec	
Cooling	25 °C	10 sec	1

3. Results

3.1. Results of LAMP-RT-PCR

Positive samples in the RT-LAMP test after adding Cyber Green (Safe Stain Cinnaclo) under UV light exhibited fluorescence properties and appeared as yellow or green, while negative samples appeared as orange to red.

In the RT-LAMP reaction of genomic RNA extraction samples, 52% of clinical samples were reported positive. The color change from orange to yellow/green indicated that the RT-LAMP method had a sensitivity of 92.8%. Also in the RT-LAMP technique without RNA extraction, the sensitivity of the method was 85.7% (Fig 3-6).

It should be noted that the oropharynx and nasopharynx samples for direct RT-LAMP testing should be performed for a maximum of 5 to 12 hours, and the more they are tested in the first sampling times. If the time is long, the mRNA will break down therefore in case of delay store the sample in the refrigerator at 4 or in the freezer at -20 °C. The specificity of the test was calculated to be 85% when tested with a sample other than Covid 19, and false reaction the were mostly due to contamination during work.

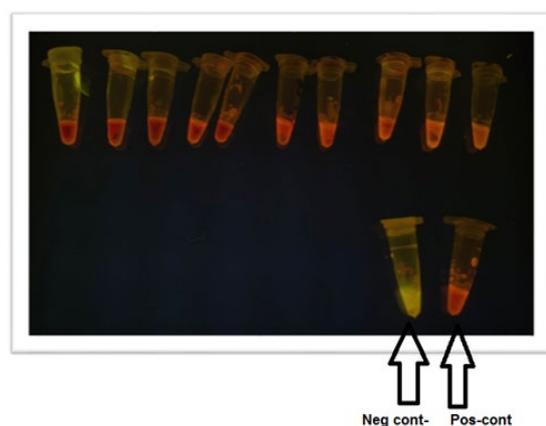


Figure 3. Negative samples of COVID-19 in RT-LAMP method with RNA extraction and using safe stain cinnaclo dye under blue light are red / orange. At the low, there is a negative control with red color and positive control with yellow color.

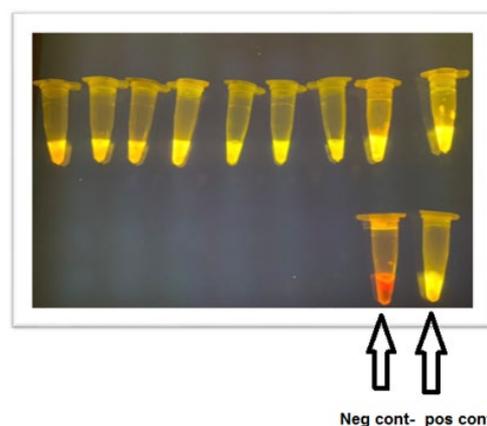


Figure 4. Positive samples of COVID-19 in RT-LAMP method by RNA extraction and using safe stain dye cinnaclo under blue light. At the low is a negative control with red color and positive control with yellow color

3.2. Results of Real Time-RT-PCR

From 200 samples collected by RT-PCR with mean Ct <30, 56% positive clinical samples and 44% negative samples were reported.

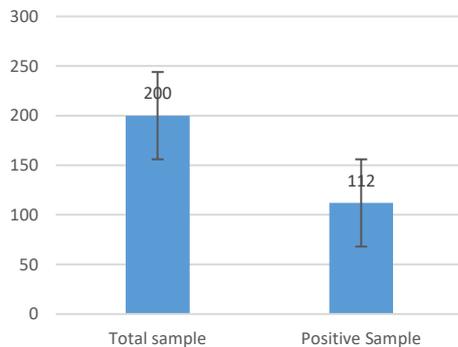


Figure 5. Real-Time RT-PCR results. The results showed that 56% of patients were reported positive by Real-Time RT-PCR

3.3. Comparison of Real-Time RT-PCR real-time and RT-LAMP Results

The comparison of Real-Time RT-PCR and RT-LAMP PCR results are shown in [figure 6](#).

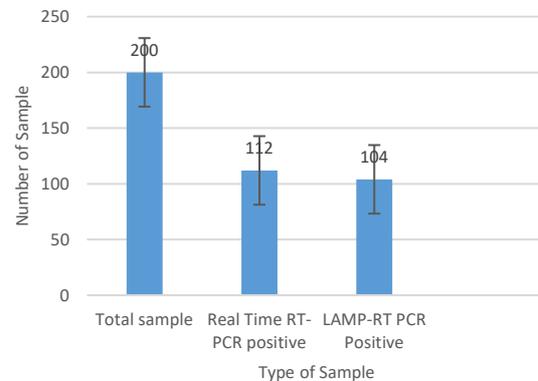


Figure 6. The comparison of Real-Time RT-PCR and RT-LAMP results shows that 56% of the samples with the Real-Time RT-PCR method and 52% of the samples with the RT-LAMP-PCR method had the Covid-19 genome.

4. Discussion

COVID-19 is the causative agent of important respiratory, digestive, and nervous systems diseases. According to the World Health Organization (WHO), more than 446 million people have been infected and more than 6 million have died by March 2022 (14, 15).

The SARS-CoV-2 virus is responsible for epidemics related to this virus. Currently, with few vaccines or specific drugs for the treatment of Covid-19, the best way to control the disease is through strong public health monitoring (16). Reverse transcription-PCR (RT-qPCR) diagnostic testing is currently considered the gold standard for diagnosing COVID-19 (9).

Despite its high sensitivity and specificity, the RT-qPCR technique requires experienced personnel, dedicated facilities, and is expensive. These factors limit its use, especially in underdeveloped countries. However, the method has great potential as it is a fast, specific, and sensitive technique (15).

The LAMP method requires several six to eight primers with high specificity and multiplies nucleic acid in less than an hour. Amplification results can be confirmed by various methods, such as turbidity due, to changes in fluorescence using intermediate dyes, DNA probes, pH, or gel electrophoresis (17-23).

Among these methods, the use of pH-based colorimetric kits is common and results are visible to the naked eye.

RT-LAMP tests have been used for the diagnosis of Covid-19; however, the performance of these

experiments was not always ideal. Using water and DNA *E.coli* as negative control instead of RNA are not appropriate and can lead to false-negative results. When human specimens were infected with other viruses, evaluate clinical specimens or the specificity of reactions decreased (24-28). It was also shown to be specific after testing with other microorganisms' DNA.

Rapid, low-cost and favorite molecular detection methods are a prerequisite for dealing with the spread of infectious diseases. Especially at the time of the outbreak of COVID-19, there is an urgent need to increase the capacity of the global test with current standard methods. One of the main advantages of the LAMP is its fast analysis. The confirmation of results for RT-LAMP is faster than RT-PCR. Turbidity or pH color can easily be used to observe amplification with the naked eye. The test speed, simplicity, and cost-effectiveness of this method make it a viable candidate for monitoring the spread of the SARS-CoV-2, as large numbers of people can use it quickly (26-28).

In addition to improving the sensitivity and specificity in the design of LAMP primers, there is a potential development trend for further use of this technique for extensive COVID-19 screening and control. Therefore, detecting COVID-19 in homes with high detection capacity may be possible. Recently, the US Food and Drug Administration (FDA) confirmed the detection of sample collected samples at home that support the possibility of testing in the RT-LAMP tube.

A lysis solution is usually required to destroy RNase to achieve such a "one-step" diagnosis in a closed tube for highly sensitive virus particles. Currently, a proteinase K containing lysis buffer is commercially available for high-efficiency virus degradation. Given the urgent need to increase screening capacity to inhibit the COVID-19 pandemic, such a home RT-LAMP approach offers an innovative way of molecular diagnosis (28-30).

5. Conclusion

Due to the sensitivity and specificity of the LAMP-PCR technique for rapid and accurate molecular identification of Covid 19, this technique can be used for large-scale screening. In this technique, due to the sensitivity of the test to contamination, it is very important to observe sterile conditions. By increasing the number of primers in this technique, the chance of identifying the virus increases. Because there is no need to extract nucleic acid, the response speed of the results increases.

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Ethics approval

This study was approved by Jamaran Hospital ethics committee. All methods were carried out in accordance with relevant guidelines and regulations.

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Author contribution

D.E., N. Y., and G.S. designed the study. D.E. performed research. N.Y performed data analysis. D. E. and N.Y. wrote the article. G. S and D.E. read and approved the final manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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