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Innovative COVID-19 Screening: RT-LAMP Assay for Spike and NSP1 Proteins

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Abstract: Coronavirus disease (COVID-19) is a serious respiratory disease that spreads through the coronavirus globally. It soon became a pandemic after its appearance in 2019 and demanded new techniques for its identification and detection. Owing to this situation, RT-LAMP appears to be a novel method for the identification of COVID-19 because of its vast applications, including cost-effectiveness and time-saving. This research highlights the use of RT-LAMP, a more sensitive test than RT-PCR, for the assessment of SARS-CoV-2, the severe acute respiratory illness. To identify the spike (S) and NSP1 protein using RT-LAMP, 170 total samples of coronavirus-suspected patients were served in this research. Health certifications and bioethical considerations were taken into consideration. After the sample was extracted from the patient's swabs, RNA was isolated, extracted, and purified. The response was then run on the RT-LAMP at the ideal temperature, and the outcomes could be observed with the unaided eye as they changed from pink to yellow. It is a simple method of determining if the test is positive or negative. For this purpose, both RT-LAMP and RT-PCR tests are used during these procedures. Genes linked with COVID-19 testing including *S*, *nsp1*, and *ORF* are suited to coronavirus testing; they have 100% specificity and low sensitivity, but *S* has more specificity and sensitivity than *nsp1* and *ORF*, respectively. Out of the 95 positive samples, 89 (93.68%) samples yielded favorable outcomes utilizing RT-LAMP, while 55 negative samples yielded 100% positive results. The present research demonstrates that RT-LAMP is less sensitive yet more selective for coronavirus detection.

Keywords: COVID-19; NSP1; Spike; RT-LAMP

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1. Introduction

The majority of individuals on the planet are infected with COVID-19, which is a worldwide health concern. It rapidly disseminated across the majority of the world's countries in just three months [1]. Four structural proteins such as spike (S) glycoprotein, membrane (M), envelope (E), and nucleocapsid (N) are critical for the body to respond to vaccinations, sixteen non-structural proteins (NSPs) are necessary for the virus to replicate, and nine auxiliary factors are also encoded in the SARS-CoV2 genome. An RNA genome with a positive single strand is

present in the COVID-19 molecular assembly. On the specific sites of the coronavirus, non-structural proteins NSP1-16 cleaving are found. The two to third (20 kb) segments of the entire coronavirus genome are composed of certain NSPs [2]. The SARS COV-2 virus's spike (S) glycoproteins attach to the receptor protein, and as a result of their engagement, the host cell's plasma membrane and the viral envelope combine, enabling the virus to enter and liberate viral RNA within the host cell and resulting in transmission [3].

Through a receptor-mediated process involving multiple steps, spike glycoprotein serves as the initial site of access for SARS-CoV-2 within the cell. The activation of the spike (S) protein occurs by its attachment to a receptor and the TM protease serine 2 enzyme, which are both found on the host cell's exterior and aid in the virus getting into the host's body. The virus's RNA is allowed to reproduce and transcribe its RNA genome once it enters the cell because of the action of polyproteins and the replicate transcriptase complex [4]. The virus has begun replicating its RNA while producing, accumulating, and packing structural proteins within the host's tissues in preparation for its discharge of those viral components. The Mpro protease, often referred to as NSP5, is responsible for transforming polyproteins into the functional components of nonstructural proteins, which is how viruses live, spread, and reproduce [5]. NSP7 and NSP8, two crucial components that play a part in template adhesion and RNA-dependent RNA polymerase activity, work together to enable this nonstructural protein to carry out its duty [6]. The polymerase function of NSP12 is increased if it forms an association with NSP7 and NSP8, but it remains insignificant when NSP12 does not create a complex. When NSP12 is in a combined state with NSP7 and NSP8, it results in a surge in RNA-dependent RNA polymerase adhesion to the RNA primer-template. Viral RNA remains safe against host cell immunity's biological activity upon multiplication [7]. Antiviral medications are made using the NSP10-NSP16 complex, and methyltransferase (MTase) boosts the activity of NSP16 [8].

The viral genomic RNA enters the recipient cell through the cytoplasm. RNA is generated by all preserved proteins in the gene 1 virus, except the NSP1 and NSP2 proteins [9]. Just one protein, NSP1, is encoded by gene 1 in just the alpha and beta coronaviruses. While the amino acid sequences of the NSP1 proteins of alpha and beta coronaviruses differ slightly, structural study of these proteins reveals greater similarities. The opposite physiological action of beta and alpha coronaviruses involves NSP1 in the suppression of host gene activity [10]. The COVID-19 virus, which is classified as group 2b, has 180 amino acids in its NSP1 protein, which participates in signalling, translation suppression, blocking type 1 IFN activation, and the start of cell cycle arrest. The primary pathogenicity viral component that can cause a pandemic in HCoV is the NSP1 protein of CoV. The most prevalent virulent SARS-CoV-2 strain, which carries a NSP1 alteration, and some forthcoming infections are caused by novel HCoV, which is extremely significant [11]. The current method for detecting COVID-19 is RT-PCR, which uses an extremely sensitive and specific nasopharyngeal swab to identify viral nucleic acid [12]. The World Health Organization (WHO) have authorized this widely used technique for COVID-19 detection. Even yet, there are certain restrictions with this method. The significant rise in COVID-19 cases for testing at certain times is mostly due to the limits of RT-PCR, which include the use of extremely sensitive machinery, competent employees, and time-consuming addresses [13].

The new method known as Loop-Mediated Isothermal Amplification (RT-LAMP) is utilized to identify COVID-19 cases with a high level of specificity and sensitivity. It also bears a resemblance to traditional PCR analyses in that it requires no unique tools, such as a thermal cycler, and compromises nucleic acid amplification while maintaining the same temperature [14]. This LAMP method for RNA/DNA amplification is incredibly quick, simple to perform, and economical. It also has the benefit of maintaining appropriate pH and temperature intervals, making it simple to analyze unprocessed specimens with this technique and seeing results with the unaided eye. Finally, all the critical parameters for RT-PCR tests, such as sensitivity and specificity are preserved.

In one procedure, the nucleic acid is detected and amplified simultaneously by preparing the specimens, a set of primers that have been specially created. All of these steps are completed and are heated to the ideal range of 60–65 °C for the RT-LAMP technique [15]. To determine the six distinct parts of the target DNA sequence, the LAMP test uses three pairs of primers: an inner pair and an outer pair. The B3c and F3c regions' respective patterns are complementary to the patterns found in the forward and backward outer primers (F3&B3). Each section contains every sequence that is going to be enhanced. The sets of primers utilized in the LAMP assay are specially made to be highly responsive to several parameters, such as the separation between DNA areas, the primer's unique thermodynamic value, and the amount and placement of nucleotide base pairs [16].

To create the appropriate primers for the RT-LAMP, we utilize an internet resource such as GenBank and the consensus sequence of 23 different identified strains of SARS-CoV-2. This allows us to conduct a rapid test for the virus in 30 minutes. By employing the general sequence, the primers used in RT-LAMP are readily successful in detecting all strains of SARS-CoV-2. Nevertheless, they are altered in the same virus structure as the Bat SARS virus [17]. Three methods are utilized to monitor the outcomes via RT-LAMP: gel-electrophoresis, colour change, and fluorescence [18].

The objective of this investigation is to quickly test for SARS-CoV2 in a way that is sensitive, particular, and economical, all without the need for expensive equipment, a sizable research facility, or highly skilled personnel. The *S* and *nsp1* genes are employed in RT-LAMP which is employed for testing for coronaviruses. Even paramedics with little laboratory training may apply for coronavirus assays conducted in any laboratory for Point-of-Care (POC) testing. The outcomes of this research's analysis are visible to the unaided eye through a hue shift.

2. Methodology

In the current study, 170 COVID-19 patients' saliva, oropharyngeal swabs, and nasal specimens were collected by trained medical personnel. Before extracting RNA from each patient's samples, the specimens were inactivated at 70°C in a dry oven with the Invitrogen PureLink RNA Mini Kit. First, Tri Reagent™ was used to homogenize tissues in a glass homogenizer that was placed in ice. This was followed by an incubation period of five to ten minutes at room temperature, during which 500 liters of isopropanol was added. Solutions of RNA precipitation were centrifuged for 10 minutes at 13,000 rpm at 4 °C. The pellet was kept while the supernatant was thrown away. After that, 1,000 L of 70% DEPC-treated ethanol was used to wash the pellet. Next, centrifuge it at 4°C for 5 minutes at 8,000 rpm. A particle was kept in tubes and allowed to dry for five to ten minutes at room temperature while the supernatant was being strained. After adding 30 L of DEPC water to the dried pellet, it was allowed to remain at ambient temperature for two to three minutes before being stored at -20°C for later use. Using a 1% agarose gel in TAE buffer, RNA validation was performed.

Through the use of gel electrophoresis on a 1% agarose gel, the integrity of the RNA was confirmed. An electric weighing balance was used to weigh 1 g of agarose gel, and 100 µL of TAE Tris-acetate EDTA was added to create a 1% gel. Allow the liquid to cool for a few minutes after it has been heating for two minutes. The combination was added to a total of 12 µL of 5 mg/mL ethidium bromide. The gel was allowed to cool for fifteen to twenty minutes after being poured into the gel caster, which was placed within the gel tank. Following the gel's solidification, the combs were taken out of the wells and replaced with an equal amount of RNA and filling colour. This was carried out for 30 minutes at 120 V. Gel spectrophotometry was used to see and store the resultant bands. The nano-drop spectrophotometer was utilized to quantify the isolated RNA. The absorb-ance of 260 and 280 nm was evaluated by filling the sensor of a nucleic acid quartz cuvette with 1 µL DEPC

water as a control. The absorbance was measured after 1 µL of isolated RNA was put onto the sensor using the same procedure. Tests were conducted at 260 and 280 nm. RNA samples with an OD ratio of 260/280 > 1.65 were deemed suitable for the production of cDNA. Although the sample volume in each tube differed, the overall volume never went above 20 µL. One step of the cDNA synthesis process was completed. RNA, 5x reaction buffer, Random Hexamer Primer, DNTPs, RNA Inhibitor, Revert Aid RT, and nuclease-free water make up the reaction solution component for cDNA synthesis, which has a total volume of 20 µL. *β-actin*, a housekeeping gene and internal control, was used to verify the product generated following cDNA synthesis. The *β-actin* gene's earlier established cDNA was amplified using PCR, and the amplicons were seen on a 2% agarose gel. Primer Explorer v5 was used in this investigation to construct multiple RT-LAMP primer sets targeting common hotspot areas on the viral genome. The forward and reverse primer sequences are given in **Table 1**.

Table 1. RT-LAMP Primer sets of β-actin gene

Target gene	Primer name	Sequence 5'–3'	Tm
β-actin gene	B-F3	AGCCTCCCGGTTTCCG	60
	B-B3	AGAAGGTGTGGTGCCAGAT	60
	B-FIP	ATCCTTCTGACCCATGCCCACCCCGTGCTCAGGGCTTCTTG	
	B-BIP	GCATCCTCACCCTGAAGTACCCCTCCATGTCGTCCCAGTTGG	
	O-LF	GCCCTGGGAAGGAAAGGA	61

Table 2. RT-LAMP Primer sets of *S* and *nsp1* genes

Target gene	Primer name	Oligo sequence 5'–3'	Tm
Spike gene (*S*)	S-F3	CTGACAAAGTTTTCAGATCCTCAG	56
	S-B3	AGTACCAAAAATCCAGCCTCTT	55
	S-FIP	TCCCAGAGACATGTATAGCATGGA ATCAACTCAGGACTTGTTCTTACC	
	S-BIP	TGGTACTAAGAGGTTTGATAACCC TGTTAGACTTCTCAGTGGAAGCA	
	S-LF	CCAAGTAACATTGGAAAAGAAA	61
	S-LB	GTCCTACCATTTAATGATGGTGTTT	60
nsp1 gene (N)	N-F3	TGCAACTATAAAGCCACG	59
	N-B3	CGTCTTTCTGTATGGTAGGATT	61
	N-FIP	TCTGACTTCAGTACATCAAACGAA TAAATACCTGGTGTATACGTTGTC	
	N-BIP	GACGCGCAGGGAATGGATAATTCC ACTACTTCTTCAGAGACT	
	N-LF	TGTTTCAACTGGTTTTGTGCTCCA	62
	N-LB	TCTTGCCTGCGAAGATCTAAAAC	61

To avoid nonspecific annealing, the reaction solution additionally contains dNTPs, which are oligonucleotide-based aptamers that function as reversible temperature-dependent inhibitors. The RT-LAMP reaction was run for almost 40 minutes at 60 °C. Each gene was amplified in a separate PCR tube because RT-LAMP is not employed as a multiplexed amplification process. Before the amplified product was put via gel electrophoresis, it was immediately inspected. The results were validated using RT-PCR. Real-time PCR (RT-PCR) was also

used to validate the results of RT-LAMP. It was carried out using primers and predetermined parameters. The tubes were used to hold the qPCR reaction solution. These tubes containing the reaction mixture were tightly closed, and then they were put in a qPCR tube rack. Applied Biosystems' Step One plus RT-PCR system was utilized to conduct the qPCR experiment. The information was statistically analyzed using SPSS 20 software. Software was used to organize the data, and various statistical tests were used to analyze it. P values less than 0.05 are regarded as statistically significant.

3. Results

170 specimens from COVID-19 patients' nasal and oropharyngeal regions were used in this investigation from the National Institute of Health (NIH) in Islamabad. All of these samples were interpreted and amplified using RT-PCR and RT-LAMP. cDNA and concatemers were demonstrated using gel electrophoresis. After analysis, it was found that 95 samples had positive RT-LAMP results, while 75 of the specimens had negative outcomes. 165 tested positive and 5 negative specimens were found in the RT-PCR findings. 95 confirmed positive specimens were found utilizing RT-LAMP with the *S* and *nsp1* genes.

Table 3. The positive and negative samples of RT-PCR & RTLAMP

Total samples (170)	Positive		Negative	
	Number of samples	Percentage	Number of samples	Percentage
RT-PCR positive	100	58.82%	70	2.94 %
RT-LAMP positive	90	52.94%	65	38.23%

Table 4. The positive results through RT-LAMP of *S* and *nsp1* gene out of 95 samples

Total samples (95)	Positive	Percentage	False-negative	Percentage
S gene	85	89.47 %	10	10.52%
nsp1 gene	81	85.26 %	14	14.73%

Table 5. The negative results through RT-LAMP of *S* and *nsp1* gene

Total samples (75)	Negative	Percentage	False-positive	Percentage
S gene	72	96 %	3	4%
N gene	69	92%	6	8%

Gel electrophoresis was used with 1% TAE buffer to evaluate the quality of RNA and concatemers, while the detected bands indicated either a positive or negative outcome. The amplification of RNA substrates is done via RT-PCR. To verify the amount and caliber of the cDNA produced from the RNA template by reverse transcriptase PCR, where the *β-actin* gene is utilized for the PCR activity in **Figure 2**. Out of 170 specimens, the RT-LAMP test yielded 95 positive and 55 negative results, which are displayed in **Figure 3** and **Figure 4**. When all the findings are compared, the accuracy of the primers is evident, but the *S* gene exhibits more precision and produces fewer adverse outcomes. Consequently, *S* has a higher specificity than the *nsp1* gene. All of the responses exhibit 90% accuracy when carried out using the gene using the appropriate primers and conditions. The *N* gene produces the highest number of false positive outcomes, while the *S* gene produces the fewest false positive findings overall.

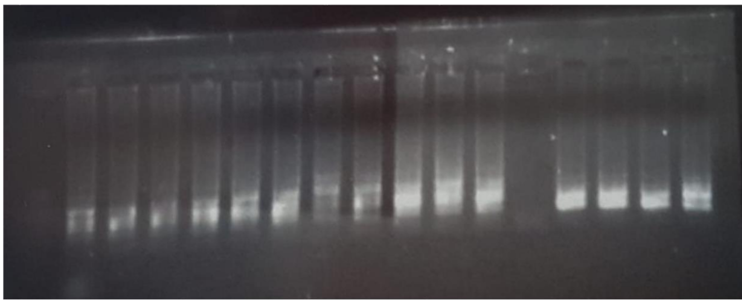
Figure 1. Gel imagining of the extracted RNA.

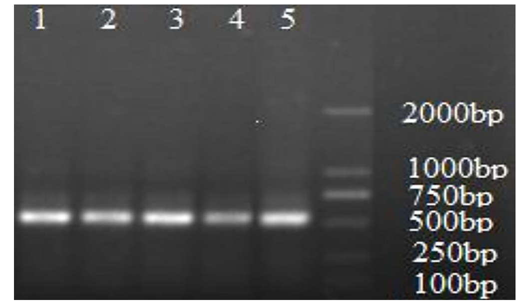

Figure 2. Amplification of cDNA by using RT-PCR for a given sample

Figure 3. Colourimetric detection of RT-LAMP products.

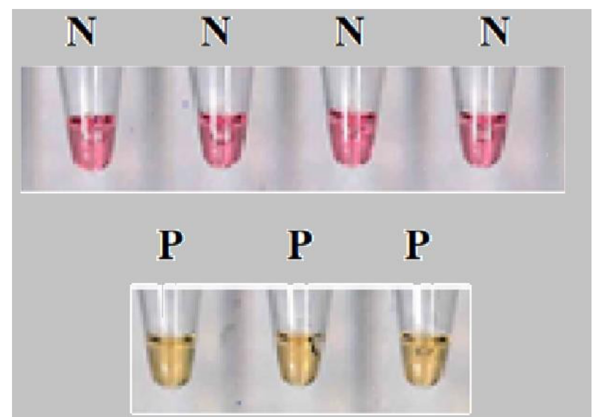

Figure 4. Positive and negative samples

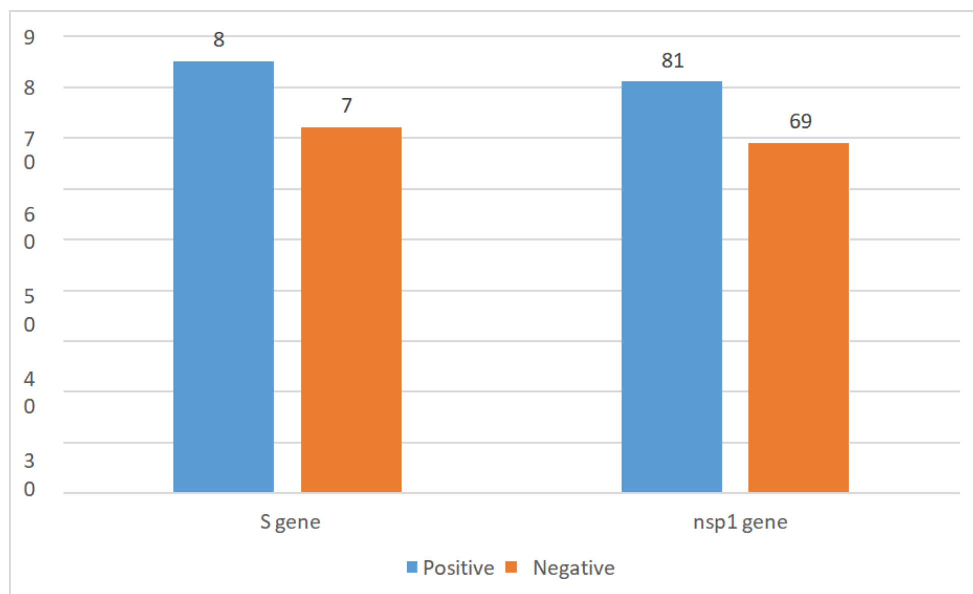

Figure 5. RT-LAMP results of *S* and *nsp1* protein.

Table 6. Comparison of *S* and *nsp1* gene-positive results

Results	Positive	False-negative
S gene	85	10
nsp1 gene	81	14

Table 7. Comparison of *S* and *nsp1* gene-negative results

Results	Negative	False-positive
S gene	72	3
N gene	69	6

In the total specimen of 170, RT-LAMP yielded 90 positive outcomes and 65 negative findings, while RT-PCR yielded 100 positive outcomes and 70 negative findings. Therefore, the comparison shows that RT-LAMP is more precise and less delicate, while RT-PCR is more accurate and less specific.

Table 8. RT-PCR and RT-LAMP results

Total Samples	Positive		Negative	
	RT-PCR	RT-LAMP	RT-PCR	RT-LAMP
170	100	90	70	65
	58.82%	52.94%	41.17%	38.23%

Specimen multiplication was used to compare the efficiency and selectivity of RT-LAMP and RT-PCR. A total of 170 samples were increased, demonstrating that while RT-LAMP is 90.00% more sensitive and 92.00% more specific than RT-PCR, RT-PCR is 98.82% more sensitive and 100% more specific. We utilize the ratio of true positive and the sum of true positive and false negative to calculate the sensitivity of the RT-LAMP. The ratio of true negative to the total of true negative and false positive is known as sensitivity.

Table 9. RT-LAMP sensitivity and specificity

RT-LAMP	Sensitivity	Specificity
170	90.00 %	92.00 %

4. Discussion

Originating in China, COVID-19 is an extremely contagious illness spreading to over 76 million individuals worldwide, with over 1.6 million deaths reported. In the twenty-first century, it was the worst scenario on earth. SARS-CoV2 is the virus that causes COVID-19, and it is distinct from all other RNA viruses [19]. The novel approach for diagnosing COVID-19 is called loop-mediated isothermal amplification (RT-LAMP), which has an excellent level of specificity and sensitivity. Like RT-PCR, it is inexpensive, simple to use, and preserves a variety of pH and temperature [20]. There was also a lot of relevant research about the assessment of the RT-LAMP's accuracy and selectivity and the outcomes can be readily seen with the naked eye by altering the colour from pink to yellow using phenol red (pH indicator). RT-LAMP is an immediate response for nucleic acid identification and amplification [21]. Consequently, the RT-LAMP approach exhibits less sensitivity than RT-PCR but a higher specificity because of certain drawbacks, such as the need for highly skilled personnel, delicate equipment, patience, and a high cost [22]. Therefore, we can apply the RT-LAMP in a particular time frame for the significant rise in COVID-19 cases.

Both RT-LAMP and RT-PCR were used in this investigation. We identified the positive, negative, and control specimens out of a total of 170 individuals. There were 95 positives, 55 negative specimens, and 20 reference specimens, according to the results of the RT-PCR. When compared to the conventional method of

RT-PCR, the 86 samples from the 95 positive samples obtained by RT-LAMP yield 90.52% sensitivity and 100% specificity. 183 clinical samples of COVID-19 were subjected to a comparable investigation utilizing the Centers for Disease Control-recommended kit for both RT-PCR and RT-LAMP. Assessing the outcomes with RT-PCR yields 100% specificity and 90.55% sensitivity, which is also the outcome of an investigation carried out in another research [23]. The FDA approved the identification and detection of coronavirus patients utilizing RT-LAMP in conjunction with the requisite cures for the tests that required an Emergency Use Authorization (EUA). SOPs that must be followed for this pandemic circumstance are also being taken care of [24]. Before drawing a decision, the negative sample was once more examined in light of the patient's clinical signs. The analysis of the positive specimen revealed that the individual has a coronavirus. The Department of Microbiology, Immunology, and Infectious Diseases at the University of Calgary in Canada conducted a study that corroborates our findings. The researchers correlated our results with RT-PCR, which yielded 97% positive outcomes and 23% negative findings, indicating low specificity and high sensitivity in comparison to RT-LAMP [25].

According to earlier research, RT-LAMP can be applied in place of RT-PCR for COVID-19 evaluation. This is particularly useful in the present pandemic scenario globally, particularly in isolated regions, as it is an affordable, user-friendly, sensitive, and time-saving method that is also utilized in various healthcare labs like Point-of-Care testing (POCT) facilities. By using RT-LAMP, COVID-19 cases can be quickly diagnosed at the Point of Care (PoC). This makes it easier to recognize this illness and helps to stop its propagation. As the name implies, the test is conducted in the home, and the risk of coronavirus transmission is still there. In a related investigation, RT-LAMP, in conjunction with the in-house LoC test was used to identify COVID-19 [26]. The point-of-care (PoC) diagnostic assay (less than 20 minutes) to identify COVID-19 from the extracted coronavirus RNA specimens. The LAMPcov research, also known as the RT-LAMP suggested investigation, depends on a phylogenetic evaluation of 8921 genes that were obtained from the GISAID and NCBI databases [27].

The investigation concludes that COVID-19 may be screened for and diagnosed using RT-LAMP in conjunction with RT-PCR every time. *S* gene primers used in the LAMP approach have a better specificity than *N* gene primers. Even though RT-PCR is the preferred instrument for many molecular biology procedures, including COVID-19 viral assessment, this study's contrast to RT-LAMP demonstrates the high diagnostic value of specificity and sensitivity. The present research found that, due to its complexity of usage, cost-effectiveness, and time-consuming nature, RT-LAMP testing for COVID-19 has a greater value of specificity and sensitivity than RT-PCR.

Authors contribution

Conceptualization: Muhammad Yousaf
Investigation: Muhammad Yousaf, Amber Khan
Analysis: Muhammad Yousaf
Writing: Muhammad Yousaf, Amber Khan

Disclosure statement

The author declares that they have no conflict of interest.

References

[1] Ciotti M, Ciccozzi M, Terrinoni A, et al., 2020, The COVID-19 Pandemic. Critical Reviews in Clinical Laboratory Sciences, 57(6): 365–388.

[2] Gorkhali R, Koirala P, Rijal S, et al., 2021, Structure and Function of Major SARS-CoV-2 and SARS-CoV Proteins. Bioinformatics and Biology Insights, 2021(15): 11779322211025876.

[3] Kakavandi S, Zare I, VaezJalali M, et al., 2023, Structural and Non-structural Proteins in SARS-CoV-2: Potential Aspects to COVID-19 Treatment or Prevention of Progression of Related Diseases. Cell Communication and Signaling, 21(1): 110.

[4] Kumar R, Srivastava Y, Muthuramalingam P, 2023, Understanding Mutations in Human SARS-CoV-2 Spike Glycoprotein: A Systematic Review & Meta-analysis. Viruses, 15(4): 856.

[5] Hu Y, Lewandowski EM, Tan H, et al., 2023, Naturally Occurring Mutations of SARS-CoV-2 Main Protease Confer Drug Resistance to Nirmatrelvir. ACS Central Science, 9(8): 1658–1669.

[6] Jiang Y, Yin W, Xu HE, 2021, RNA-dependent RNA Polymerase: Structure, Mechanism, and Drug Discovery for COVID-19. Biochemical and Biophysical Research Communications, 2021(538): 47–53.

[7] Reshamwala SM, Likhite V, Degani MS, et al., 2021, Mutations in SARS-CoV-2 NSP7 and NSP8 Proteins and Their Predicted Impact on Replication/Transcription Complex Structure. Journal of Medical Virology, 93(7): 4616–4619.

[8] Thomas J, Ghosh A, Ranjan S, et al., 2024, Cheminformatics Approach to Identify Andrographolide Derivatives as Dual Inhibitors of Methyltransferases (NSP14 and NSP16) of SARS-CoV-2. Scientific Reports, 14(1): 9801.

[9] Zhong C, She G, Zhao Y, et al., 2024, Swine Acute Diarrhea Syndrome Coronavirus *nsp1* Suppresses IFN-λ1 Production by Degrading IRF1 via Ubiquitin-Proteasome Pathway. Veterinary Research, 55(1): 45.

[10] Mei M, Cupic A, Miorin L, et al., 2024, Inhibition of mRNA Nuclear Export Promotes SARS-CoV-2 Pathogenesis. Proceedings of the National Academy of Sciences, 121(22): e2314166121.

[11] Maurina SF, O'Sullivan JP, Sharma G, et al., 2023, An Evolutionarily Conserved Strategy for Ribosome Binding and Host Translation Inhibition by β-coronavirus Non-structural Protein 1. Journal of Molecular Biology, 435(20): 168259.

[12] Kim H, Hong H, Yoon SH, 2020, Diagnostic Performance of CT and Reverse Transcriptase Polymerase Chain Reaction for Coronavirus Disease 2019: A Meta-analysis. Radiology, 296(3): e145–e155.

[13] Binny RN, Priest P, French NP, et al., 2023, Sensitivity of Reverse Transcription Polymerase Chain Reaction Tests for Severe Acute Respiratory Syndrome Coronavirus 2 Through Time. The Journal of Infectious Diseases, 227(1): 9–17.

[14] Nwe MK, Jangpromma N, Taemaitree L, 2024, Evaluation of Molecular Inhibitors of Loop-mediated Isothermal Amplification (LAMP). Scientific Reports, 14(1): 5916.

[15] Zhang X, Zhao Y, Zeng Y, et al., 2023, Evolution of the Probe-based Loop-mediated Isothermal Amplification (LAMP) Assays in Pathogen Detection. Diagnostics, 13(9): 1530.

[16] Park JW, 2022, Principles and Applications of Loop-Mediated Isothermal Amplification to Point-of-care Tests. Biosensors, 12(10): 857.

[17] Soroka M, Wasowicz B, Rymaszewska A, 2021, Loop-mediated Isothermal Amplification (LAMP): The Better Sibling of PCR?. Cells, 10(8): 1931.

[18] Choi G, Moehling TJ, Meagher RJ, 2023, Advances in RT-LAMP for COVID-19 Testing and Diagnosis. Expert Review of Molecular Diagnostics, 23(1): 9–28.

[19] Isath A, Malik AH, Goel A, et al., 2023, Nationwide Analysis of the Outcomes and Mortality of Hospitalized COVID-19 Patients. Current Problems in Cardiology, 48(2): 101440.

[20] Sen S, Bhowmik P, Tiwari S, 2024, Versatility of Reverse Transcriptase Loop-mediated Isothermal Amplification

(RT-LAMP) From Diagnosis of Early Pathological Infection to Mutation Detection in Organisms. Molecular Biology Reports, 51(1): 1–17.

[21] da Silva SJR, de Magalhães JJF, Matthews Q, et al., 2024, Development and Field Validation of a Reverse Transcription Loop-Mediated Isothermal Amplification Assay (RT-LAMP) for the Rapid Detection of Chikungunya Virus in Patient and Mosquito Samples. Clinical Microbiology and Infection, 30(6): 810–815.

[22] Pu R, Liu S, Ren X, et al., 2022, The Screening Value of RT-LAMP and RT-PCR in the Diagnosis of COVID-19: Systematic Review and Meta-analysis. Journal of Virological Methods, 2022(300): 114392.

[23] Juscamayta-Lopez E, Valdivia F, Horna H, et al., 2021, A Multiplex and Colourimetric Reverse Transcription Loop-mediated Isothermal Amplification Assay for Sensitive and Rapid Detection of Novel SARS-CoV-2. Frontiers in Cellular and Infection Microbiology, 2021(11): 653616.

[24] Newman CM, Ramuta MD, McLaughlin MT, et al., 2021, Initial Evaluation of a Mobile SARS-CoV-2 RT-LAMP Testing Strategy. Journal of Biomolecular Techniques, 32(3): 137.

[25] Domnich A, Orsi A, Panatto D, et al., 2021, Comparative Diagnostic Performance of a Novel Reverse Transcription Loop-mediated Isothermal Amplification (RT-LAMP) Kit for the Rapid Detection of SARS-CoV-2. Pathogens, 10(12): 1629.

[26] Sagar V, Singh MP, Kaur G, et al., 2023, LAMP-based Point-of-Care Nucleic Acid-Based Detection Method Can Be Useful for Quick Decision-Making for Diagnosis of Acute COVID-19 Emergency Cases in Hospital Settings. COVID, 3(6): 914–923.

[27] Rodriguez-Manzano J, Malpartida-Cardenas K, Moser N, et al., 2021, Handheld Point-of-care System for Rapid Detection of SARS-CoV-2 Extracted RNA in under 20 min. ACS Central Science, 7(2): 307–317.

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