Jundishapur Journal of Microbiology
Volume 18 · Issue 8 · e160288

OPEN PEER REVIEW

Enhancing Rapid Detection of COVID-19: Optimization of RT-LAMP Assays for Improved Sensitivity and Specificity

Shohreh Fahimirad, Ali Ganji, Houshang Alizadeh, Behzad Khansarinejad, Hamid Abtahi

SUBMITTED	REVISED	ACCEPTED	DOI
20 Feb 2025	26 May 2025	3 Jul 2025	10.5812/jjm-160288

About this report

This open peer review (OPR) report shows the public comments made on this article during peer review, by the reviewers, the associate editor and the editor-in-chief, for every revision round, together with the authors' replies. Comments appear only with the consent of the people who wrote them. Reviewers who chose to sign their report are named; the others are shown as "Referee". Comments are edited for language only, and confidential remarks to the editors are not included.

More about open peer review: brieflands.com/knowledgebase/opr

Contents

1 **Original submission** Referee 1 2
2 **Revision 1** Associate Editor, Editor-in-Chief 3
Authors' reply to reviewers 4
Reviewer and editor comments 11

1 Original submission

1 comment · 21 Apr 2025

Referee 1 Reviewer

REJECT 21 Apr 2025

Dear editor
In this study, the author has tried to optimize the LAMP-PCR diagnostic method for detecting COVID-19 by making changes in factors such as PK concentration, time, or temperature to achieve higher sensitivity and specificity.

-At first glance, it is striking that there is no innovation in the subject under review, and even considering the author's intended goals, it seems that the author has failed to achieve these goals, such as increasing the sensitivity of the test.
-The introduction should briefly explain the principles of the LAMP test so that the reader understands its difference from the regular PCR test. It is also appropriate to mention the most important challenge of this test that has prevented it from being used as a routine laboratory procedure in the diagnosis of infectious diseases, namely the possibility of contamination with the PCR product due to the high concentration of the product generated in this test.
-The sensitivity reported for this test (about 84%) is low and is not even comparable to the common real-time PCR test.
-Also, given that the aim of the study was to optimize the test, no details of these optimizations in temperature, time, PK concentration, or betaine concentration were given in the description of the method, and these details were not mentioned in the results. How was the optimization carried out?
-The photo of the gel used is of very poor quality.
-The discussion is short and should be more focused on the failure to achieve the design goal of optimizing the sensitivity of the LAMP test compared to the common real-time PCR test.
Considering the above points, it seems that this study is unfortunately not suitable for publication in the Jundishapur Journal of Microbiology.

2

Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Dear Editor of the Jundishapur Journal of Microbiology,

Please find enclosed the revised manuscript entitled:

“Enhancing Rapid Detection of COVID-19: Optimization of RT-LAMP Assays for Improved Sensitivity and Specificity”

First of all, we would like to thank the editorial board and the reviewers for their constructive comments that substantially improved our manuscript.

We have considered all the comments and revised the manuscript accordingly (changes displayed in red), and here we provide answers to each issue raised by the reviewers:

Reviewers’ comments

Reviewer #1

Comment no. 1

In this study, the author has tried to optimize the LAMP-PCR diagnostic method for detect Covid 19 by making changes in factors such as PK concentration, time, or temperature to achieve higher sensitivity and specificity.

-At first glance, it is striking that there is no innovation in the subject under review, and even considering the author's intended goals, it seems that the author has failed to achieve these goals, such as increasing the sensitivity of the test

Clarification no.1

We appreciate the reviewer’s observation. Although LAMP-based assays for SARS-CoV-2 detection have been previously reported, our study offers practical value by systematically optimizing key reaction parameters—specifically proteinase K concentration, incubation

temperature, and reaction time—to enhance the performance of an accessible, extraction-free RT-LAMP assay. This work is particularly relevant for resource-limited settings, where traditional RT-PCR is often impractical due to its cost, infrastructure demands, and long turnaround times. By refining the conditions under which RT-LAMP operates, our aim was not to propose a wholly novel technique, but rather to improve the reliability and usability of an existing method for real-world application. While the improvement in sensitivity may be modest, it represents a step toward a more deployable and user-friendly diagnostic solution, particularly valuable in decentralized testing environments where speed, simplicity, and affordability are crucial.

Comment no.2

The introduction should briefly explain the principles of the LAMP test so that the reader understands its difference from the regular PCR test. It is also appropriate to mention the most important challenge of this test that has prevented it from being used as a routine laboratory procedure in the diagnosis of infectious diseases, namely the possibility of contamination with the PCR product due to the high concentration of the product generated in this test.

Clarification no.2

We thank the reviewer for this constructive comment. In response, the introduction has been revised to include a concise yet informative explanation of the fundamental principles of LAMP, highlighting its key differences from conventional PCR methods. Specifically, we have clarified that LAMP operates under isothermal conditions using multiple primers to target distinct regions of the gene, which allows for rapid and specific amplification without thermal cycling. Additionally, we have addressed the major limitation of LAMP namely, the high risk of contamination due to the substantial quantity of amplified product generated during the reaction.

This addition helps contextualize the rationale behind our study and enhances the reader's understanding of both the advantages and limitations of LAMP in the diagnostic setting.

Comment no.3

the sensitivity reported for this test (about 84%) is low and is not even comparable to the common real-time PCR test.

Clarification no.3

We acknowledge the reviewer's concern regarding the reported sensitivity of approximately 84%, which is lower than that of conventional RT-PCR. However, it is important to highlight that this sensitivity was achieved using direct nasopharyngeal swabs without RNA extraction, which significantly simplifies the workflow, reduces processing time and cost, and enhances applicability in point-of-care or resource-limited settings. While this approach may modestly reduce sensitivity due to the presence of inhibitors or variable viral loads, it demonstrates a promising balance between accessibility, speed, and operational simplicity. Our study aims to advance such simplified protocols to expand diagnostic reach, particularly where RT-PCR is not feasible.

Comment no.4

Also, given that the aim of the study was to optimize the test, no details of these optimizations in temperature, time, PK concentration, or betaine concentration were given in the method, and these details were not mentioned in the results. How was the process of optimization?

Clarification no.4

We appreciate the reviewer's comment and understand the need for greater detail regarding the optimization process. In our study, we focused on optimizing key parameters such as temperature, incubation time, proteinase K (PK) concentration, and betaine concentration to enhance the sensitivity and specificity of the RT-LAMP assay. The optimization process involved systematic testing of these factors to identify conditions that yielded the most consistent and reliable results. Specific details regarding the ranges of concentrations tested for PK and betaine, as well as the temperature and time conditions evaluated, will be included in the revised methods section. We will also provide a more comprehensive description of how these parameters were adjusted based on performance metrics such as signal intensity and reaction efficiency. The results of these

optimizations will be further clarified in the revised manuscript to ensure transparency in the methodology.

As requested, the detailed summary of these optimized conditions has been summarized in **Table 2,** which will be added to the results section for greater clarity and transparency regarding the optimization process.

Comment no.5

The photo of the gel used is of very poor quality.

Clarification no.5

Thank you for your feedback. We have addressed the issue of image quality by replacing the original gel image with a higher-quality version to more clearly illustrate the results. The updated image has been included in the revised manuscript to ensure better visual clarity and to meet the required standards for publication.

Comment no.6

The discussion is short and should be more focused on the failure of the design goal to optimize the sensitivity of the LAMP test compared to the common real-time PCR test

Clarification no.6

We will expand the discussion to address the shortcomings in sensitivity and compare our findings with existing literature. We will also emphasize the improvements achieved in the assay's usability and rapid response time despite the lower sensitivity.

Reviewer #2

Comment no. 1

-Is the aim of this study to design a new lamp method or to improve the quality of an established lamp method?

Clarification no.1

The primary objective of this study was to optimize an established LAMP-PCR diagnostic method for COVID-19 detection, rather than to develop a completely new methodology. Our focus was on enhancing the performance of the existing assay by fine-tuning key parameters, such as primer concentration, incubation time, temperature, and other critical reagents, to improve both sensitivity and specificity. This optimization was aimed at refining the assay to ensure more accurate and reliable results, particularly in resource-limited settings where rapid, point-of-care testing is essential. By adjusting these factors, we sought to overcome existing limitations and improve the practical application of the LAMP method for widespread use in COVID-19 diagnostics.

Comment no. 2

- What is the justification for the lower sensitivity and specificity when using pharyngeal swab specimens directly?

Clarification no.2

The observed lower sensitivity when using pharyngeal swabs directly could be attributed to several factors, including the presence of inhibitors in the sample, variations in sample composition, or differences in viral load. Pharyngeal swabs, especially when processed without RNA extraction, may contain substances that inhibit the PCR amplification process, potentially leading to false negatives or reduced sensitivity. Additionally, the variability in viral load among individuals, particularly in cases with lower viral titers, could contribute to the decreased detection sensitivity. We will revise the manuscript to provide a more thorough explanation of these factors and justify the observed differences in performance. Further, we will discuss how the optimization steps were designed to mitigate these challenges and improve the sensitivity of the assay, even when using pharyngeal swabs directly.

Comment no. 3

How was the inhibitory effect of guanidine hydrochloride managed in the PCR reaction?

Clarification no.3

The potential inhibitory effect of guanidine hydrochloride in the PCR reaction was managed by systematically optimizing its concentration in the RT-LAMP assay. During the optimization process, we tested a range of guanidine hydrochloride concentrations to determine the most effective concentration that enhanced assay sensitivity without compromising the amplification efficiency. By carefully controlling the concentration of guanidine hydrochloride (40 mM in our

final optimized protocol), we were able to mitigate its inhibitory effects on the PCR reaction. We will provide additional details in the manuscript regarding the concentration ranges tested and the steps taken to minimize any negative impact on the reaction, ensuring optimal performance of the assay.

Comment no. 4

-How was the optimum concentration of added compounds determined?

Clarification no.4

The optimum concentrations of added compounds, such as primers, betaine, and guanidine hydrochloride, were determined through a systematic optimization process. In this process, we conducted a series of experiments where we tested a range of concentrations for each compound while monitoring their effects on the assay performance. The concentrations were adjusted based on key performance metrics, including signal intensity, reaction efficiency, and sensitivity. For example, primer concentrations were varied, and the reaction was assessed for amplification efficiency and specificity. Similarly, the effects of betaine and guanidine hydrochloride were evaluated by testing different concentrations and determining their impact on the assay's sensitivity and overall performance. We will provide additional details on this optimization process in the methods section to enhance transparency and clarify how each compound's optimal concentration was determined.

Comment no. 5

-It is best to compare and evaluate the results obtained from the existing LAMP method with a validated commercial kit.
The discussion should be more comprehensive and complete.

Clarification no.5

We appreciate the suggestion to compare our results with a validated commercial LAMP kit. However, due to international sanctions against Iran and the high costs associated with acquiring commercial LAMP kits for COVID-19, it was not feasible for the authors to perform such a comparison. Despite this limitation, we focused on systematically optimizing an existing RT-LAMP protocol through experimental adjustments in sample preparation, reagent concentrations, and reaction conditions to enhance its performance and applicability.

To strengthen the scientific contribution of our work, the Discussion section has been completely revised to provide a more comprehensive and detailed analysis. It now includes a clearer acknowledgment of the limitations, a more thorough comparison with existing methods (including commercial RT-PCR kits), and an expanded evaluation of the assay's practical relevance in resource-limited settings. This revision aims to better contextualize our findings and highlight both the challenges and potential of deploying optimized RT-LAMP assays in constrained environments.

2 Revision 1

2 comments · 3 Jul 2025 – 3 Jul 2025

Associate Editor

ACCEPT 3 Jul 2025

Dear EIC
The manuscript was completely revised based on the reviewers' and associate editor's comments, and it is acceptable for publication in JJM.
Regards

Editor-in-Chief

ACCEPT 3 Jul 2025

I am pleased to inform you that your manuscript has been accepted for publication in the JJM. Your accepted manuscript will now be transferred to our production department. If we need additional information from you during the production process, we will contact you directly. Please remember to always include your manuscript number whenever inquiring about your manuscript. We will create a proof which you will be asked to check. When you receive the proof, please check all names and affiliations very carefully, as these cannot be corrected once the article is published.
We appreciate you submitting your manuscript to JJM and hope you will consider us again for future submissions.