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OPEN PEER REVIEW

An In-house PCR Workflow for Rapid Detection of *Candida* spp. from Blood Cultures of Septicemia Suspected Patients

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SUBMITTED	REVISED	ACCEPTED	DOI
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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, Referee 2, Associate Editor, Editor-in-Chief 2
2 **Revision 1** Referee 2, Referee 1, Associate Editor, Editor-in-Chief 4
Authors' reply to reviewers 5
Reviewer and editor comments 7
3 **Revision 2** Referee 1, Associate Editor, Editor-in-Chief 8
Authors' reply to reviewers 9
Reviewer and editor comments 13
4 **Revision 3** Editor-in-Chief 14
Authors' reply to reviewers 15
Reviewer and editor comments 19

1 Original submission

4 comments · 2 Aug 2025 – 3 Aug 2025

Referee 1 Reviewer

MAJOR REVISION 2 Aug 2025

1. Given that only Candida albicans has been identified and all information is on this yeast species, it is best to refer to this Candida species throughout the entire text of the article.
2. In the discussion section of the article, provide an adequate explanation of the superiority of HWP1 over other gene targets (such as ITS or 5.8S rDNA).
3. Time to positivity in cultures was not reported, missing a chance to correlate molecular and conventional timelines.
4. The influence of potential PCR inhibitors (e.g., heme, proteins) from blood samples was not quantified or addressed. Explain your reasons.
5. The study doesn't investigate whether the C. albicans isolates were genetically identical or from diverse clades. Please explain.
6. The limit of detection is reported numerically but lacks a detailed explanation or modeling (e.g., probit regression or standard curve analysis).
7. The table numbers should be corrected. There are two tables labeled Table 1.
8. The electrophoresis figure lacks a clear size marker or scale.
9. Patient inclusion criteria for "suspected sepsis" are not described.
10. There is redundant repetition between the abstract and results sections.
11. The sample is described as "whole blood" but was actually culture fluid.
12. Terms like "Interquartile Range" are used without explanation for non-specialist readers.

Referee 2 Reviewer

MAJOR REVISION 2 Aug 2025

Chief Reviewer: Accept with major revision
Dear Editor/Author
Thank you for everything.
In my opinion, it needs a major revision.
Below are some comments for each section of the paper:

***Abstract
1- The conclusion is not appropriate.
2- It is better to include the words "PCR, Rapid Detection" in the keywords.
3- It is better to remove the words "Positive Blood Culture, Candidemia" from the keywords.

***Introduction
4- The prevalence rate of candidemia in patients with septicemia worldwide and in Iran should be reported.
5- Explain the importance of candidemia in the deterioration of septicemic patients and the treatment process.

*** Method
6- How was the sample size determined?
7- How was the quality of the extracted DNA checked?
8- Explain the internal control in the DNA extraction protocol.
9- Explain the internal control in the PCR amplification protocol.

***Results

10- Since the sample size was 200, please show the power of the test if possible.

*** Discussion and Conclusions

11- The conclusion shall be made on the basis of your findings. You may not talk about definitely meaningful advances in the molecular detection of candidemia in septicemia-suspected patients, since you have not followed up your patients and did not observe the outcome of the other molecular detection methods.

Associate Editor

MAJOR REVISION 3 Aug 2025

Dear Authors

According to the reviewers' comments, your article requires major revision.

Editor-in-Chief

MAJOR REVISION 3 Aug 2025

Dear Authors

According to the reviewers' and associate editor's comments, your article requires major revision. When revising your manuscript, please consider all issues mentioned in the reviewers' comments carefully: please outline every change made in response to their comments and submit the required files.

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.

Chief Reviewer: Accept with major revision

Dear Editor/Author

Thanks for all

In my opinion, it needs a major revision.

Below are some comments for each section of the paper:

***Abstract

1- The conclusion is not appropriate.
R: The conclusion section of the abstract has been revised in accordance with the esteemed reviewer's feedback and highlighted.
2- It is better to include the words "PCR, Rapid Detection" in the keywords.
R: These additional words have been highlighted and removed based on the esteemed reviewer's feedback.
3- It is better to remove the words "Positive Blood Culture, Candidemia" from the keywords.
R: These additional words have been highlighted and removed based on the esteemed reviewer's feedback.

*****Introduction**

4- The prevalence rate of the Candidemia in Septicemia patients in the world and Iran should be reported.
R: This issue has been discussed in the discussion section.
5- Explain the importance of candidemia in the deterioration of septicemic patients and the treatment process.
R: This issue has been discussed in the discussion section.

***** Method**

6- How was the sample size determined?
The sample size was determined informally with statistical consultation, and this has been referenced in the manuscript and highlighted.
7- How was the quality of the extracted DNA checked?
R: The quality of the DNA was assessed using the Nanodrop method and by examining the OD, and it was found to be within an acceptable range.
8- Explain about internal control in DNA extraction Protocol.
R: Beta-macroglobulin was used as the internal control. This has been mentioned in the text and highlighted.
9- Explain about internal control in PCR Amplification Protocol.

R: Beta-macroglobulin was used as the internal control. This has been mentioned in the text and highlighted.

*****Results**

10- Since the sample size was 200, please show the power of the test If possible.
R: It was not possible.

***** Discussion and Conclusions**

11- The conclusion shall be made on the basis of your findings. You may not talk about definitely meaningful advances to the molecular detection of candidemia in septicemia-suspected patients, since you have not followed up your patients and did not observe the outcome of the other molecular detection Methods.
R: Significant advancements in the molecular diagnosis of Candida are achieved through scientific progress, representing a scientific pathway. However, the correlation between early and accurate diagnosis and timely treatment of patients undoubtedly requires follow-up and monitoring of patients, which was not conducted in our study and is, in fact, beyond the scope of our research.

2 Revision 1

4 comments · 7 Aug 2025 – 9 Aug 2025

Referee 2 Reviewer — ACCEPT — 7 Aug 2025

Hello Dear Editor
In my opinion, this article is publishable.

Referee 1 Reviewer — MINOR REVISION — 9 Aug 2025

We have reviewed your submission and noticed that the reviewers' comments/questions have not been addressed. Please provide detailed responses to all reviewer queries to facilitate the further evaluation of your manuscript.
Thank you for your cooperation.

Associate Editor — MINOR REVISION — 9 Aug 2025

Dear Authors
Your article needs to be revised. One of the reviewer's comments has not been addressed and is not reflected in the revised manuscript. Please respond fully to all reviewer comments and revise the manuscript accordingly based on their feedback.

Editor-in-Chief — MINOR REVISION — 9 Aug 2025

Dear Authors
Your article needs minor revision. One of the reviewer's comments has not been addressed and is not reflected in the revised manuscript.
When revising your manuscript, please consider all issues mentioned in the reviewers' comments carefully: please outline every change made in response to their comments and submit the required files.

3

Revision 2

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.

Reviewer 0:

1. Given that only *Candida albicans* has been identified and all information is on this yeast species, it is best to consider this *candida* throughout the entire text of the article.

R: Thank you for your valuable feedback. While it is true that only *Candida albicans* was identified in this study, it is important to note that this was not one of the primary objectives of our research. The primers designed in this study are intended to identify the genus *Candida*, which encompasses multiple species. Our initial assumption was based on the *Candida* genus, and the identification of *C. albicans* only emerged after analyzing the results of our research. Therefore, there was no preconceived bias towards the genus or species of *Candida albicans* at the outset of the study or during the manuscript preparation.

2. In the discussion section of the article, provide an adequate explanation of the superiority of HWP1 over other gene targets (such as ITS or 5.8S rDNA).

R: Thank you for emphasizing this important point, dear Reviewer. Yes, this aspect has been addressed in the text and has been highlighed accordingly.

3. Time to positivity in cultures was not reported, missing a chance to correlate molecular and conventional timelines.

R: In the Methods, it is mentioned that suspicious blood culture cases, which exhibit one of the signs indicatives of a positive blood culture, render the blood culture vial susceptible to positivity as soon as these signs are observed. Consequently, the diagnostic pathway, including subculture and diagnostic tests as well as antibiogram, should be performed promptly. For automated culture systems, this timeframe is a minimum of 10 hours, while for traditional blood cultures, it is at least 24 hours.

4. The influence of potential PCR inhibitors (e.g., heme, proteins) from blood samples was not quantified or addressed. Explain your reasons.

R: Since the extraction method utilized the Pars Tous kit, the PCR inhibitors have been removed or their effects minimized through various stages of the kit. Therefore, there was no interference in the PCR reaction. It is highlighted in text.

5. The study doesn’t investigate whether the *C. albicans* isolates were genetically identical or from diverse clades. Please explain.

R: The genetic relationship or similarity among the identified *Candida* species requires more extensive genetic investigations, which falls outside the scope of this study. This research focused on the identification of *Candida* species from suspicious positive blood cultures.

6. The limit of detection is reported numerically but lacks detailed explanation or modeling (e.g., probit regression or standard curve analysis).

R: Thank you for mentioning this important point, Following statistical consultation for this manuscript, the issue has been incorporated into the manuscript and highlighted accordingly.

7. The table numbers should be corrected. There are two Table 1.

R: it is corrected.

8. The electrophoresis figure lacks a clear size marker or scale.

R: It is revised and highlighted.

9. Patient inclusion criteria for “suspected sepsis” are not described.

R: We sincerely thank the esteemed reviewer for their insightful comment. It should be noted that the term "suspected sepsis" is applied to blood culture samples exhibiting macroscopic characteristics suggestive of a positive culture, which may arise from non-infectious conditions, such as hemolysis due to sampling errors. Patients with suspected sepsis are identified by clinicians based on clinical suspicion, and their samples are submitted to the laboratory for a battery of tests, including CRP, CBC, and blood culture. The determination of a patient’s clinical condition falls outside the scope of this study and is within the purview of the attending physician. This article focuses solely on samples with macroscopic features of a positive blood culture, conducting detailed molecular analyses alongside conventional diagnostic methods, with results validated against the gold standard of culture.

10. Redundant repetition between abstract and results sections.

R: We appreciate the reviewer’s feedback, and the issue has been revised accordingly. However, as the abstract’s results section inherently includes a concise summary, a brief reference to this point has been retained.

11. The sample is described as "whole blood" but was actually culture fluid.

R: It is revised and Highlighted in the text.

12. Terms like "Interquartile Range" are used without explanation for non-specialist readers.

R: The statement, along with a brief explanation, has been highlighted in the manuscript.

Chief Reviewer: Accept with major revision

Dear Editor/Author

Thanks for all

In my opinion, it needs a major revision.

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3 Revision 2

3 comments · 11 Aug 2025 – 12 Aug 2025

Referee 1 Reviewer

ACCEPT 11 Aug 2025

Dear Editor,
I confirm that the authors have adequately answered the questions posed and that the answers are acceptable.

Associate Editor

MINOR REVISION 12 Aug 2025

Dear Authors
Thank you for improving the quality of the article. However, some grammatical issues remain in the manuscript. Further editing is recommended to address these issues.

Editor-in-Chief

MINOR REVISION 12 Aug 2025

Dear Authors
According to the associate editor's comments, your article requires minor revision. Please outline every change made in response to her comments and submit the required files.

4

Revision 3

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

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4 Revision 3

1 comment · 18 Aug 2025

Editor-in-Chief

ACCEPT 18 Aug 2025

Dear Authors
We are pleased to inform you that your article has been accepted for publication in the MEJRHS. We hope to receive other qualified articles from your group.