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PEER-REVIEW REPORT

Name of journal: *World Journal of Virology*

Manuscript NO: 88164

Title: Development of a multiplex polymerase chain reaction assay for detection of hepatitis C virus, hepatitis B virus, and human immunodeficiency virus 1

Provenance and peer review: Invited manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 05620806
Position: Peer Reviewer
Academic degree: BSc, MSc, PhD
Professional title: Postdoctoral Fellow
Reviewer's Country/Territory: United States
Author's Country/Territory: Egypt

Manuscript submission date: 2023-09-16

Reviewer chosen by: Yu-Lu Chen

Reviewer accepted review: 2023-10-23 14:58

Reviewer performed review: 2023-11-04 14:10

Review time: 11 Days and 23 Hours

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Novelty of this manuscript	[ ] Grade A: Excellent [Y] Grade B: Good [ ] Grade C: Fair [ ] Grade D: No novelty
Creativity or innovation of this manuscript	[ ] Grade A: Excellent [Y] Grade B: Good [ ] Grade C: Fair [ ] Grade D: No creativity or innovation

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Scientific significance of the conclusion in this manuscript	[ ] Grade A: Excellent [Y] Grade B: Good [ ] Grade C: Fair [ ] Grade D: No scientific significance
Language quality	[ ] Grade A: Priority publishing [ ] Grade B: Minor language polishing [Y] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [Y] Major revision [ ] Rejection
Re-review	[Y] Yes [ ] No
Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous
	Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

The manuscript appears to be well-structured and provides a detailed explanation of the development and evaluation of a multiplex PCR assay for the simultaneous detection of HCV, HBV, and HIV-1. While the manuscript presents a valuable contribution to the field of diagnostic virology, there are several shortcomings and areas for improvement that should be addressed. With some revisions and additional details, it can be even more informative and impactful. Here are some specific comments and suggestions: 1. The abstract needs major restructuring and language formatting for better clarity and putting the message through concisely. 2. Consider making the title more specific and informative. It should clearly convey the focus of the study, such as "Development and Evaluation of a Multiplex PCR Assay for Simultaneous Detection of HCV, HBV, and HIV-1." 3. In the introduction, provide a bit more context on the global impact of HCV, HBV, and HIV-1. Mention some statistics or the consequences of these infections to emphasize their importance. 4. Consider including a brief description of the equipment and software used in the laboratory experiment (e.g., the PCR machine and data analysis software). 5. Include units for measurements where relevant, temperature in degrees

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Celsius needs to be written in correct format. 6. Provide more details about the actual experimental results, such as the concentration of primers and the specifics of the LOD calculations. 7. 'Purified viral genomes (whether RNA or DNA) were added as templates into reverse transcription (RT) reaction which was carried out using Invitrogen™ SuperScript™ III Reverse Transcriptase', can the authors please elaborate on this? 8. The statement 'except for a tiny melt curve peak at a lower melting temperature' needs to be discussed properly. 9. Expand on the implications and potential applications of the developed multiplex PCR assay. How could this method be used in real-world scenarios, and what are the potential benefits? 10. Discussion lacks in-depth analysis and interpretation. Consider discussing the practical implications of the research. What are the potential advantages and limitations in clinical or research settings? 11. Discuss any limitations or challenges faced during the study or potential sources of error. Were there any difficulties in designing the primers or conducting the experiments that could affect its practical application? 12. Clarify the significance of the correlation between %E and LOD. Explain why this correlation is important and what it means for the practical application of the assay. 13. Consider adding a statement about future directions. What are the next steps or potential improvements for this research? 14. It would be beneficial to compare the performance of your multiplex PCR assay with existing methods for detecting HCV, HBV, and HIV-1. How does it compare in terms of sensitivity, specificity, and cost-effectiveness? Providing a benchmark for your assay's performance can strengthen your conclusions. 15. Some of the references that are mentioned as recent date back to 2018, needs to be updated. 16. The figures provided are low resolution and not very clear, hence limiting the understanding of the results. 17. Proofread the manuscript for grammar, punctuation, typographical errors, and clarity (Eg. 'dimmers'). There are a few sentences that could be rephrased for better readability. While the manuscript presents an innovative approach to simultaneous detection of HCV, HBV,

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and HIV-1, addressing the above shortcomings will enhance the clarity and impact of the research. Providing more context, data, and analysis will make the manuscript more informative and accessible to a broader scientific audience.