**Response to Editorial and Reviewers Comments in bold**

**Editorial comments:**  
Changes to be made by the Author(s) regarding the manuscript:  
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

**We have tried our best to make this manuscript free of spelling or grammar issues.**

2. Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account. The Figure must be cited appropriately in the Figure Legend, i.e. “This figure has been modified from [citation].”

**Permission has been taken from Journal of general virology for the use of figure 2 and 3. Permission document has been uploaded. Figure legends have been updated as suggested.**   
3. Please upload each Figure individually to your Editorial Manager account as a .png, .tiff, .svg, .eps, .psd, or .ai file.

**Each figure uploaded separately as .png**

4. Figure 1: Please use SI abbreviations for all units (°C, h) and include a space between numbers and their units. Please make the number in CO2 as a subscript.

**Figure 1 corrected**  
5. Please provide an email address for each author.

**Email of each author included**

6. Please spell out each abbreviation the first time it is used.

**Checked the abbreviation and corrected them**

7. Please use SI abbreviations for all units: L, mL, µL, h, min, s, etc.

**All the units are changed to SI abbreviations**

8. Please include a space between all numbers and their corresponding units: 15 mL, 37 °C, 60 s; etc.

**Space included between all the numbers and their corresponding units**

9. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents. For example: Trizol, Qiagen, Nanodrop, Bio-Rad iCycler, QuantiFast®, etc.

**We have tried to make the language as generic as possible by simply assigning the name of Qiagen kit buffers according to their functions, as the original compositions of the buffers is classified.**

10. Please revise the protocol to be a numbered list: step 1 followed by 1.1, followed by 1.1.1, etc. Please refrain from using bullets, dashes, or indentations.

**Numbering corrected**  
11. Please revise the protocol text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).

**Changed as suggested**

12. Please revise the protocol to contain only action items that direct the reader to do something (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” Please include all safety procedures and use of hoods, etc. Please move the discussion about the protocol to the Discussion.

**Changed as suggested**

13. The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. Please simplify the Protocol so that individual steps contain only 2-3 actions per step and a maximum of 4 sentences per step. Use sub-steps as necessary. Please move the discussion about the protocol to the Discussion.

**We understand that protocol should be based on discrete steps. We have tried to make short and precise paragraphs.**

14. Lines 161-163: Please remove the embedded table from the manuscript. All tables should be uploaded separately to your Editorial Manager account in the form of an .xls or .xlsx file. Each table must be accompanied by a title and a description after the Representative Results of the manuscript text.

**Table removed from the manuscript and .xls file attached to the Editorial manager.**

15. Please add more details to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. For example:  
Lines 165-166: Please mention how to obtain and monitor melt curve and amplification value. What values are considered to be healthy?

**Information related to the amplification values and melt cure has been added to manuscript**

Line 168: How to calculate the ΔΔCt values?

**Formula has been added to the manuscript**

16. Please reference figures showing the experimental set-up in the Protocol.

**We made this figure to give a visual demonstration of step involved in this protocol specifically for this manuscript and has not been published before.**

17. Please include single-line spaces between all paragraphs, headings, steps, etc.

**Revised as suggested**

18. After you have made all the recommended changes to your protocol (listed above), please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol.

**Protocol paragraphs highlighted**  
19. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense.

**Done as suggested**  
20. Please include all relevant details that are required to perform the step in the highlighting. For example: If step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be highlighted.

**Done as suggested**  
21. Please describe Figure 3 in more detail in Representative Results.

**More details of figure 3 has been added**  
22. As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:  
a) Critical steps within the protocol  
b) Any modifications and troubleshooting of the technique  
c) Any limitations of the technique  
d) The significance with respect to existing methods  
e) Any future applications of the technique

**We have highlighted all the critical steps in main protocol. And we have discussed both significance and future applications of the techniques in the discussion.**

23. References: Please do not abbreviate journal titles.

**Changed as suggested**

24. Please revise the table of the essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials in separate columns in an xls/xlsx file. Please remove trademark (™) and registered (®) symbols. Please provide lot numbers and RRIDs of antibodies, if available.

**Revised as suggested**

**Reviewers' comments:**  
  
**Reviewer #1:**  
  
Manuscript Summary:  
This manuscript describes a simple but effective, RT-QPCR based protocol to assess the dengue serum caused ADE on Zika infection in human macrophages or primary cell lines. This protocol is significant and timely because ZIka virus is causing world-wide human health concerns. There are a lot of efforts in developing antivirals against Zika infection and this protocol may facilitate the process of testing the potential ADE of antibody-based anti-Zika vaccines. This protocol will also be interested especially to the related new investigators who recently started to study Zika and dengue related research topics.  
  
Major Concerns:  
None.  
  
Minor Concerns:  
The authors may need to state the strain name of Zika virus they used, and which Zika gene they amplified with their qPCR primers.

**Information about strain and gene used for qPCR primers has been included in the manuscript**  
**Reviewer #2:**  
  
Manuscript Summary:  
A good manuscript worth for JOVE, however minor corrections are required.  
  
Major Concerns:  
1. Line 31 it appears that (ZIKV), which uses Aedes aegypti and Aedes albopictus as its primary and secondary transmission vector, where As per Grard et al., 2007, Aedes albopictus is primary vector for Zika virus involved in the Gabonese outbreaks.

**The information along with reference regarding *Aedes Albopictus* has been added to the manuscript.**

2. Line 51 write full form of abbreviation PRNT.

**The full form of PRNT has been added to the manuscript.**

3. A little-bit brushing in language correction is required.

**We have tried our best to improve the language of the manuscript.**  
  
Minor Concerns:  
  
Add few latest references on ADE e.g. Khandia et al. (2018) Modulation of Dengue / Zika Virus Pathogenicity by Antibody-Dependent Enhancement and Strategies to Protect against Enhancement in Zika Virus Infection. Front. Immunol. doi: 10.3389/fimmu.2018.00597.

**The abovesaid reference has been added**