03-22-16

We are grateful to the editors for constructive suggestions. Please find point-by-point replies (indented blue text) and modified/added text has been changed to red in the main article.

Editorial comments:

1. Editor modified the formatting of the manuscript and made minor copy-edits. In keeping with the JoVE format, a few of your protocol steps may have been revised to the imperative tense and any step that could not be written in the imperative tense may have been added as a “Note”. Please maintain the current formatting throughout the manuscript. You can find the updated manuscript attached to this e-mail.

Relevant text has been modified accordingly.

2. In the Author Affiliations section of the manuscript, please provide an email address for each author.

We added email addresses for each author on Page 1.

3. Please adjust the numbering of your protocol section to follow JoVE’s instructions for authors, 1. should be followed by 1.1) and then 1.1.1) if necessary. Please make sure that all steps are lined up at the left margin with no indentations and there is a one line space between each protocol step (done for you). Please note that in the remainder of this letter, steps are referred to using the JoVE format, for e.g. in section 1, step 4 is referred to as step 1.4.

We tried to use your recommended format. Please see the new section “setting up replicating and non-replicating MIC90 assays”, with subheadings 4, 4.1, and 4.2.

4. Please define all abbreviations when they are first used, e.g. OADC.

To our knowledge, all abbreviations have been corrected to indicate what they mean the first time they appear in the text.

5. Please add more details to the following protocol steps

a) 1.7 – it is not clear what is meant by “repetitive injuries”. Please revise.

Repetitive injuries such as carpal tunnel and intense thumb pain are very common when performing high-throughput style assays in the laboratory. To simplify the protocol, we simply described using the p1000 electronic pipettor and removed text about repetitive injuries.

b) 2.1 – What are the concentrations of albumin, dextrose, NaCl, oleic acid and catalase? How is the inoculation done and what is the volume of the culture? What are the culture conditions (temperature, agitation etc.)?

All concentrations have been added to the text.

c) 2.2 – how is the experiment set up? Please provide step-wise details written in the imperative tense, as if you are telling someone how to do the technique (i.e. "Do this", "Measure that" etc.). Please also simplify this step into sub-steps (e.g. 2.2.1, 2.2.2 etc.) so that individual steps contain only 2-3 related actions per step.

Relevant text has been modified accordingly.

6. After you have made all of the recommended changes to your protocol (listed above), please re-evaluate the length of your protocol section. There is a 10-page limit for the protocol text, and a 3- page limit for filmable content. If your protocol is longer than 3 pages OR if there steps in protocol that will NOT be filmed, please highlight 2.75 pages or less of text (which includes headings and spaces) to identify which steps should be visualized to tell the most cohesive story of your protocol steps. Please see JoVE’s instructions for authors for more clarification. Remember that the non-highlighted protocol steps will remain in the manuscript and therefore will still be available to the reader.

Our protocol falls within the recommended 3 page limit.

7. JoVE is unable to publish manuscripts containing commercial sounding language, including trademark or registered trademark symbols (TM/R) and the mention of company brand names before an instrument or reagent. Please remove all commercial sounding language from your manuscript. Examples of commercial sounding language in your manuscript are “alamarBlue™”, “Ziploc” etc. All commercial products should be sufficiently referenced in the table of materials/reagents.

We have removed text containing “AlamarBlue”, “Ziploc”, and “Kimwipe”.

8. Please revise the legend for figure 5 so that all parts of the figure (A-D) are mentioned.

The Fig5 legend has been revised to include parts A-D.

9. Please expand your discussion to cover: 1) modifications and troubleshooting, 2) critical steps in the protocol.

We have added a final paragraph in the discussion to explore modifications, troubleshooting and critical steps in our protocol.

10. Please upload Table 1 as an Excel file.

We have uploaded the original Excel file of Table 1.

11. Please ensure that all materials used in the Protocol appear in the Table of Materials, e.g. OADC.

The Table of Materials has been significantly expanded to include all reagents.

12. Please remove the trademark (™) symbol(s) from the table of reagents/materials.

All trademark symbols have been removed.