July 20, 2016

Dear Editor:

As requested in your email from July 1, 2016 I’ve reviewed manuscript JoVE55151 (*Membrane Diffusion Chambers and Above Ground Mesocosms for the Study of Microbial Responses to Groundwater Geochemistry*) and addressed each of the reviewer’s/editor’s comments as listed in email. There were a few comments which I felt were too ambiguous but tried to address what I thought the deficiency(ies) the comment was addressing. If I misinterpreted any of these comments, I would like the opportunity to properly address those comments with additional guidance from the editorial staff.

The changes to the revised manuscript are recorded within the manuscript using the Track Changes option in MS Word. Hopefully, these changes have made the manuscript a better product while bringing the format more in line with the publication requirements of JoVE.

My responses to each of the reviewer’s/editor’s comments as listed in the email are included in this document.

Thanks and I look forward to hearing back from you and your staff,

John Lisle, PhD

US Geologic Survey

St. Petersburg Center for Coastal & Marine Research

600 Fourth Street-South

St. Petersburg, FL 33701

**Author’s Responses to Editorial Comments**

**JoVE55151**

Membrane Diffusion Chambers and Above Ground Mesocosms for the Study of Microbial Responses to Groundwater Geochemistry (Author: John Lisle)

*1. Please re-write steps of your protocol section in imperative tense, as if you are telling someone how to do the technique (i.e. "Do this", "Measure that" etc.). Please try to avoid usage of phrases such as “should be”, “could be”, “would be” and write in the active/imperative style.*

**Author’s Response:** The manuscript was reviewed and changes were made as suggested.

*2. Please simplify steps of your protocol section so that individual steps contain only 2-3 actions per step. As written currently, the steps are too long to review in-depth and comment succinctly. (See sections 5, for example).*

**Author’s Response:** A complete review of the manuscript was conducted and several of the steps in the protocol which could be sub-divided into smaller steps are now listed as sub-steps. These changes have each step and sub-step containing 2-3 separate actions as requested.

*3. 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 and replace it with a more generic term as much as possible throughout the entire manuscript. All commercial products should be sufficiently referenced in the table of materials/reagents. Examples of commercial sounding language in your manuscript are “ATCC #BAA–1159”, etc.*

**Author’s Response:** The reference to the commercial source of the bacterial culture has been deleted. It’s not clear how you would prefer the names of media, which are proprietary products with unique names (e.g., mTEC agar), described in the protocol. Are you OK with the product name being listed in the protocol as long as the company name is not included?  
  
*4. Step 4.1.9.1 should be a NOTE.*

**Author’s Response:** “NOTE” has been added to this step.  
  
*5. Please provide a reference for step 7.1.*

**Author’s Response:** The appropriate citation has been added to step 7.1.

*6. In the representative results section please remove the numbering. Representative results should not be written like a protocol it should discuss specific results.*

**Author’s Response:** All numbering has been removed from the Representative Results section.  
  
*7. Please remove the embedded Table from the manuscript. All tables should be uploaded to the Editorial Manager site in the form of Excel files. A description of the table should be included with the Figure legends.*

**Author’s Response:** The tables have been removed from the body of the manuscript, as requested, and place holder notation added. The table titles have been added to the Figure Legends file.   
  
*8. Please remove the embedded figures from the manuscript. Figure legends, however, should remain within the manuscript text, directly below the Representative Results text.*

**Author’s Response:** Figure 5, the only figure embedded in the manuscript version I have, has been deleted. You requested this figure legend be left in place, but this was not required for the other figures. The legend for this figure is also listed in the Figure Legends file.   
  
*9. Please expand your representative results in the context of the technique you describe; i.e. how do these results show the technique, suggestions about how to analyze the outcome etc. This text should be written in paragraph form under a "Representative Results" heading and should refer to all of the results figures. You may include the figure captions under this heading but the captions and figure text must be separate entities.*

**Author’s Response:** Based on the example papers sent and the description of what is required for writing this section of a JoVE paper on your website, I think the current format of the Representative Results meets those criteria and the general ones mentioned in Comment #9. I did remove the numbering within this section as requested in Comment #6. If there is a specific issue with the way I’ve written this section I’ll be more than willing to make the changes but please be more specific with identifying the deficiencies.

*10. Each figure or data table must have an accompanying legend including a short title, followed by a short description of each panel and/or a general description. All figures showing data must include measurement definitions and error bars (if applicable). Please include the figure legends as part of the manuscript text (not part of the figure file) directly below the representative results text.*

**Author’s Response:** The figure legends have been inserted into the manuscript, in addition to being listed in the Figure Legends file. The table titles have also been listed in the Figure Legends file (see Editor’s Comment #8 and author’s response). Are you also asking for a detailed legend for the tables as well? Tables are normally constructed in a way that the title and column and row labels provide the information necessary for the reader to interpret the data so a detailed legend is not required.

*11. If your figures and tables are original and not published previously, please ignore this comment. For figures and tables that have been published before, please include phrases such as “Re-print with permission from (reference#)” or “Modified from..” etc. And please send a copy of the re-print permission for JoVE’s record keeping purposes.*

**Author’s Response:** All figures, tables and data sets in this manuscript have not been previously published.

*12. Please make sure that the “Discussion” is written under the following sections.  
a. Critical steps within the protocol.  
b. Modifications and troubleshooting.  
c. Limitations of the technique.  
d. Significance of the technique with respect to existing/alternative methods.  
e. Future applications or directions after mastering this technique.*

**Author’s Response:** It’s not clear to me if you have cited issues for each of these sections within the manuscript or is this just a list of items to check during my final edits and you have no specific comments. If there are specific issues or deficiencies please provide the line numbers for those passages. In case you are expecting a direct response to each item in this comment:

(a) Critical steps within the protocol: The critical steps in the protocol have been identified by the formatting and additional comments that in my opinion tell the reader these steps are critical for successfully completing the task.

(b) Modifications and troubleshooting: Throughout the manuscript I have added comments or steps and sub-steps that briefly describe possible modifications and identify steps and sub-steps which would be a problem if not properly executed. Are you asking for a section within the Discussion that re-lists and discusses these items?

(c) Limitations of the technique: Considering the focus of the manuscript is the design and construction of a type of mesocosm to study microbial responses under natural conditions, I don’t think there is a limitation. This is because if you’ve the funds, you can make any and all of the items described in this protocol from materials that can withstand the most extreme geochemical conditions that a human can access.

(d) Significance of the technique with respect to existing/alternative methods: There are no alternative methods to the one described in this manuscript. There are alternative designs for the diffusion chambers but these are differences in sizes, shapes and materials but these are not applicable to the environmental conditions described for my systems.

(e) Future applications or directions after mastering this technique: There are several other applications for the diffusion chambers and the above ground mesocosm, which I’ve noted in the manuscript (e.g., lines 667-682). I don’t think there is a higher level of expertise or skill that can be developed on the experience gained from successfully fabricating and deploying the mesocosm and diffusion chambers as described in this manuscript.

*13. IMPORTANT: Please copy-edit the entire manuscript for any grammatical errors you may find. The text should be in American-English only. This editing should be performed by a native English speaker (or professional copyediting services) and is essential for clarity of the protocol and the manuscript. Please thoroughly review the language and grammar prior to resubmission.* *Your JoVE editor will not copy-edit your manuscript and any errors in your submitted revision may be present in the published version.*

**Author’s Response:** I’m not sure if this is a general comment added to all of the Editor’s Comment documents or a specific comment on the writing style and structure of this manuscript. If there’s a major issue with the English style and structure used in this manuscript please be more specific with examples, denoted by line numbers.