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July 29, 2020

To the Editors and Reviewers of *Journal of Visualized Experiments*,

We are submitting our revised manuscript (JOVE61749R1) and thank the Reviewers for their efforts to improve the manuscript. We have addressed each comment to the best of our abilities in the paper and in a separate point-by-point response letter.

On behalf of all authors, I state again that there were no financial, personal, or professional interests that could be construed to have influenced the writing of this paper. All authors are aware and approve of this submission, its content, authorship, and the order of authorship.

We look forward to a thorough and positive review and appreciate the opportunity to submit our work to *Journal of Visualized Experiments*.

Best Regards,

Michelle C. Callegan, Ph.D.  
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## JoVE61749R1 (07/29/2020) RESPONSE TO REVIEWERS

We thank the Reviewers for their thorough and positive review of our JoVE submission. We address each comment below and denote where in the revised manuscript those changes can be found.

### **Editorial Comments:**

- *...proofread the manuscript to ensure that there are no spelling or grammatical errors.*  
Correct as suggested.

- *Avoid punctuating the title.*

Corrected as suggested, page 1.

- *Please list a minimum of 6 keywords/phrases.*  
Keywords and phrases amended, page 1.

- ***Protocol Language:*** *Please ensure that all text in the protocol section is written in the imperative voice/tense as if you are telling someone how to do the technique...1) Some examples NOT in the imperative: 1.11*

Addressed as suggested in 1.11 and throughout.

- ***Protocol Detail:*** *... Please ensure that all specific details (e.g. button clicks for software actions, numerical values for settings, etc) have been added to your protocol steps. ...*

Addressed as suggested throughout.

*1) Please include an ethics statement before your numbered protocol steps indicating that the protocol follows the animal care guidelines of your institution.*

Ethics statement added, page 3.

- ***Protocol Highlight:*** *Please highlight ~2.5 pages or less of text (which includes headings and spaces) in yellow, to identify which steps should be visualized to tell the most cohesive story of your protocol steps...*  
Relevant text has been highlighted in Sections 1-7.

- ***Discussion:*** *JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form (3-6 paragraphs): 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.*

The Discussion covers these areas.

• **Figures:**

1) Fig 2: Add scale bars to all micrographs.

Added as suggested.

• **References:**

1) Please spell out journal names.

Corrected as suggested.

• **Commercial Language:** 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. 1) ... replace all commercial sounding language in your manuscript with generic names that are not company-specific.

Corrected as suggested throughout.

• **Table of Materials:** Please sort in alphabetical order.

Corrected as suggested.

**Reviewer #1:**

**Minor Concerns:**

*For method 7.7, wouldn't the concentration be  $4.5 \times 10^5$  CFU per 10 uL (rather than per mL) since 10 uL was plated?*

For bacterial track dilution, the multiplication of a dilution factor with the respective colonies represent the CFU/mL. That is why 45 colonies at 4th dilution represents  $45 \times 10^4$  CFU/mL or  $4.5 \times 10^5$  CFU/mL.

**Reviewer #2:**

**Major Concerns:** *My only concern was that the authors only describe intravitreal injection of Bacillus or bacterial species and its quantification can only be applied for bacterial infections. The qualification of fungal spores/hyphae would vary and would not be applicable in this context. Hence the authors should change their title to specify only Mouse Model of BACTERIAL Endophthalmitis.*

We agree and have changed the title as suggested.

**Minor Concerns:**

*It would also be useful for Point 3.2- that the authors specify the McFarland concentration used for qualification as initial load and overnight growth may vary among clinical strains.*

We did not use a McFarland standard to quantify the concentration stated in the protocol. Point 3.2 (page 5) states that the growth of a *Bacillus* overnight achieves  $2 \times 10^8/10$  uL. We included the dilution needed to achieve 200 CFU/uL of *Bacillus*. We discussed the variation in the growth of other species in the Discussion (full paragraph #1 on page 11).

Respectfully submitted,

*Michelle Callegan*