May 16, 2017



Editor,

Journal of Visualized Experiments

Dear Editor,

Please consider our revised manuscript entitled “**Dissection of the Mouse Pancreas for Histological Analysis and Metabolic Profiling**” for publication in the *Journal of Visualized Experiments*. Below, you will find our detailed responses to the reviewer’s comments.

**Reviewer #1:**

1) Reviewer comment: “Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.”

**Response:** Manuscript was proofread to ensure no spelling errors or grammatical errors were present. If found, they were corrected.

2) Reviewer Comment: “**Introduction:** Paragraph 2 is lacking appropriate references.”

**Response:** Additional references were added in paragraph 2 of the Introduction to provide adequate citations.

3) Reviewer Comment: “**Title/abstracts:**As there is no histology or metabolic profiling presented, these must be edited to reflect the manuscript and video content OR results/data showing these should be added.”

**Response:** Representative results and figures have been added showing histology and metabolic profiling data.

4) Reviewer Comment: “**Protocol Language:** Please edit the language so that ALL text in the protocol section is written in the imperative tense as if you are telling someone how to do the technique (i.e. “Do this”, “Measure that” etc.) Any text that cannot be written in the imperative tense may be added as a “Note”, however, notes should be used sparingly and actions should be described in the imperative tense wherever possible.  
1) Examples NOT in imperative tense: 1.1, 1.1.2, 1.1.3, 1.2, 1.3, 1.4, 1.5, etc.  
2) Some long steps were split up.”

**Response:** 1) Language was edited to imperative tense or a “Note” was added. 2) Long steps were split up.

5) Reviewer Comment: “**Protocol Detail:** Please add more details to the following protocol steps.  
1) 1.6: Mention isofluorane%.  
2) 1.8: How do you ensure euthanasia?  
3) 1.9: How much isoflurane do you use? What %? It appears that the mouse still alive in 1.9.1. If so, it is confusing to call the glass jar a euthanasia jar, instead perhaps call it anesthesia jar?”

**Response:** 1) Added 99.9%. 2) “Euthanasia” chamber was changed to “anesthesia” chamber. Euthanasia is discussed in a later section 2.3.5. 3) Sentence was changed to say, “Place the head inside the tube lined with a surgical pad soaked with a few drops of isoflurane (99.9%)”. Euthanasia was changed to read anesthesia.

6) Reviewer Comment: “**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: 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.”

**Response:** Discussion was edited to include sections titled: “significance with respect to existing methods, limitations of the technique, critical steps within the protocol, modifications and troubleshooting, and future applications”.

7) Reviewer Comment: “**Figures:**:  
1) Fig 1-10: Please include scale bars on all images to provide context to the magnification used.  
2) Fig 9,10 : Please include arrows to indicate spleen and pancreas.”

**Response:** Scale bars have been added to figures 1-10. On figures 9 and 10, arrows have been included to indicate spleen and pancreas.

8) Reviewer Comment: “**Figure/Table Legends:**:  
1) Fig 3: It may be best not to call this a “euthanasia chamber as the mouse is euthanized much later in the procedure.”

**Response:** The word “Euthanasia” was changed to “Anesthesia” in the title and caption.

9) Reviewer Comment: “**References:**Please make sure that your references comply with JoVE instructions for authors. Citation formatting should appear as follows: (For 6 authors or less list all authors. For more than 6 authors, list only the first author then *et al.*): [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. *Source*. **Volume** (Issue), FirstPage – LastPage, doi:DOI (YEAR).]  
1) Please abbreviate all journal titles, and also edit the references to match the suggested format.”

**Response:** References were edited to match the suggested format.

10) Reviewer Comment: “**Table of Materials:**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/software in separate columns in an xls/xlsx file. Please include items such as mouse strain.”

**Response:** Mouse strain information was added to the materials list.

11) Reviewer Comment: “Please define all abbreviations at first use.”

**Response:** All abbreviations have been defined at first use.

12) Reviewer Comment: “Please use standard abbreviations and symbols for SI Units such as µL, mL, L, etc., and abbreviations for non-SI units such as h, min, s for time units. Please use a single space between the numerical value and unit.”

**Response:** Standard abbreviations and symbols have been added/changed.

13) Reviewer Comment: “If your figures and tables are original and not published previously or you have already obtained figure permissions, please ignore this comment. If you are re-using figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."”

**Response:** Figures and tables have not been previously published and are original- not action was taken for this comment.

**Veterinary Reviewer:**

1) Reviewer Comment: “Isoflurane anesthesia delivered via open drop method is acceptable, however, care should be taken to ensure the mouse does not come into contact with the isoflurane liquid, or the soaked gauze, as this can be irritating and cause discomfort. There are methods designed to ensure the mouse is only exposed to the vapors and not the liquid isoflurane. This should be addressed, see article for an easy way to accomplish this: Taylor DK, Mook DM. Isoflurane Waste Anesthetic Gas Concentrations Associated with the Open-Drop Method. Journal of the American Association for Laboratory Animal Science: JAALAS. 2009;48(1):61-64.”

**Response:** To ensure the animal does not come in contact with the isoflurane, a barrier needs to be placed between the pad soaked with a few drops of isoflurane and the animal. Manuscript was edited to read that a paper towel over the top of the pad will serve as this barrier.

2) Reviewer Comment: “The instructions say to use a “soaked surgical pad” with isoflurane – the use of isoflurane should be minimized and from an occupational health perspective and personnel exposure perspective, the least possible volume that will provide anesthesia should be used. It is unnecessary to “soak” a gauze pad, therefore, with isoflurane. This should be addressed in the text – the video already says (more correctly) “soaked with a few drops of isoflurane”

**Response:** Manuscript was edited to say soaked with a few drops of isoflurane.

3) Reviewer Comment: “1.6 says isoflurane should be used only in the “vent hood.” – scavenging of waste anesthetic gas should be addressed in more detail, as not all “vent hoods” provide enough scavenging. The article above and others are readily available upon searching to address appropriate waste gas scavenging.

**Response:** The scavenging of waste anesthetic gas, regarding isoflurane usage, has been address and referenced.

4) Reviewer Comment: “Text: page 4, section 1.9 states “place the head inside the falcon tube lined with the isoflurane soaked surgical pad and perform a foot pinch to ensure discomfort is not being experienced” – this would better be described as in the video, where the voiceover says “mouse cannot experience discomfort” – an even better description would be “mouse is unresponsive to stimuli.”

**Response:** In section 1.9 the words, “discomfort is not being experienced” was change to “the mouse is unresponsive to stimuli.”

5) Reviewer Comment: “The video voiceover describes a cervical dislocation after the mouse is anesthetized and removed from the isoflurane jar. If that is done, there is no need to put the mouse’s head in a tube with isoflurane during the dissection, as the mouse has been euthanized, and therefore represents an unnecessary occupational exposure to isoflurane.”

**Response:** Following transfer from the anesthesia chamber to the dissection table, the mouse is still alive up until the terminal blood draw was performed. After the terminal blood draw, the heart was detached as a secondary method to ensure euthanasia. Therefore, the head of the mouse remained in the tube until euthanasia was performed. At this point the dissection of the pancreas was carried out. This is accurately reflected in the video and article in the revised manuscript.

We hope that our revised manuscript is now considered suitable for publication in the Journal of Visualized Experiments.

Sincerely,

Michael A. Kennedy

Professor and Eminent Scholar

Department of Chemistry and Biochemistry

Miami University

Oxford, OH 45056