DU_LOGO_Blue for letter head BIOLOGY Gen

December 20, 2018

Dear Dr. Vineeta Bajaj,

Thank you for your email of December 13, 2018 that requested corrections and revisions to our manuscript JoVE59295 "Validating tail vein injections in rat with near infrared labelled agents; a new best practice."

We have made all the requested corrections, which I will list below. Also, Figures 1 and 2 are provided as .tif (flattened) at 300 dpi. If you need, I will be happy to provide the original assembly of the figures as layered .psd.

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**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.

**Authors Response:** All authors have again proofed the manuscript.

2. Please note that Standard Access is checked in the uploaded ALA, while in the Questionnaire Responses Open Access is selected. Please be consistent.

**Authors Response:** We will be open access and will make the documentation consistent.

3. Please revise the title to avoid the use of semicolon.

**Authors Response:** The title has been revised to “A new best practice for validating tail vein injections in rat with near infrared labelled agents.”

4. Abstract: Please do not include references here.

**Authors Response:** References have been removed from the abstract section and the text has been revised

5. 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. You may use the generic term followed by “(see table of materials)” to draw the readers’ attention to specific commercial names. Examples of commercial sounding language in your manuscript are: LI-COR Pearl®, LI-COR Biosciences, Caliper, Xenogen IVIS, Perkin Elmer, VisEn Medical FMT, D100012G Research Diet, Inc., BrightSite™ IRDye®, SAIVI™, Hilltop Animals, SURFLO®, etc.

**Authors Response:** Commercial language has been excluded from the manuscript; in it’s place, generic terms such as “small animal imager” and “see table of materials” have been used.

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

**Authors Response:** All personal pronouns have been removed from the manuscript.

7. 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. However, notes should be used sparingly and actions should be described in the imperative tense wherever possible. Please move the discussion about the protocol to the Discussion.

**Authors Response:**

* + The Protocol contains only action items that direct the reader to do some specific action. These actions are described in the imperative tense in complete sentences. Any text this is not imperative has been added as a “Note.” Notes have been used sparingly.
  + Phrases such as “could be,” “should be,” and “would be,” have been removed.
  + All safety procedures have been included.
  + Discussion about the protocol has been moved to the Discussion.

8. 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.

**Authors Response:** The Protocol has been simplified to contain only 2-3 actions per step with a maximum of 4 sentences per step. Sub-steps have been used when necessary.

9. 1.3: Please mention how proper anesthetization is confirmed.

**Authors Response:** The text has been edited to indicate that proper anesthetization is confirmed via tail pinch.

10. 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.

**Authors Response:** Aspects of the Protocol (including headings and spacing) have been highlighting in order to identify essential steps for video production.

11. 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. Please do not highlight any steps describing anesthetization and euthanasia.

**Authors Response:** Highlighted regions of the protocol are complete sentences, and include at least one action that is written in imperative tense. As requested, no steps describing anesthetization and euthanasia are included.

12. 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.

**Authors Response:** All relevant sub-steps are included in highlighted sections where they are required for video production.

13. Discussion: Please discuss critical steps within the protocol.

**Authors Response:** The Discussion section has been edited to provide more depth on critical steps in the Protocol.

14. Please remain neutral in tone when discussing commercial products. The accompanying video cannot become an advertisement.

**Authors Response:** We have adjusted the text to remain neutral when discussing commercial products; all commercial products will be addressed with a generic term to avoid any sense of promotion.

15. References: Please do not abbreviate journal titles.

**Authors Response:** The reference list has been edited to included full journal titles.

16. Table of Materials: Please sort the items in alphabetical order according to the name of material/equipment.

**Authors Response:** As requested, items have been sorted in alphabetic order according to the name of the material/equipment.

**Reviewers' comments:**  
  
Reviewer #1:  
  
Manuscript Summary:  
The manuscript is very useful and will help to avoid common and widespread problems with tail vein injections. A few points might be addressed and added to strengthen the manuscript.

**Authors Response:** We appreciate the positive comments from this reviewer.

Major Concerns:  
None  
  
Minor Concerns:  
  
1. Please explain why tail injection is a preferred way for the delivery of the probes. Are there any other points of entries can be used?

**Authors Response:**  There are other point of entry for venous injection, which we describe in the opening of the introduction.

2. What is the typical time when the mouse or rat with a subcutaneous tail injection can be reused for imaging?

**Authors Response:** This question goes beyond the scope of this work and will not be discussed in the manuscript. There are a number of variables that can influence this, which would be contingent on the nature and design of an individual experiment. That being said, in the case of a successful injection, the animal could likely be re-injected (in the contralateral vein) within hours. An unsuccessful injection that leave a subcutaneous bolus should be removed from the study and should not be re-used.

3. Some companies offer devices to facilitate tail vein injections, i.e Rotating Tail Injector: <https://www.braintreesci.com/prodinfo.asp?number=RTI>, or Tailveiner: <https://www.medilumine.com/mouse-and-rat-restrainers-for-tail-vein-injections/> . Could you comment on these?

**Authors Response:**

4. There are also publications of using automatic tail vein injectors, not sure if there are commercially available. This information might also be added to the discussion

**Authors Response:** Automatic tail vein injectors have been commented on in the discussion section.

Reviewer #2:  
  
Manuscript Summary:  
Optimizing tail vein injections is very important for many experimental procedures. In this study, the authors describe a method of tail vein injections in rat with near infrared labelled agents. And they summarized the detailed procedures and notes in every step of tail vein injections. It is helpful for the researchers to deal with similar experimental procedures.

**Authors Response:** The authors appreciate these supportive observations.

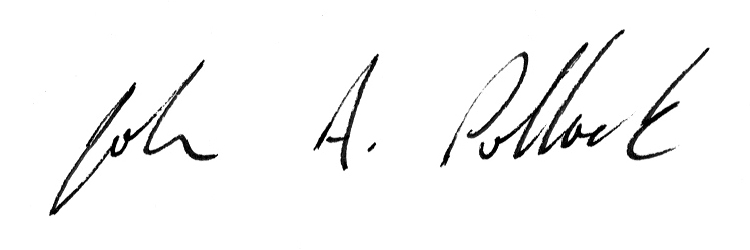
Major Concerns:  
Just minor concerns, that is, in the discussion section, the author should give more discussion about their improvements and notes about experimental procedures rather than statements of experiment.

**Authors Response:** The authors have added to the discussion commentary on how this method offers improvement.

Some more papers can be referenced for improving, for examples,  
Fluorescent chemical probes for accurate tumor ……, CHEMICAL SOCIETY REVIEWS, 2017, Volume: 46, Issue: 8, Pages: 2237-2271;  
Ratiometric Near-Infrared Fluorescent Probe for ……, ANALYTICAL CHEMISTRY, 2018, Volume: 90, Issue: 6, Pages: 4054-4061;  
A Ratiometric Near-Infrared Fluorescent Probe for ……, ADVANCED FUNCTIONAL MATERIALS, 2017, Volume: 27, Issue: 28, Article Number: UNSP 1700769

**Authors Response:** We appreciate these recommendations and have read these papers and find them useful and appropriate; and they are now cited in the discussion.

Sincerely,



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