

Title: A simple, visualized approach to assess mouse peritoneal macrophage phagocytosis using EGFP-expressing Escherichia coli

URL: <https://www.jove.com/video/58751/title?status=a60757k>

Were animals used humanely and was the appropriate anesthesia or analgesia provided for potentially painful procedures?

The article and presentation primarily discuss *in vitro* work, with the animal portion only providing a source of fresh macrophage cells. The authors do state that primary peritoneal macrophages are ideal for their purpose over established cultured cell lines.

It is very difficult to answer this question for this presentation and article as very little information is provided regarding the animal care.

There is a statement that the procedures were approved by the institution’s Animal Care and Use Committee. My questions and comments for the text part 2. Mouse peritoneal macrophage isolation and primary culture section follow:

1. The health status of the mice and husbandry conditions are not mentioned. A brief paragraph to state type of housing, feed used, health status, etc. is standard in animal studies. Murine pathogens can also alter macrophage function so that healthy SPF mice are important in this type of procedure. Strongly suggested.

Reply: The information has been added in the revision. Sixteen-month-old (body weight 30-35 g) and 8-week-old (20-25 g) SPF male C57BL/6 mice were obtained from the SPF animal center of Dalian Medical University. All mice were kept in the animal house with access to food and water ad libitum. The temperature was kept at 20-24 °C, humidity was 40-70 %, and lighting was 12 hours light / 12 hours dark. Animals were allowed to acclimate to the environment for at least 7 days before the experiment. All procedures were performed under the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals, and the protocols were approved by the Animal Care and Use Committee of Dalian Medical University.

1. Anesthesia is induced with Sevoflurane dripped directly into an induction box. This agent is designed as an inhalant anesthetic. Accepted veterinary standard is to deliver anesthesia with this agent using a precision vaporizer, which can deliver the gas into the induction box. An exhaust scavenging system from the box to either a downdraft table or carbon cannister would also be expected. If the anesthetic is used as shown in this presentation, there needs to be separation between the mouse and the liquid form of the anesthetic using a platform or other method to avoid direct contact. In the video it appears the liquid was simply put in the box with the mouse, not even on a cotton ball; and this is not generally an acceptable practice. No protection was provided for the human doing the procedure who might be exposed on opening the box. At a minimum discuss in the text.

Reply: Thanks for this critical suggestion. Although the process of inducing anesthesia was carried out in a fume hood, we agreed that this procedure was not a general practice. We decided to remove this part in case of misleading the beginner.

1. Why is the mouse single housed? Mice are social animals and current practice is to group house them unless there is a scientific justification for single housing. Add justification to the text (or delete this completely in text and video).

Reply: This information has been removed in the text and video in the revision.

1. What monitoring was done for the animals? Are any symptoms seen with the agent injected and what would be done if they occurred? The model is inducing peritonitis, which has potential to be painful. There should be a statement that no adverse effects are noted within the 3-5-day period mice are held, or a statement of what symptoms might indicate early euthanasia or what analgesic treatment might be used. This needs to be addressed in the text.

Reply: Revised. A brief statement was added to the text.

1. Why this method of euthanasia – sevoflurane anesthesia followed by cervical dislocation? Preferably I would say to euthanize the mouse by an approved method; then say that in this case sevoflurane followed by cervical dislocation was used. Also, it is stressful to mice to be combined with mice not from the same cage during anesthesia and euthanasia. The video shows two mice in the induction box together for euthanasia while the mice were single housed; that is not generally accepted practice. Please justify why this specific method is required. This can be added in the text.

Reply: Revised. The sevoflurane had multiple effects like muscle relaxation, analgesia ,and sedation. The muscle relaxation effect of sevoflurane made it easy to perform the cervical dislocation and minimized the suffering of the animals.

Please provide additional comment, if necessary.

Other Comments on the text:

Line 119: I believe the word “voiding” should read “avoiding”

Revised.

Line 125 +: Throughout the section 10 to an exponent should have superscript for the exponents

Revised.

Line 118 (and later if applicable): What temperature is “cold” with reference to PBS?

Revised.

Line 142: When referring to a dark humid place and room temperature, please state the range of humidity required and what temperature is considered room temperature; at least with first usage.

Revised.

Table 2 was not present in the text I received and was flashed up very briefly in the video. This table is needed to understand the groups used.

Revised.

Line 233: what is meant by “shortly wash”?

Revised.

Line 234: “which binding” is an awkward phrase. I think it means which bind or were binding.

Revised.

Please be specific in your comments. If possible, divide your comments into 2 categories:   
a) Absolutely not acceptable - for serious errors and deviations from the animal research standards.   
b) Improvement requires - for minor deviations, missing parts, etc....   
  
For each comment, please specify if the changes in video are required, or if only changes in the complementary text are necessary. **Obviously, changes in the video are more difficult so it is important to note if changes in the text are sufficient.** Please use the chart below to provide details on each issue (replace examples listed):

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **#** | **Time in the video** | **comment** | **Change in video required**  **Yes/No** | **Change in text is sufficient**  **Yes/No** | **Suggested Changes** |
| Example | 2:20 – 2:34 | Name of drug used for anesthesia is not mentioned | No | Yes |  |
| 1 | 3:43 | Induction box for anesthesia with liquid Sevoflurane added directly into the box with the mouse. Not on a downdraft table or in a fume hood. | Yes | At a minimum discuss safety precautions | See comments on text. |
| 2 | 4:12 | Housing 1/cage is not scientifically justified | Yes if not justified | Yes if justification can be added. | See comments on text |
| 3 | 4:20 | Why show two anesthetized mice in the box? | Not beneficial to the presentation |  | Already showed anesthetization and this is the same but now two mice are in the box together which if being housed singly is not generally acceptable. |

For #1: This part has been remove in the video.

For #2: Revised according to the comments.

For #3: Revised in the video.