

IACUC Use Only

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Procedure Number : LAH11-03

Genus (if other than mus):

Procedure Type: Major Surgery Minor Surgery Non-Surgical

Procedure Name: Isoflurane Anesthesia in Rodents

1. Procedure Description

Include exact details of any/all chemical, biological, radiation, or physical agents as well as route(s)/ dose(s)/ volume(s)/ frequency and duration

For procedures requiring general anesthesia an alternative to the use of injectable anesthetic drugs is the use of inhalant anesthetic gas. For many procedures it is the preferred method of anesthesia as it permits greater control over the depth and duration of anesthesia with a rapid recovery time. Depending on the anesthesia system, single or multiple animals may be anesthetized at the same time.

Personnel using isoflurane to induce anesthesia in animals at The Jackson Laboratory must be properly trained and demonstrate proficiency under the supervision of a surgical trainer, designated by the Lab Animal Health Services staff.

Basic principle

Oxygen (O₂) flows through a vaporizer over a liquid anesthetic creating a gas mixture of the anesthetic agent and O₂. The anesthetic gas is delivered to the animal in an induction chamber or through a breathing circuit. The breathing circuit is attached to a nose cone or endotracheal (ET) tube for inspiration by the animal.

Equipment needed

1.) Anesthetic machine

- a) Precision vaporizer (Figure 1A): accurately adjusts the concentration of anesthetic delivered to the animal. Different liquid anesthetics cannot be used interchangeably in the same vaporizer. Isoflurane can only be used in an isoflurane vaporizer. In addition, all vaporizers are certified annually to ensure that they are operating properly.
- b) Liquid anesthetic agent: volatile liquid most commonly used in rodent inhalant anesthesia is isoflurane.
- c) O₂ source: typically compressed oxygen in a cylinder (Figure 2A). High volume users may want to consider using an oxygen concentrator.
- d) O₂ regulator (Figure 2B): used to reduce the high pressure O₂ from the tank to ~50 pounds per square inch (psi).
- e) O₂ flowmeter (Figure 1B): used to deliver the anesthetic gas to the animal at the desired flow rate.
- f) Induction chamber (Figure 3C): permits anesthesia of the animal without physical restraint. The maximum number of animals that can be maintained in the chamber depends on the size of the animal and the size of the chamber. Each animal placed within the chamber should have enough space to theoretically place all 4 feet on the floor at the same time when awake. In addition, there should also be enough space in the chamber for

anesthetized animals to rest on the bottom of the chamber without stacking partially or fully on top of one another.

- g) Breathing circuit: a non-rebreathing or Bain circuit is commonly used (Figure 3A). The breathing circuit delivers the anesthetic gas to the animal through a nose cone (Figure 3D), rodent face mask, or ET tube*. Nose cones and face masks may vary somewhat in design; however, it is important to use one that fits snugly around the animal to limit leakage of anesthetic gas.
 - h) Waste gas scavenging system: eliminate waste anesthetic gases (WAGs) from the work area to minimize exposure to personnel. WAGs may be absorbed into specialized charcoal canisters or are directly vented out of the work area. Operation of the scavenging system on some anesthetic systems (active scavenging) may vary depending on manufacturer.**
- 2.) Sterile ophthalmic ointment (such as Puralube): used for lubrication of the eyes. Application of the ointment is required during anesthesia to prevent corneal desiccation.
 - 3.) Supplemental heat source and thermometer: As rodents suffer a significant drop in body temperature while under anesthesia,^{1,2} thermal support is required during recovery from anesthesia and during procedures that exceed 15 minutes. Supplemental heat can be provided by use of a circulating hot water blanket, a slide warmer, or other such device. When using any supplemental heating device, check the temperature at rodent level prior to use and frequently thereafter with a thermometer.
 - 4.) Clean cage: Used to provide a warm and clean environment for recovery of animals after anesthesia.
 - a. When using a heating source located underneath the recovery cage (ie. circulating water blanket), no bedding should be used.
 - b. If the heat source is located above the recovering mouse (ie. incandescent bulb), the cage should contain clean bedding.

Procedure

Equipment set-up

1. Set up the gas anesthesia system. The operating manual should be kept with the machine or be easily available for reference. The operator should know how to set up their anesthesia machine for the proper use of their system.
2. Check the certification date on the precision vaporizer. If the vaporizer has not been certified within the last year, an alternate anesthesia system should be used.
3. Check that there is sufficient O₂ on hand. If using the small “E” tank for O₂ it is best to have a back-up on hand. When the pressure on a tank reaches 500 psi (Figure 2) it should be replaced.
4. Set O₂ regulator on the tank to 50 psi (Figure 2B).
5. Check the level of isoflurane in the vaporizer (Figure 1C). An anti-spill device is recommended to reduce personnel exposure to vapors when refilling the vaporizer.
6. Check that all tubing is connected and flow valve (stopcock - Figure 3B) is open to the induction chamber and closed to the breathing circuit nose cone.
7. Check scavenging system.
 - a. Charcoal canister: Weigh charcoal canister and record weight on canister. Attach canister(s) to filter waste gas from induction chamber and breathing circuit (Figure 3). Refer to Guidelines for Proper Use of Isoflurane Vaporizer Charcoal Filter Canisters to ensure proper positioning of the canister during use.

- b. Other scavenging system: If performing procedure in a certified chemical fume hood, ensure the hood is on. For other active systems, follow manufacturer instructions for operation.
8. Set up supplemental heat source where the procedure will be performed if the procedure will exceed 15 minutes.
 - a. To avoid overheating the animal, you must check the temperature at the animal level prior to placing the animal on the heated surface and frequently thereafter. The temperature at the level of the animal should be maintained at a temperature range of 80-100°F (26.6-38°C).
9. Turn O₂ flow on and set flow meter (Figure 1B) to 1 liter/min (LPM). To adjust the flow meter, turn the flow meter knob (Figure 1D) to place the middle of the ball in the flow meter to the desired number setting.

Anesthesia induction

10. Place the rodent(s) inside the induction chamber and close the lid. If the induction chamber is heated, check the temperature at the animal level prior to placing the animal on the heated surface and frequently thereafter. The temperature at the level of the animal should be maintained at a temperature range of 80-100°F (26.6-38°C).
11. Turn the vaporizer dial (Figure 1 arrow) to 3%. As the rodent goes from awake to anesthetized the respiratory rate will become slower and rhythmic and movement will stop. Within 2-3 minutes, the rodent will become recumbent and non-responsive to movement of the induction chamber.
12. When using passive scavenging, depress and hold the O₂ flush valve (Figure 1E) for 10 seconds to fill the chamber with O₂ to displace the isoflurane gas from the induction chamber to the scavenging system. This will minimize exposure of personnel to WAGs as the chamber is opened.

*This step may be omitted when using active scavenging** or when the anesthesia system is located inside a certified chemical fume hood.*

CAUTION: While an animal is on a breathing circuit, the stopcock to the breathing circuit should always be closed when using the O₂ flush. Never use the O₂ flush to provide O₂ to an animal on a nose cone or ET tube.
13. Immediately remove the animal(s) from the induction chamber. If there are no other animals in the induction chamber close the stopcock to the chamber.

Anesthesia maintenance and animal monitoring

14. Apply sterile eye lubricant to each eye of the animal. *To avoid damage to the eye or contamination of the lubricant, during application do not touch the tip of the tube to the surface of the eye or skin.*
15. Place the animal on the breathing circuit. Secure the nose cone or face mask around the animal's muzzle. Ensure that the animal's eyes do not make direct contact with any part of the nose cone/face mask. *Since mice are obligate nasal breathers and cannot breathe through the mouth, it is not essential to include the mouth in the nose cone unless required by the design of the nose cone/face mask in order to prevent leakage of anesthetic gas.*
16. Open the stopcock to the breathing circuit and adjust O₂ flow rate.
 - a. The O₂ flow rate may be adjusted down to 0.6 LPM if maintaining the animal(s) on a breathing circuit only.
 - b. If both the induction chamber and breathing circuit are being utilized simultaneously, the O₂ flow rate should remain at 1 LPM.
17. Monitor animal(s) for depth of anesthesia.
 - a. Every 15 minutes all anesthetized animals, including any animals remaining in the induction chamber, must be monitored.

- b. Respirations should remain rhythmic and slower than when awake and should not change in response to a noxious stimuli (e.g., surgical manipulation, toe pinch).
 - i. Fast respiratory rate, spontaneous movement or movement in response to toe pinch or a noxious stimuli: Anesthesia depth is too light. The percent of isoflurane should be increased
 - ii. Respiratory rate becomes very slow or the animal is gasping: Anesthesia depth is too deep. The percent of isoflurane should be decreased.

CAUTION: Adjustments to the flow of isoflurane will affect all circuits receiving gas. If animals are being maintained under anesthesia in both the induction box and on the breathing circuit, it is important to remember that adjusting the anesthetic gas flow for one animal will automatically change the flow delivered to the other animal.

18. When the procedure is complete, close the stopcock to the breathing circuit.
 - a. If additional animals remain in the induction chamber then the next animal can be removed from the chamber and placed on the breathing circuit as outlined above starting at step #14.
 - b. As one animal is removed from the induction chamber an additional animal may be placed in the chamber at the same time. If you have several animals in the chamber they should be identified such that they are moved to the breathing circuit in the same order as they were placed into the induction box.
19. If no other animals are to be anesthetized, the anesthesia system must be shut down.
 - a. The isoflurane vaporizer must be turned to "0".
 - b. Open the stopcock to both the breathing circuit and induction chamber and hold down the O₂ flush valve** for at least 5 seconds to flush any remaining anesthetic gas into the scavenging system.
 - c. Set the O₂ flow meter to 0 LPM.

Anesthesia recovery

20. Check the temperature inside the warmed recovery cage at rodent level **prior** to placing the animal in the cage and frequently thereafter with a thermometer. The cage should be maintained at a temperature range of 80-100°F (26.6-38°C). The animal should be provided a means to move away from the heat source once awake. This can be accomplished by providing supplemental heat to only ½ of the cage. If the animal will be recovered outside of a cage, it should not be placed directly on a heating device. Clean material (e.g., paper towel) should be placed between the rodent and the heated surface.
 - a. When using a heating source located underneath the recovery cage (ie. circulating water blanket) no bedding should be used.
 - b. If the heat source is located above the recovering mouse (ie. incandescent bulb), the cage should contain clean bedding.
21. Place the animal in the clean warmed recovery cage. The animal should begin to awaken in ~2 minutes.
22. While the rodent is recovering from anesthesia you or your designee*** must stay in the procedure room or an adjacent animal room until the rodent has recovered. Check on the animal at least every 15 minutes to ensure the animal is recovering normally.
23. Once the animal is able to walk around the cage it may be returned to the home cage and taken back to the animal room.

* For intubation of a rodent with an endotracheal tube refer to LAH11-01.

**If using an active waste gas scavenging system follow manufacturer instructions for operation.

***Designee: one who has been approved to perform a procedure or has received training in anesthesia and recovery by a LAHS surgical trainer.

Notes:

- Anesthesia is administered “to effect” this means that the amount of anesthetic gas delivered to the rodent must be sufficient to achieve and maintain a surgical plane of anesthesia (no response to noxious stimuli, e.g., no movement when toe pinch) throughout a surgical procedure. The percent of isoflurane and O₂ flow rate given in these guidelines is a recommendation for the “average” rodent under “average” conditions. It is the responsibility of the person administering the anesthesia to periodically check that the animal is sufficiently anesthetized for the procedure performed.
- Some analgesics, e.g. buprenorphine, may reduce the percent of isoflurane required to achieve a plan of surgical anesthesia³.

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Figure 1

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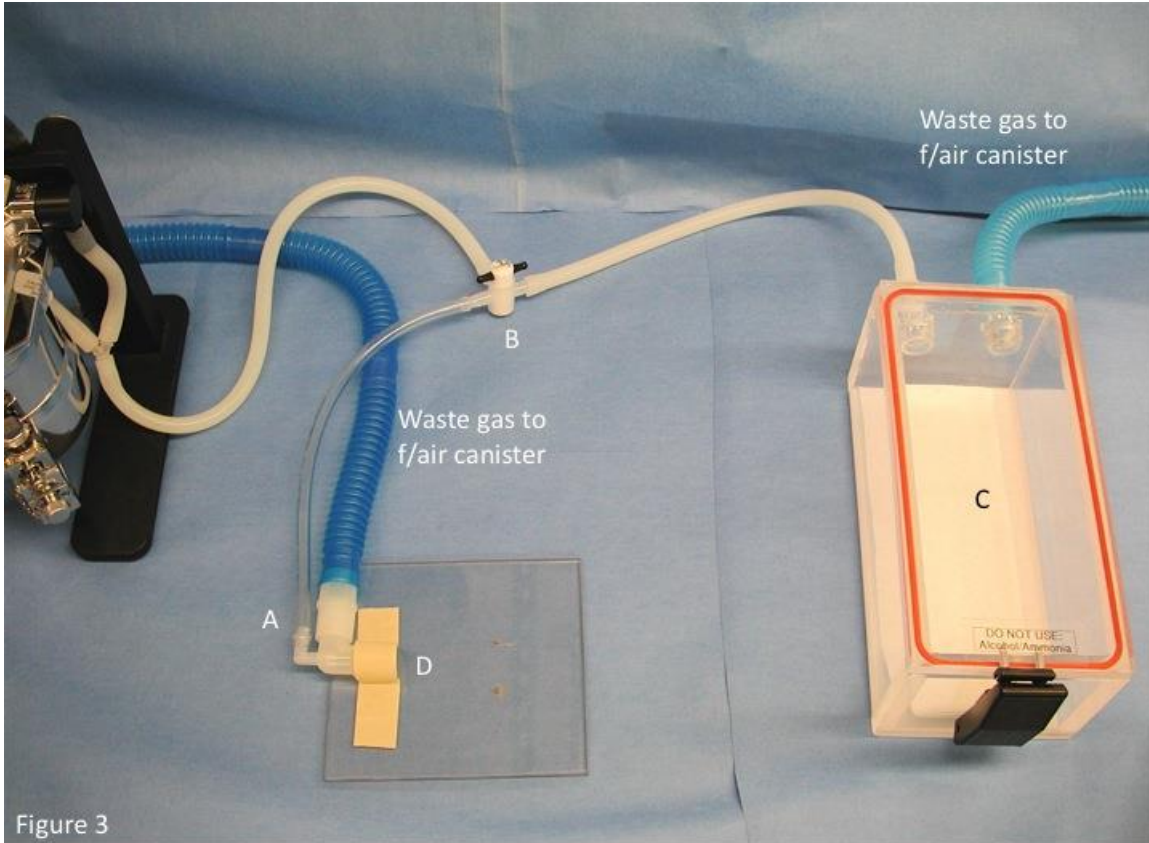


Figure 3

2. Anesthetic/Analgesic Regimen

- a. Please list all anesthetics/analgesics used in this procedure in the following table.

If not applicable, please check here NA

Example:

Anesthetic Agent	Diluents Used	Dose & Route of Administration (e.g. 1mg/kg I.V.)	Volume
Isoflurane	Oxygen	Inhalation to effect ~2%	
OR Tribromoethanol	Sterile PBS	400 mg/kg IP	0.2ml/10g body weight

Anesthetic Agent	Diluents Used	Dose & Route of Administration (e.g. 1mg/kg I.V.)	Volume
Isoflurane	Oxygen	Inhalation to effect	

Analgesic Agent	Dose & Route of Administration (e.g. 1mg/kg I.V.)	Volume

- b. Supportive care while animal recovers from anesthesia:

See steps #20- 23

3. Post Procedure Care

Describe post procedure care, including frequency of observations, schedule for removal of sutures/clips, etc...

If applicable, refer to the *Standards for Rodent Survival Surgery at the Jackson Laboratory* for post-procedure care guidelines.

4. References if applicable:

- 1) Caro, Adam C., F. Claire Hankenson, and James O. Marx. "Comparison of Thermoregulatory Devices Used during Anesthesia of C57BL/6 Mice and Correlations between Body Temperature and Physiologic Parameters." *Journal of the American Association for Laboratory Animal Science: JAALAS* 52.5 (2013): 577.
- 2) Taylor, Douglas K. "Study of two devices used to maintain normothermia in rats and mice during general anesthesia." *Journal of the American Association for Laboratory Animal Science* 46.5 (2007): 37-41.
- 3) Criado, A. B., et al. "Reduction of isoflurane MAC with buprenorphine and morphine in rats." *Laboratory animals* 34.3 (2000): 252-259.