1. PURPOSE

This Standard Operating Procedure (SOP) describes methods for anesthetizing mice.

2. RESPONSIBILITY

Principal Investigators (PIs) and their research staff, veterinary care staff.

3. INTRODUCTION

3.1. Mice are not routinely fasted prior to anesthesia due to their inability to vomit.
3.2. Rodents can be anesthetized with either inhalant gas or injectable drugs. The use of inhalant gas is the preferred method of anesthesia, whenever possible.
3.3. Heat loss is rapid in anesthetized rodents. Keep animals warm by covering them and providing a heat source until the animal has recovered from anesthesia. Care should be taken to not overheat or burn the animal; do not place animals directly in contact with the heat source, use a drape or other material as a barrier.
3.4. Never leave an anesthetized animal unattended. Continuously monitor anesthetized animals until fully recovered; sternal and moving in the cage.

4. MATERIALS

4.1. Material or equipment to provide or conserve body heat: heating disc, warming pad or warm-water circulating pad. Do not use electric heating pads unless specifically designed for use with laboratory rodents.
4.2. Ophthalmic ointment (natural tears)
4.3. Gas anesthesia machine (calibrated within the last 12 months) with adequate gas scavenging system or filter
4.4. Induction chamber constructed of a see-through material (glass, polycarbonate, etc.)
4.5. Rodent anesthesia nosecone or mask
4.6. Isoflurane
4.7. Ketamine (100 mg/mL) *Controlled Drug
4.8. Xylazine (20 mg/mL)
4.9. Acepromazine (10 mg/mL)
4.10. Atipamezole (5 mg/ml)
4.11. 2,2,2-Tribromoethanol (Avertin)
4.12. Tertiary amyl alcohol
4.13. Sterile isotonic saline (0.9% saline) or sterile water for injection
4.14. Crushed ice or ice pack

5. PROCEDURES FOR ADULT MICE

5.1. Isoflurane anesthesia:

5.1.1. Induction:

5.1.1.1. Adjust the oxygen flowmeter to 0.8 to 1.5 L/min.
5.1.1.2. Place the animal in the induction chamber
5.1.1.3. Adjust the isoflurane vaporizer to 3% to 5%.
5.1.2. Maintenance:

5.1.2.4. Remove the animal from the induction chamber and use a nosecone or mask connected to the Bain circuit.
5.1.2.5. Adjust the flowmeter to 0.4 to 0.8 L/min.
5.1.2.6. Adjust the isoflurane vaporizer to 2 to 2.5%.
5.1.2.7. Apply ophthalmic ointment (natural tears) to both eyes to prevent dryness and damage to the cornea. Reapply as needed.
5.1.2.8. Continuously monitor the animal during anesthesia and adjust the level of isoflurane as needed according to monitored parameters:
   5.1.2.8.1. Presence of reflexes/response to stimuli (pedal withdrawal reflex)
   5.1.2.8.2. Respiratory rate and breathing pattern
   5.1.2.8.3. Mucous membrane color surrounding the nose and mouth (should remain pink)

5.1.3. Recovery:

5.1.3.1. Keep the animal on oxygen until it starts to recover.
5.1.3.2. Transfer animal to their cage once it begins to move and allow to recover fully (sternal position).
5.1.3.3. Provide supplemental heat during the recovery period.

5.2. Ketamine/Xylazine/Acepromazine anesthesia:

5.2.1. Injectable anesthetic dose can vary with the sex, the age, the strain, and the body condition of the animal.
5.2.2. Contact your veterinarian for advice on the appropriate dose prior to use.
5.2.3. Recommended anesthetic dose: ketamine 100 mg/kg, xylazine 10 mg/kg, acepromazine 3 mg/kg.
5.2.4. When working with a new mouse strain, administer 75% of the recommended dose. If pedal withdrawal reflexes are still present after 5 minutes, administer the remaining 25% of the recommended dose. An additional 25% of the recommended dose may be administered if pedal withdrawal reflexes remain present after 5 minutes. Do not exceed 125% of the recommended dose.
5.2.5. Prepare the solution the day before or shake it thoroughly before use.
5.2.6. To prepare the solution, in a sterile vial or bottle with a rubber stopper, mix:
   - 1 mL of ketamine (100 mg/mL)
   - 0.5 mL xylazine (20 mg/mL)
   - 0.3 mL acepromazine (10 mg/mL)
   - 8.2 mL of sterile isotonic saline or sterile water for injection.
5.2.7. Indicate expiration date on vial or bottle (maximum 6 months). The final concentration of the mixture is: ketamine 10 mg/mL, xylazine 1 mg/mL, acepromazine 0.3 mg/mL.
5.2.8. Solution should be protected from light and stored at room temperature.
5.2.9. Administer 0.1 mL/10g body weight intraperitoneally for the recommended dose.
5.2.10. Apply ophthalmic ointment (natural tears) to both eyes to prevent dryness and damage to the cornea. Reapply as needed.
5.2.11. After 5 minutes, monitor anesthetic depth by verifying the pedal withdrawal reflex.
5.2.12. Duration of anesthesia is approximately 20 minutes.
5.2.13. After 20 minutes, a half dose may be administered as needed.
5.2.14. Administration of atipamezole at the end of the procedure may improve respiration and speed up the recovery. Atipamezole is the antidote for xylazine.
   5.2.14.1. Recommended dose: 1-2 mg/kg.
5.2.14.2. Prepare a 1:10 atipamezole solution in sterile isotonic saline or sterile water for injection. The final concentration of the mixture is 0.5 mg/mL.

5.2.14.3. Administer 0.02-0.04 mL/10g body weight subcutaneously or intraperitoneally.

5.2.15. Provide supplemental heat and monitor until recovery (sternal position).

5.3. Ketamine/Xylazine anesthesia:

5.3.1. Injectable anesthetic dose can vary with the sex, the age, the strain, and the body condition of the animal.

5.3.2. Contact your veterinarian for advice on the appropriate dose prior to use.

5.3.3. Recommended anesthetic dose: ketamine 100 mg/kg, xylazine 10 mg/kg.

5.3.4. When working with a new mouse strain, administer 75% of the recommended dose. If pedal withdrawal reflexes are still present after 5 minutes, administer the remaining 25% of the recommended dose. An additional 25% of the recommended dose may be administered if pedal withdrawal reflexes remain present after 5 minutes. Do not exceed 125% of the recommended dose.

5.3.5. Prepare the solution the day before or shake it thoroughly before use.

5.3.6. To prepare solution, in a sterile vial or bottle with a rubber stopper, mix:

- 1 mL of ketamine (100 mg/mL)
- 0.5 mL xylazine (20 mg/mL)
- 8.5 mL of sterile isotonic saline or sterile water for injection.

5.3.7. Indicate expiration date on vial or bottle (maximum 6 months). The final concentration of the mixture is: ketamine 10 mg/mL, xylazine 1 mg/mL.

5.3.8. Administer 0.1 mL/10g body weight intraperitoneally or subcutaneously for the recommended dose.

5.3.9. Apply ophthalmic ointment (natural tears) to both eyes to prevent dryness and damage to the cornea. Reapply as needed.

5.3.10. After 5 minutes, monitor anesthetic depth by verifying the pedal withdrawal reflex.

5.3.11. Duration of anesthesia is approximately 20 minutes.

5.3.12. After 20 minutes, a half dose may be administered as needed.

5.3.13. Administration of atipamezole at the end of the procedure may improve respiration and speed up the recovery. Atipamezole is the antidote for xylazine.

5.3.13.1. Recommended dose: 1-2 mg/kg.

5.3.13.2. Prepare a 1:10 atipamezole solution in sterile isotonic saline or sterile water for injection. The final concentration of the mixture is 0.5 mg/mL.

5.3.13.3. Administer 0.02-0.04 mL/10g body weight subcutaneously or intraperitoneally.

5.4. 2,2,2-Tribromoethanol (TBE or Avertin) anesthesia:

5.4.1. Anesthetic dose is 250 to 500 mg/kg.

5.4.2. Avertin administration can result in sensitization of the animal; thus, it is recommended to be given only on a single occasion.

5.4.3. Avertin stock solution:

5.4.3.1. In a sterile container add 25g of 2,2,2-tribromoethanol to 15.5 mL tertiary amyl alcohol and dissolve by heating to 50°C and stirring until completely dissolved.

5.4.3.2. Store protected from light (wrapped in foil or in an amber container) in the refrigerator or freezer.

5.4.4. Label as “Avertin Stock Solution” and indicate expiration date (up to 1 year).

5.4.5. Avertin working solution:

5.4.5.1. In a sterile container, mix 0.5 mL of the stock solution in 39.5 mL of sterile isotonic saline.

5.4.5.2. Heat solution to 40°C then shake well until completely dissolved. Mixture should be clear.

5.4.5.3. Filter the working solution through 0.2 micron filter.
5.4.4. Store protected from light (wrapped in foil or in an amber container) at 4°C.
5.4.5. Label as “Avertin 20 mg/mL” and indicate expiration date (maximum 4 months).
5.4.6. Inject 0.1-0.25 mL of working solution/10g body weight, intraperitoneally.
5.4.7. Apply ophthalmic ointment (natural tears) to both eyes to prevent dryness and damage to the cornea. Reapply as needed.
5.4.8. Duration of anesthesia is approximately 20 minutes.
5.4.9. Provide supplemental heat and monitor until recovery (sternal position).

6. PROCEDURES FOR NEONATAL MICE

6.1. Hypothermia:
   6.1.1. Use only in animals less than 7 days of age.
   6.1.2. Provides immobilization and mild analgesia for short, minor procedures.
   6.1.3. Protect pup in a glove or paper-lined tube to avoid damage to the skin.
   6.1.4. Induction:
      6.1.4.6. Immerse pup in ice water or crushed ice for 3 to 4 minutes.
   6.1.5. Maintenance:
      6.1.5.1. Place pup on a paper-covered ice pack.
      6.1.5.2. Use a fiber optic surgical lamp if necessary as incandescent lamps will warm the animal and interfere with anesthesia.
      6.1.5.3. Duration of anesthesia is approximately 10 minutes.
   6.1.6. Recovery:
      6.1.6.1. Remove animal from ice pack and allow to warm.
      6.1.6.2. Recovery time can be up to 1 hour.

6.2. Isoflurane anesthesia:
   6.2.1. Neonates require higher concentration of isoflurane than adults (maintenance at 3-4%). See section 5.1 for detailed procedure.

7. REFERENCES


### SOP REVISION HISTORY

<table>
<thead>
<tr>
<th>DATE</th>
<th>NEW VERSION</th>
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<tr>
<td>2016.01.15</td>
<td>Addition of Rodent Procedure Log (annex)</td>
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</table>
| 2016.03.16 | 5.2.1 Injectable anesthetic dose can vary with the sex, the age, the strain, and the body condition of the animal.  
5.2.2 Contact your veterinarian for advice on the appropriate dose prior to use. |
| 2020.05.15 | 4.5 Rodent anesthesia nosecone                                               |
| 2020.05.15 | 5.1.1.1. Place the animal in the induction chamber. Adjust the oxygen flowmeter to 0.8 to 1.5 L/min.  
5.1.1.2. Adjust the oxygen flowmeter to 0.8 to 1.5 L/min. Place the animal in the induction chamber. |
| 2020.05.15 | 5.1.2.1. Use the nosecone or mask connected to the Bain circuit.             |
| 2020.05.15 | 5.1.3.1. Turn off the isoflurane vaporizer, **flush the system** and keep the animal on oxygen. |
| 2020.05.15 | 5.1.3.3. Provide supplemental heat during the recovery period.               |
| 2020.05.15 | 5.2.3. Recommended anesthetic dose: ketamine 100mg/kg, xylazine 10mg/kg, acepromazine 3mg/kg. |
| 2020.05.15 | 5.2.4. When working with a new mouse strain, administer 75% of the recommended dose. If pedal withdrawal reflexes are still present after 5 minutes, administer the remaining 25% of the recommended dose. An additional 25% of the recommended dose may be administered if pedal withdrawal reflexes remain present after 5 minutes. Do not exceed 125% of the recommended dose. |
| 2020.05.15 | 5.2.5. Label as "Mouse Cocktail" and indicate expiration date on vial or bottle (maximum 6 months). The final concentration of the mixture is: ketamine 10mg/mL, xylazine 1mg/mL, acepromazine 0.3mg/mL. |
| 2020.05.15 | 5.2.8. Mixed cocktail should be protected from light and stored in a cool place at room temperature. |
| 2020.05.15 | 5.2.9. Administer 0.05-0.1mL/10g body weight intraperitoneally for the recommended dose. |
| 2020.05.15 | 5.2.13.1. Recommended dose: 1-2 mg/kg.  
5.2.13.2. Prepare a 1:10 atipamezole solution in sterile isotonic saline or sterile water for injection. The final concentration of the mixture is 0.5mg/mL.  
5.2.13.3. Administer 0.02-0.04mL/10g body weight subcutaneously or intraperitoneally. |
| 2020.05.15 | 5.2.14. Provide supplemental heat and monitor until recovery (sternal position). |
| 2020.05.15 | 5.3.9. Provide supplemental heat and monitor until recovery (sternal position). |
| 2020.05.15 | 7. REFERENCES                                                                |
| 2020.12.07 | 4.1. Material or equipment to provide or conserve body heat: heating disc, warming pad or warm-water circulating pad. Do not use electric heating pads unless specifically designed for use with laboratory rodents. (e.g. gauze pads, heating disc or pad, warm-water circulating pad) |
| 2020.12.07 | 5.1.2.5. Continuously monitor the animal during anesthesia and adjust the level of isoflurane as needed according to monitored parameters:  
5.1.2.5.1. Presence of reflexes/response to stimuli (pedal withdrawal reflex)  
5.1.2.5.2. Respiratory rate and breathing pattern  
5.1.2.5.3. Mucous membrane color surrounding the nose and mouth (should remain pink) |
| 2020.12.07 | 5.1.11. After 5 minutes, monitor anesthetic depth by verifying the pedal withdrawal reflex. |
| 2022.01.13 | 5.2.11. Remove the animal from the induction chamber and use a nosecone or mask connected to the Bain circuit. |
| 2022.01.13 | 5.1.3.1. Turn off the isoflurane vaporizer, move the animal away from the nosecone momentarily to flush the anesthetic system. Return the animal to the nosecone and keep the animal on oxygen until it starts to recover. |
5.3. Ketamine/Xylazine anesthesia:

5.3.1. Injectable anesthetic dose can vary with the sex, the age, the strain, and the body condition of the animal.

5.3.2. Contact your veterinarian for advice on the appropriate dose prior to use.

5.3.3. Recommended anesthetic dose: ketamine 100 mg/kg, xylazine 10 mg/kg.

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5.3.5. Prepare the solution the day before or shake it thoroughly before use.

5.3.6. To prepare solution, in a sterile vial or bottle with a rubber stopper, mix: 1 mL of ketamine (100 mg/mL), 0.5 mL xylazine (20 mg/mL), 8.5 mL of sterile isotonic saline or sterile water for injection.

5.3.7. Indicate expiration date on vial or bottle (maximum 6 months). The final concentration of the mixture is: ketamine 10 mg/mL, xylazine 1 mg/mL.

5.3.8. Administer 0.1 mL/10g body weight intraperitoneally or subcutaneously for the recommended dose.

5.3.9. Apply ophthalmic ointment (natural tears) to both eyes to prevent dryness and damage to the cornea. Reapply as needed.

5.3.10. After 5 minutes, monitor anesthetic depth by verifying the pedal withdrawal reflex.

5.3.11. Duration of anesthesia is approximately 20 minutes.

5.3.12. After 20 minutes, a half dose may be administered as needed.

5.3.13. Administer atipamezole to improve respiration or speed up the recovery if needed. Atipamezole is the antidote for xylazine.

5.3.13.1. Recommended dose: 1-2 mg/kg.

5.3.13.2. Prepare a 1:10 atipamezole solution in sterile isotonic saline or sterile water for injection. The final concentration of the mixture is 0.5 mg/mL.

5.3.13.3. Administer 0.02-0.04 mL/10g body weight subcutaneously or intraperitoneally.

Instructions: Complete this log for rodent procedures requiring anesthesia, analgesia or post-procedure care (ex. surgeries, experimental infection). Keep the log in the housing room while active and in your files for 3 years for future review by the Quality Assistant and/or the FACC. Cages should be identified with a Post-Procedure cage card.

### ANALGESIA
- carprofen: mouse: 20 mg/kg, rat: 5-10 mg/kg, SC, every 24 hrs
- buprenorphine: mouse: 0.1 mg/kg SC or IP every 4-8 hrs; rat: 0.05 mg/kg, SC or IP, every 8-12 hrs
- buprenorphine slow release 1 mg/kg SC
- lidocaine/bupivacaine (local analgesic)

### ANESTHESIA
- isoflurane 2-2.5%
- ketamine/xylazine/acepromazine*:
  - mouse: 100 mg/kg (K)- 10 mg/kg (X)- 3 mg/kg (A) IP
  - rat: 50 mg/kg (K)- 5 mg/kg (X)- 1 mg/kg (A); IP or IM
- buprenorphine slow release 1 mg/kg SC

### OTHER AGENTS ADMINISTERED
- lidocaine/bupivacaine (local analgesic)

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Comments/footnotes:
*Dose can vary with the sex, the age, the strain, and the body condition of the animal.

Revised: 2022-06-13
**ANALGESIA**

- carprofen: mouse: 20 mg/kg, rat: 5-10 mg/kg, SC, every 24 hrs
- buprenorphine: mouse: 0.1 mg/kg SC or IP every 4-8 hrs; rat: 0.05 mg/kg, SC or IP, every 8-12 hrs
- buprenorphine slow release 1 mg/kg SC

Initial the appropriate boxes when completed

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