

1. PURPOSE

This Standard Operating Procedure (SOP) describes the anesthesia procedure for conducting terminal lung physiology measurements in mice and rats using the Flexivent apparatus.

2. RESPONSIBILITY

Principal investigator (PI) and their research staff and students.

3. MATERIALS

- 3.1. Animal weighing scale
- 3.2. Xylazine (20 mg/mL)
- 3.3. Pentobarbital *Controlled drug
- 3.4. Neuromuscular blocking agent, e.g., Rocuronium bromide
- 3.5. Ophthalmic ointment (natural tears)
- 3.6. Endotracheal tube, 18G - 20G
- 3.7. 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.
- 3.8. Flexivent®, computer-controlled small animal ventilator

4. PROCEDURES

- 4.1. Mice are not routinely fasted prior to anesthesia due to their inability to vomit.
- 4.2. Monitor animals closely during induction and maintenance of general anesthesia. Monitoring must be documented.
 - 4.2.1. Never leave an anesthetized animal unattended.
 - 4.2.2. Monitor animal every 5 minutes:
 - 4.2.2.1. Anesthetic depth: absence of reflexes, e.g., pedal, absence of movement, muscle relaxation.
 - 4.2.2.2. Respiratory function: respiratory rate, thoracic wall movements. When species-adapted equipment is available include oxygen saturation (SpO₂), end-tidal carbon dioxide (ETCO₂).
 - 4.2.2.3. Body temperature: rectal temperature when possible.
- 4.3. Maintain records of each anesthesia procedure and include:
 - 4.3.1. Date and time of procedure
 - 4.3.2. Principal investigator and Animal Use Protocol (AUP)
 - 4.3.3. Species and animal's identification
 - 4.3.4. Animals' weight
 - 4.3.5. Name, dose, route, and time of administration of each drug
 - 4.3.6. Description of the procedure
 - 4.3.7. Measurements of the animal's anesthetic depth and vital signs
 - 4.3.8. Time of recovery
 - 4.3.9. Name of the individual monitoring the animal and of the surgeon

- 4.4. Weigh each animal individually and calculate the appropriate dose of anesthetics.
- 4.5. Mouse model:
 - 4.5.1. Xylazine 10 mg/kg and pentobarbital 50 mg/kg for procedures lasting up to 60 minutes. Administer intraperitoneally, mixed in the same syringe.
 - 4.5.2. Ketamine 100 mg/kg, xylazine 10 mg/kg, and acepromazine 3 mg/kg for procedures lasting up to 20 minutes. Administer as per SOP 110.
- 4.6. Rat model:
 - 4.6.1. Xylazine 5 mg/kg and pentobarbital 50 mg/kg for procedures lasting up to 60 minutes. Administer intraperitoneally, mixed in the same syringe.
 - 4.6.2. Ketamine 50 mg/mL, xylazine 5 mg/mL, and acepromazine 1 mg/mL for procedures lasting up to 20 minutes. Administer as per SOP 111.
- 4.7. Leave the animals in their home cage for 2-3 minutes.
- 4.8. Verify the absence of pedal reflex by pinching the toes and/or footpads with a pair of forceps.
- 4.9. Once the reflex is completely lost, perform the tracheotomy.
 - 4.9.1. Make a skin incision in the throat area. Gently separate the submaxillary gland and the muscle layer covering the trachea to expose it.
 - 4.9.2. Secure the trachea and make a small incision in the trachea without sectioning it, cutting between two rings of cartilage nearest the larynx.
 - 4.9.3. Insert the endotracheal tube into the incision and advance it gently inside the trachea.
 - 4.9.4. Secure the cannula in place using sutures. The attachment should form an airtight seal around the cannula.
- 4.10. Connect the endotracheal tube to the Flexivent.
- 4.11. Administer the neuromuscular blocking agent as described in the Animal Use Protocol.
- 4.12. Proceed to lung function measurements.
- 4.13. Euthanize animal according to SOP immediately after disconnecting from the ventilator.

SOP REVISION HISTORY

DATE	NEW VERSION
2024.03.15	This Standard Operating Procedure (SOP) describes the anesthesia procedure for conducting terminal lung physiology measurements in mice and rats using the Flexivent apparatus.
2024.03.15	3.1. Animal weighing scale
2024.03.15	3.2. Pentobarbital (54.7mg/mL) *Controlled drug
2024.03.15	3.4. Lidocaine 2%
2024.03.15	3.6. Endotracheal tracheal tube, 18G – 20G
2024.03.15	3.7. 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.
2024.03.15	4.1. Mice are not routinely fasted prior to anesthesia due to their inability to vomit.
2024.03.15	4.2. Monitor animals closely during induction and maintenance of general anesthesia. Monitoring must be documented. 4.2.1. Never leave an anesthetized animal unattended. 4.2.2. Monitor animal every 5 minutes: 4.2.2.1. Anesthetic depth: absence of reflexes, e.g., pedal, absence of movement, muscle relaxation. 4.2.2.2. Respiratory function: respiratory rate, thoracic wall movements. When species-adapted equipment is available include oxygen saturation (SpO ₂), end-tidal carbon dioxide (ETCO ₂). 4.2.2.3. Body temperature: rectal temperature when possible.

2024.03.15	<p>4.3. Maintain records of each anesthesia procedure and include:</p> <p>4.3.1. Date and time of procedure</p> <p>4.3.2. Principal investigator and Animal Use Protocol (AUP)</p> <p>4.3.3. Species and animal's identification</p> <p>4.3.4. Animals' weight</p> <p>4.3.5. Name, dose, route, and time of administration of each drug</p> <p>4.3.6. Description of the procedure</p> <p>4.3.7. Measurements of the animal's anesthetic depth and vital signs</p> <p>4.3.8. Time of recovery</p> <p>4.3.9. Name of the individual monitoring the animal and of the surgeon</p>
2024.03.15	<p>4.5. Mouse model:</p> <p>4.5.1. Xylazine 10 mg/kg and pentobarbital 50 mg/kg for procedures lasting up to 60 minutes. Administer intraperitoneally, mixed in the same syringe.</p> <p>4.5.2. Ketamine 100 mg/kg, xylazine 10 mg/kg, and acepromazine 3 mg/kg for procedures lasting up to 20 minutes. Administer as per SOP 110.</p>
2024.03.15	<p>4.6. Rat model:</p> <p>4.6.1. Xylazine 5 mg/kg and pentobarbital 50 mg/kg for procedures lasting up to 60 minutes. Administer intraperitoneally, mixed in the same syringe.</p> <p>4.6.2. Ketamine 50 mg/mL, xylazine 5 mg/mL, and acepromazine 1 mg/mL for procedures lasting up to 20 minutes. Administer as per SOP 111.</p>
2024.03.15	<p>4.9.1. Make a skin incision in the throat area. Gently separate the submaxillary gland and the muscle layer covering the trachea to expose it.</p> <p>4.9.2. Secure the trachea and make a small incision in the trachea without sectioning it, cutting between two rings of cartilage nearest the larynx.</p> <p>4.9.3. Insert the endotracheal tube into the incision and advance it gently inside the trachea.</p> <p>4.9.4. Secure the cannula in place using sutures. The attachment should form an airtight seal around the cannula.</p>
2024.03.15	4.11. Administer the neuromuscular blocking agent as described in the Animal Use Protocol.
2024.03.15	4.12. Proceed to lung function measurements for up to a maximum of 45 minutes after injection of xylazine-pentobarbital.
2024.03.15	4.13. Euthanize animal according to SOP prior to immediately after disconnecting from the ventilator.