1. PURPOSE

This Standard Operating Procedure (SOP) describes the anesthesia procedure for conducting 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. Xylazine (20mg/mL)
3.2. Pentobarbital (54.7mg/mL) *Controlled drug
3.3. Lidocaine 2%
3.4. Neuromuscular blocking agent, e.g., rancuronium
3.5. Ophthalmic ointment (natural tears)
3.6. Tracheal tube
3.7. Flexivent®, computer-controlled small animal ventilator

4. PROCEDURES

4.1. Weigh each animal individually and calculate the appropriate dose of anesthetics.
4.2. Never leave an anesthetized animal unattended.
4.3. Administer intraperitoneally, mixed in the same syringe:
   4.3.1. Mouse model: xylazine 10 mg/kg + pentobarbital 50 mg/kg
   4.3.2. Rat model: xylazine 5 mg/kg + pentobarbital 50 mg/kg
4.4. Apply ophthalmic ointment (natural tears) to both eyes to prevent dryness and damage to the cornea.
4.5. Leave the animals in their home cage for 2-3 minutes.
4.6. Verify the absence of pedal reflex by pinching the toes and/or footpads with a pair of forceps.
4.7. Once the reflex is completely lost, perform the tracheotomy.
4.8. Connect the tracheal tube to the Flexivent.
4.9. Administer the neuromuscular blocking agent.
4.10. Proceed to measurements for up to a maximum of 45 minutes after injection of xylazine-pentobarbital.
4.11. Euthanize animal according to SOP prior to disconnecting from the ventilator.

SOP REVISION HISTORY

<table>
<thead>
<tr>
<th>DATE</th>
<th>NEW VERSION</th>
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<tbody>
<tr>
<td>2017.05.02</td>
<td>3.4 Rancuronium bromide (1mg/mL) Neuromuscular blocking agent, e.g., rancuronium</td>
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<tr>
<td>2017.05.02</td>
<td>4.8 Administer rancuronium bromide the neuromuscular blocking agent.</td>
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<td>2019.03.27</td>
<td>4.10. Euthanize animal according to SOP prior to disconnecting from the ventilator.</td>
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