1. PURPOSE

This Standard Operating Procedure (SOP) describes methods for anesthetizing non-human primates (NHPs).

2. RESPONSIBILITY

Veterinary care staff

3. INTRODUCTION

3.1. Perform a thorough physical exam and obtain an accurate body weight.

3.2. Withhold food (not water) for 12 hours prior to anesthesia in order to reduce the risk of aspiration of stomach contents. Juveniles or small species such as marmosets should only be fasted 4 to 6 hours to help avoid hypoglycemia.

3.3. Keep animals warm by providing a heat source until the animal has recovered from anesthesia. Care should be taken to not overheat or burn the animals.

3.4. Never leave an anesthetized animal unattended.

4. MATERIALS

4.1. Material or equipment to provide or conserve body heat (e.g. warm-water circulating pad)

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. Tight-fitting mask

4.5. Isoflurane

4.6. Sevoflurane

4.7. Glycopyrrolate (0.2mg/mL)

4.8. Intra-venous catheter

4.9. Ketamine (100mg/mL) *Controlled drug

4.10. Diazepam (5mg/mL) *Controlled drug

4.11. Xylazine (20mg/mL)

4.12. Dexmedetomidine (0.5mg/mL)

4.13. Atipamezole (5mg/mL)

4.14. Midazolam (5mg/mL) *Controlled drug

4.15. Thiopental sodium (Pentothal) powder *Controlled drug

4.16. Propofol (10mg/mL)

4.17. Alfaxalone (10mg/ml)

4.18. Sterile isotonic saline (0.9% saline) or Lactated Ringer’s Solution (LRS)

4.19. Xylocaine spray

4.20. Sterile lubricant (e.g., water soluble jelly)

4.21. Endotracheal tubes, cuffed

4.22. Laryngoscope

4.23. Plain gauze rolls
5. PROCEDURES

5.1. Premedication:
   
   5.1.1. Can be used for longer procedures to control salivation.

   5.1.2. Administer glycopyrrolate, intramuscularly

   5.1.2.1. Macaque: 0.004 to 0.008mg/kg

   5.1.2.2. Marmoset: 0.005 to 0.01mg/kg

   5.1.3. Glycopyrrolate should not be administered in conjunction with Alpha-2 agonists, e.g., xylazine and dexmedetomidine.

5.2. Injectable anesthesia:

   5.2.1. Can be used alone for short, non-invasive procedures.

   5.2.2. Used for induction prior to use of isoflurane anesthesia for smooth and rapid induction and to facilitate intubation.

   5.2.3. Anesthesia protocols:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>Route</th>
<th>Duration of Effect</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketamine</td>
<td>10 to 15mg/kg</td>
<td>15 to 20mg/kg</td>
<td>IM</td>
<td>15 to 30 minutes</td>
</tr>
<tr>
<td>Ketamine/Diazepam</td>
<td>15mg/kg</td>
<td>15 to 20mg/kg</td>
<td>IM</td>
<td>30 minutes to 1 hour</td>
</tr>
<tr>
<td></td>
<td>1mg/kg</td>
<td>1mg/kg</td>
<td></td>
<td>Can be mixed in the same syringe.</td>
</tr>
<tr>
<td>Ketamine/Xylazine</td>
<td>7mg/kg</td>
<td>10 to 20mg/kg</td>
<td>IM</td>
<td>30 minutes to 2 hours</td>
</tr>
<tr>
<td></td>
<td>0.6mg/kg</td>
<td>3mg/kg</td>
<td></td>
<td>Can be mixed in the same syringe.</td>
</tr>
<tr>
<td>Ketamine/Dexmedetomidine</td>
<td>5 to 10mg/kg</td>
<td>10 to 20mg/kg</td>
<td>IM</td>
<td>30 minutes to 2 hours</td>
</tr>
<tr>
<td></td>
<td>0.05mg/kg</td>
<td>1mg/kg</td>
<td></td>
<td>Can be mixed in the same syringe.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Can be reversed with atipamezole by administering the same volume of Dexmedetomidine used.</td>
</tr>
<tr>
<td>Ketamine/Midazolam</td>
<td>8mg/kg</td>
<td>10mg/kg</td>
<td>IM</td>
<td>30 to 45 minutes</td>
</tr>
<tr>
<td></td>
<td>0.2mg/kg</td>
<td>1mg/kg</td>
<td></td>
<td>Can be mixed in the same syringe.</td>
</tr>
<tr>
<td>Pentothal</td>
<td>5mg/kg</td>
<td>IV, slowly</td>
<td>15 minutes</td>
<td>May produce apnea. Administer slowly to effect.</td>
</tr>
<tr>
<td>Propofol</td>
<td>Loading dose:</td>
<td>Loading dose:</td>
<td>IV, slowly</td>
<td>Until discontinued</td>
</tr>
<tr>
<td></td>
<td>2 to 5mg/kg</td>
<td>7 to 8mg/kg</td>
<td>0.9mg/kg/min</td>
<td>May produce apnea.</td>
</tr>
<tr>
<td></td>
<td>Infusion: 0.3</td>
<td>Infusion: 0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>to 0.4 mg/kg/min</td>
<td>mg/kg/min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alfaxolone</td>
<td>7-10mg/kg</td>
<td>IM</td>
<td>30-45 minutes</td>
<td>May cause respiratory depression.</td>
</tr>
</tbody>
</table>

5.3. Intravenous (IV) catheter placement:

   5.3.1. To provide IV fluid therapy and venous access during surgery.

   5.3.2. Insert IV catheter in either the cephalic or saphenous vein and secure.

5.4. IV fluid administration:

   5.4.1. Administer isotonic saline (0.9% saline) or Lactated Ringer’s Solution at a rate of 10mL/kg/hour.
5.5. Endotracheal intubation:

5.5.1. Placement of an endotracheal tube is recommended for maintaining open airways.

5.5.2. Cuffed endotracheal tubes are preferred as they reduce the possibility of aspiration of saliva or stomach contents.

5.5.3. Intubation:

5.5.3.1. Lubricate endotracheal tube with sterile lubricant.

5.5.3.2. With the animal in sternal recumbency, extend the neck and head so that they are in a straight line.

5.5.3.3. While holding the upper jaw, pull the tongue forward and down so that the epiglottis is visible.

5.5.3.4. Use the laryngoscope to disengage the epiglottis from the soft palate, exposing the glottis and vocal chords.

5.5.3.5. Spray the laryngeal folds with 2% xylocaine to help decrease laryngospasm (spasmodic closing and opening of the glottis).

5.5.3.6. Insert the endotracheal tube (with the convex side facing upwards) gently into the proximal larynx.

5.5.3.7. Gently rotate the endotracheal tube 180° and apply gentle pressure to insert into the trachea. Confirm proper placement by checking for the animal’s breath as it exits the endotracheal tube during exhalation.

5.5.3.8. Secure the endotracheal tube by tying a piece of gauze around the tube then behind the animal’s head.

5.5.3.9. Inflate the cuff of the endotracheal tube.

5.5.3.10. Verify adequate ventilation of both lungs by auscultation.

5.6. Isoflurane anesthesia:

5.6.1. Induction (if injectable anesthetics not previously administered):

5.6.1.1. Use a tight-fitting mask.

5.6.1.2. Adjust the oxygen flowmeter to 0.8 to 1.5 L/min.

5.6.1.3. Adjust the isoflurane vaporizer to 3% to 5%.

5.6.2. Maintenance:

5.6.2.1. Use the endotracheal tube or mask connected to the Bain circuit.

5.6.2.2. Adjust the flowmeter to 0.8 to 1.5 L/min.

5.6.2.3. Adjust the isoflurane vaporizer to 1.0 to 2.0% (dose to effect).

5.6.2.4. Apply ophthalmic ointment (natural tears) to both eyes to prevent dryness and damage to the cornea.

5.6.2.5. When not under assisted ventilation, animal should be manually ventilated or “bagged” every 5-10 minutes to ensure proper air exchanges.

5.6.3. Recovery:

5.6.3.1. Turn off the isoflurane vaporizer but keep the animal on oxygen for 2 to 5 minutes or longer if oxygen saturation levels are low.

5.6.3.2. Remove the endotracheal tube as soon as the animal shows signs of impending arousal, i.e., when reflexes begin to return.

5.6.3.3. Monitor the animal in his home cage to ensure it regains full consciousness and able to stand in a sternal position.

5.7. Sevoflurane anesthesia:

5.7.1. Induction (if injectable anesthetics not previously administered):

5.7.1.1. Use a tight-fitting mask.

5.7.1.2. Adjust the oxygen flowmeter to 0.8 to 1.5 L/min.
5.7.1.3. Adjust the vaporizer to 5% to 8%.

5.7.2. Maintenance:

5.7.2.1. Use the endotracheal tube or mask connected to the Bain circuit.

5.7.2.2. Adjust the flowmeter to 0.8 to 1.5 L/min.

5.7.2.3. Adjust the vaporizer to 1.0 to 3.0% (dose to effect).

5.7.2.4. Apply ophthalmic ointment (natural tears) to both eyes to prevent dryness and damage to the cornea.

5.7.2.5. When not under assisted ventilation, animal should be manually ventilated or “bagged” every 5-10 minutes to ensure proper air exchanges.

5.7.3. Recovery:

5.7.3.1. Turn off the vaporizer but keep the animal on oxygen for 2 to 5 minutes or longer if oxygen saturation levels are low.

5.7.3.2. Remove the endotracheal tube as soon as the animal shows signs of impending arousal, i.e., when reflexes begin to return.

5.7.3.3. Monitor the animal in his home cage to ensure it regains full consciousness and able to stand in a sternal position.

6. REFERENCES

### SOP REVISION HISTORY

<table>
<thead>
<tr>
<th>DATE</th>
<th>NEW VERSION</th>
</tr>
</thead>
</table>
| 2016.02.22 | 1.1.1. Administer glycopyrrolate, intramuscularly  
            1.1.1.1. Macaque: 0.004 to 0.008 mg/kg  
            1.1.1.2. Marmoset: 0.005 to 0.01 mg/kg |
| 2016.02.22 | 5.4 Pentothal route of administration: IV slowly |
| 2016.02.22 | 5.6.2.3. Adjust the isoflurane vaporizer to 1.0 to 2.0% (dose to effect). |
| 2016.02.22 | 5.6.3.1. Turn off the isoflurane vaporizer but keep the animal on oxygen for 2 to 5 minutes or longer if oxygen saturation levels are low. |
| 2016.02.22 | 5.6.3.2. Remove the endotracheal tube as soon as the animal shows signs of impending arousal, i.e., when reflexes begin to return. |
| 2016.02.22 | 5.7. Sevoflurane anesthesia:  
            5.7.1. Induction (if injectable anesthetics not previously administered):  
            5.7.1.3. Use a tight-fitting mask.  
            5.7.1.4. Adjust the oxygen flowmeter to 0.8 to 1.5 L/min.  
            5.7.1.5. Adjust the isoflurane vaporizer to 5% to 8%.  
            5.7.2. Maintenance:  
            5.7.2.6. Use the endotracheal tube or mask connected to the Bain circuit.  
            5.7.2.7. Adjust the flowmeter to 0.8 to 1.5 L/min.  
            5.7.2.8. Adjust the isoflurane vaporizer to 1.0 to 3.0% (dose to effect).  
            5.7.2.9. Apply ophthalmic ointment (natural tears) to both eyes to prevent dryness and damage to the cornea.  
            5.7.3. Recovery:  
            5.7.3.10. Turn off the isoflurane vaporizer but keep the animal on oxygen for 2 to 5 minutes or longer if oxygen saturation levels are low.  
            5.7.3.11. Remove the endotracheal tube as soon as the animal shows signs of impending arousal, i.e., when reflexes begin to return. |
| 2017.01.25 | Addition of: 4.10 Diazepam and 4.13 Midazolam |
| 2017.01.25 | 5.1. Premedication (can be used for longer procedures): |
| 2017.01.25 | 5.2.3. Anesthesia protocols: |
| 2017.01.25 | (Table of injectable anesthetic protocols)  
            Doses for marmosets  
            Ketamine, 10 to 15mg/kg  
            15 to 20mg/kg, IM, 15 to 30 minutes  
            Ketamine – Diazepam, 15mg/kg – 1mg/kg, 15 to 20mg/kg – 1mg/kg, IM, 30 minutes to 1 hour, Can be mixed in the same syringe.  
            Ketamine – Dexmedetomidine, 5 to 10mg/kg - 0.05mg/kg, 10 to 20mg/kg – 1mg/kg, IM, 30 minutes to 2 hours, Can be mixed in the same syringe.  
            Ketamine – Midazolam, 8mg/kg – 0.2mg/kg, 10mg/kg – 1mg/kg, IM, 30 to 45 minutes, Can be mixed in the same syringe. |
| 2017.01.25 | 6. REFERENCES  
| 2017.03.29 | 5.1. Premedication:  
            5.1.1. Can be used for longer procedures to control salivation. |
| 2017.03.29 | 5.1.3. Glycopyrrolate should not be administered in conjunction with Alpha-2 agonists, e.g., xylazine and dexmedetomidine. |
| 2018.05.24 | 2. Principal Investigators (PIs) and their research staff, Veterinary care staff |
| 2020.05.13 | 3.3 Keep animals warm by providing a heat source until the animal has recovered from anesthesia. Care should be taken to not overheat or burn the animals. |
| 2020.05.13 | 4.13. Atipamezole (5mg/mL) |
| 2020.05.13 | 5.2.3. Ketamine/Dexmedetomidine: Can be reversed with atipamezole by administering the same volume of Dexmedetomidine used |
| 2020.05.13 | 5.5.3.6. Insert the endotracheal tube (with the convex side facing upwards) gently past the vocal folds into the trachea (this can be timed with exhalation) gently into the proximal larynx.  
            5.5.3.7. Gently rotate the endotracheal tube 180° and apply gentle pressure to insert into the trachea. Confirm proper placement by checking for the animal’s breath as it exits the endotracheal tube during exhalation.  
            5.5.3.8. Secure the endotracheal tube by tying a piece of gauze around the tube then behind the animal’s head.  
            5.5.3.9. Inflate the cuff of the endotracheal tube  
            5.5.3.10. Verify adequate ventilation of both lungs by auscultation. |
| 2020.05.13 | 5.6.2.5. When not under assisted ventilation, animal should be manually ventilated or “bagged” every 5-10 minutes to ensure proper air exchanges. |
| 2020.05.13 | 5.6.3.3. Monitor the animal in his home cage to ensure it regains full consciousness and able to stand in a sternal position. |
| 2020.05.13 | 5.7.2.5. When not under assisted ventilation, animal should be manually ventilated or “bagged” every 5-10 minutes to ensure proper air exchanges. |
| 2020.05.13 | 5.7.3.3. Monitor the animal in his home cage to ensure it regains full consciousness and able to stand in a sternal position. |
| 2020.12.07 | 5.2.3. Alfaxalone - Dose for marmosets: 7-10mg/kg, IM, duration: 30-45 minutes, May cause respiratory depression. |