Florida Atlantic University
Institutional Animal Care and Use Committee

SOP

Rodent Anesthesia, Surgery and Analgesia

**Performance Standard:** To minimize the risks associated with anesthesia and surgical procedures and reduce post-operative pain. This is accomplished by ensuring animals are appropriately assessed and monitored during pre-, intra-, and post-procedural periods and that appropriate analgesic regimens are provided. To assure proper documentation is provided for confirmation of quality care.

**Background/Purposes:** Florida Atlantic University (FAU) is committed to the improvement of human and animal health through research and the advancement of science. It is FAU policy to meet or exceed all laws and regulations governing animal care and use in research, teaching and testing. Humane treatment and well-being of laboratory animals are considered highest priority. This SOP in conjunction with FAU Policy 10.4.6 is intended to outline the pre-, intra-, and post-procedural requirements for rodents that undergo general anesthesia for experimental (i.e. imaging, ultrasound) and/or surgical procedures at FAU.

**Responsibilities:**

1. **Researcher/Investigator:**
   a. Describe all anesthetic/surgical procedures and the methods used to monitor animals during pre-, intra-, and post-procedural periods in the animal protocol including analgesic provisions that will be given to the animal post-surgery.
   b. Ensure appropriate training of personnel and documentation of procedures in accordance with this and other relevant SOPS and policies.
   c. Ensure that all anesthesia, analgesia and surgical procedures are followed and documented per approved protocols.
   d. Assure animals receive adequate post-surgical/procedural care.

2. **IACUC:**
   a. Review and approve protocols/amendments to protocols.
   b. Assure adequate training of personnel.
   c. Inspect animal facilities/laboratories at least semiannually to ensure records are maintained and approved procedures are followed.
   d. Review/report animal welfare issues.
3. Research Integrity:
   a. Serve as liaison between the IACUC and PIs to ensure surgical locations and procedures are consistent with the regulatory requirements.
   b. Keep relevant training records

4. Attending veterinarian and/or designee:
   a. Provide guidance/oversight on surgery programs and post-surgical care.
   b. Provide consultation services to investigators on the appropriate choice of anesthetic and analgesic agents.
   c. Assist with training personnel and participate in procedures as required to ensure animal health and well-being. Verify proficiency of personnel in particular approved experimental procedures.
   d. Provide support regarding proper maintenance of equipment.

SOP Outline:

A. SPECIFIC REQUIREMENTS FOR SURVIVAL PROCEDURES

1. Pre-procedural planning
   a. Pre-procedural planning is critical to the success of surgical and anesthetic procedures in animals. This plan which is detailed in an approved IACUC protocol will include input from all members of the surgical team, including the surgeon/anesthetist, veterinarian, animal care staff and investigator.
   b. The plan has to identify personnel, their roles and experience in those roles so training requirements can be identified and addressed; equipment and supplies required for the procedures planned; the location where the procedure will be performed; and preoperative animal health assessment and post-operative care.

2. Preparation of the surgical arena
   a. The surgical suites and procedure rooms available within or in conjunction to a vivarium should be used whenever feasible. If the procedure has to be performed outside an animal facility, the justification has to be provided in the protocol and approved by the IACUC. The area of a research laboratory used for surgery should be located in a part with the least amount of traffic.
   b. There should be an area specifically designated for surgery where no other procedures are performed during the surgical procedure. The surgical area must be distinctly separate from the surgical prep area.
c. The surgical “table” must be constructed of a non-porous material that can be disinfected using appropriate agents (see Appendix A: Table 1). Lab benches or tables work well for this purpose. A surface disinfectant will be available at all times in the specific area(s) within the vivarium.

d. Disinfect the surgical area and equipment prior to the surgery.

e. The area immediately surrounding the surgical area should also be wiped down prior to surgery to decrease dust borne contaminates in the area. Surgical suites and procedure rooms in animal facilities will be kept sanitary by VS staff according to relevant SOPs.

3. Sterilization of surgical instruments

   a. Surgical instruments must be sterile.
   
   b. Sterilization of the instruments can be achieved in a number of ways with heat sterilization via steam (autoclave) being the preferred method. (See Appendix A: Table 2)
   
   c. Chemical sterilants:
      
      i. Chlorine dioxide is not documented as being toxic to animal tissue, but it will corrode stainless steel instruments.
      
      ii. Aldehydes must be thoroughly rinsed off of instruments with sterile saline or sterile water before use.
      
      iii. Peracetic Acid is a very powerful oxidant. It is compatible with most materials. Needs to be made fresh.
      
      iv. Ethylene Oxide is a regulated chemical carcinogen therefore any use at FAU must be approved by EH&S.
      
      v. Alcohol is not a sterilant and will not be approved as such by the IACUC.
   
   d. Sterilized instruments stored in sterile unopened packages must be labeled with the date of sterilization and have 6 month shelf live if stored properly.
   
   e. In rodents, a sterile surgical pack can be used consecutively in a group of animals provided a hot bead sterilizer is used to sterilize the cutting end of the instruments prior to use between animals. Care must be taken to remove blood and debris before sterilizing using this method.

4. Pre-surgical/procedural evaluation of the animal

   a. Evaluate the animal to insure their health. The animal should be alert, have a smooth hair coat and clear eyes.

   b. Withholding food is not necessary in rodents unless specifically mandated by the protocol or surgical procedure. Water should not be withheld unless required by the protocol. It needs to be described in the IACUC protocol.
5. **Preparation of the animal**

   a. The surgical prep area must be distinctly separate from the rodent surgical area. Clip or shave the surgical site with enough border area to keep hair from contaminating the incision site. This can also be done the day before surgery. An ophthalmic lubricating ointment (i.e. artificial tears) should be applied to the eyes to prevent drying during the procedure.

   b. The surgical site should be cleansed and disinfected with germicidal scrub (see Appendix A: Table 3). Start with a concentrated disinfectant scrub solution, being careful to scrub from the center of the site toward the periphery, and follow by rinsing with an alcohol, dilute disinfectant or sterile saline solution. This process should be repeated three times. The animal should not be excessively doused with liquid as this will contribute to heat loss. Note that alcohol will contribute significantly to hypothermia if used too liberally.

   c. Move the animal to the surgical area and make sure to position the patient correctly. Stabilize the animal in the correct position with rolled up gauze or alike and secure the limbs with tape in a way not to constrict blood circulation. Apply a final disinfectant spray to the surgical site.

   d. It is recommended that the area be covered with a sterile surgical drape (paper, adhesive, or gauze sponges). This not only helps prevent stray hair from entering the surgical field, but also provides an area on which to lay sterile instruments during surgery.

6. **Preparation of the Surgeon**

   a. The surgeon must wear clean laboratory garments (lab coat or scrubs), a hair cover and surgical mask. Clean scrubs are available in each vivarium. This must be donned prior to hand washing.

   b. The surgeon should thoroughly scrub his/ her hands with a bactericidal scrub (See Appendix A: Table 3) then must don sterile surgical gloves just prior to starting the surgery, being careful to put them on aseptically. (Standard laboratory gloves are not acceptable alternatives.)

   c. Gloves, drapes, and instruments should be replaced with new sterile components if the sterile field is compromised.

7. **Monitoring the animal during surgery and providing proper anesthesia**

   a. Evaluation of anesthetic depth is important. Techniques for monitoring this state vary slightly with the agent used. Absence of a response to pinching the
toe is one indicator of the appropriate level of anesthesia. The palpebral reflex should also be used. Caution: The zone between enough anesthesia and too much is very narrow in rodents.

b. Maintaining body temperature is very important during any survival surgical or experimental anesthetic procedure. External heat sources such as recirculating water heating pads or chemical heating pads can be safely used to provide supplemental heat. However, heat lamps and commercial household heating pads are not permitted.

c. During long surgeries, warmed (25°C-27°C) sterile fluids (0.9% NaCl or Lactated Ringer’s solution) should be provided via subcutaneous, intravenous, or intraperitoneal routes to warm the animal, rehydrate and compensate for blood loss. A microwave is available in designated areas in the animal facilities. Use caution not to overheat fluids.

d. All tissues exposed for extended periods should be kept moist with the same warmed fluids used for hydration.

8. Closure of the body cavity

a. Closure of the body cavity is typically accomplished in at least two layers, with an absorbable suture used for muscle/peritoneum inner layer(s), and a non-absorbable, non-wicking suture or surgical staples used for the skin layer. An absorbable suture may be used for a subcuticular closure (See Appendix A: Table 4). Tissue glue is an alternative for closing skin, especially for small incisions with little tension.

b. Holding tissue layers such as skin must be sutured in an interrupted pattern. All external skin closure suture or staples should be removed within 7-14 days.

9. Providing appropriate post-surgical/procedural care

a. The animal recovering, in or out of its cage, must be kept warm using an external heat source as described in 7b. A clean cage needs to be used for recovering animals to minimize contamination of the incision site.

b. Animals should be observed/monitored regularly until completely recovered. Rotating the animals a couple of times until they can maintain sternal recumbency, and are capable of purposeful movement is necessary.

c. Once the animals are returned to the housing room, a Surgery Post-OP card (see Appendix B) should be placed on the cage clearly indicating the date of surgery.

d. PI should monitor animal for signs indicative of postoperative complications as indicated in the IACUC approved protocol. (Examples include the following: lethargy, labored or abnormal breathing, unkempt appearance, increased or decreased movement, dehydration, self-mutilation, abnormal
posturing, redness or swelling around the surgical site or partial/total opening of the wound.) The monitoring methods, treatments, and humane endpoints used should be clearly stated in the approved animal protocol.

10. Provision of Analgesics

Pre-emptive or intra-operative/procedural analgesia and most often post-operative analgesia are required whenever pain is expected. Any exception to use of analgesics must be scientifically justified in the protocol and approved by the IACUC.

11. Documentation requirements

a. Documentation must be maintained by the research staff and needs to be kept in the animal housing room until the end of the post-operative period (i.e. when sutures are removed). Clip boards will be provided by VS. After completing, keep the records at least until the end of the study.

b. Documentation should include PI name, IACUC protocol number, species, experimental procedure performed, date of the procedure, administration of anesthetic and analgesic agents, including dose, volume injected, route, and time of administration beside any complications encountered during or after the procedure. This can be recorded on the Rodent Surgery and Post-Procedural Record Forms available at [http://www.fau.edu/research/forms.php](http://www.fau.edu/research/forms.php). If the monitoring will be recorded for a group of rodents or individual animals will depend on the invasiveness of the procedure and/or impact on the well-being of the animal and will be decided on a case by case basis during the discussion with the veterinarian. Records can be adjusted to the needs of an individual study in collaboration with the veterinarian. All records must be available on demand from the IACUC, outside agencies (e.g. AAALAC), and the AV.

c. The cage of the animal(s) that underwent surgery has to be flagged with a red cage card stating the day of surgery (See Appendix B) and kept until the sutures/staples are removed. Only animals with the same group ID and/or surgical date should be kept in the same cage for the time from surgery until the end of the post-surgical period.

B. SPECIFIC REQUIREMENTS FOR NON- SURVIVAL PROCEDURES

1. Non-survival surgeries do not require separate designated surgical areas. They should still be conducted in an uncluttered, clean area.
2. Anesthesia is required for all surgical and experimental procedures and depth of anesthesia assessed and monitored to avoid pain/distress.
3. A lab coat and gloves are required for non-survival surgeries/procedures.
4. Short non-survival surgical procedures such as transcardial perfusion and harvesting of tissues during surgery do not require shaving of the surgical site unless otherwise dictated by the specific research aim.
5. Therapeutic drugs given during the procedure must be recorded in the comment section of the Rodent Surgery and Post-Procedural Records Form(s) (http://www.fau.edu/research/forms.php).
### Table 1: Hard Surface Disinfectants

<table>
<thead>
<tr>
<th>NAME</th>
<th>EXAMPLES *</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quaternary Ammonium</td>
<td>Roccal®, Cetylcide®</td>
<td>Rapidly inactivated by organic matter. Compounds may support gram-negative bacteria.</td>
</tr>
<tr>
<td>Chlorine</td>
<td>Sodium hypochlorite (Clorox® 10% solution)</td>
<td>Corrosive depending on the product. Presence of organic matter reduces activity. Chlorine dioxide must be fresh; kills vegetative organisms within 3 minutes of contact.</td>
</tr>
<tr>
<td></td>
<td>Chlorine dioxide (Clidox®, Alcide®, Vimoba®)</td>
<td></td>
</tr>
<tr>
<td>Aldehydes</td>
<td>Glutaraldehyde (Cidex®, Cide Wipes®)</td>
<td>Rapidly disinfects surfaces. Toxic. OSHA has set exposure limits.</td>
</tr>
<tr>
<td>Phenolics</td>
<td>Lyso1®, TBQ®</td>
<td>Less affected by organic material than other disinfectants.</td>
</tr>
<tr>
<td>Chlorhexidine</td>
<td>Nolvasan®, Hibiclens®</td>
<td>Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses.</td>
</tr>
</tbody>
</table>

* The use of brand names as examples does not indicate a product endorsement. Always follow manufacturer’s instructions.

### Table 2: Methods of Initial Instrument Sterilization

<table>
<thead>
<tr>
<th>AGENTS</th>
<th>EXAMPLES *</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical: Steam sterilization (moist heat)</td>
<td>Autoclave</td>
<td>Effectiveness dependent upon temperature, pressure and time (e.g., 121°C for 15 min. vs. 131°C for 3 min).</td>
</tr>
<tr>
<td>Physical: Dry Heat</td>
<td>Hot Bead Sterilizer²</td>
<td>Make sure instruments are clean and free of debris before using this method for effective sterilization</td>
</tr>
<tr>
<td>Ionizing radiation</td>
<td>Gamma Radiation</td>
<td>Requires special equipment.</td>
</tr>
<tr>
<td>Hydrogen Peroxide</td>
<td>Sterad®</td>
<td>Not useful for delicate items.</td>
</tr>
<tr>
<td>Chlorine¹</td>
<td>Chlorine Dioxide (Clidox®, Alcide®)</td>
<td>A minimum of 6 hours required for sterilization. Presence of organic matter reduces activity. Must be freshly made.</td>
</tr>
<tr>
<td>Aldehydes¹</td>
<td>Formaldehyde (6% sol.), Glutaraldehyde</td>
<td>Many hours required for sterilization. Corrosive and irritating. Consult safety representative on proper use. Only 12 min contact time required. Compatible with most materials.</td>
</tr>
<tr>
<td></td>
<td>Dialdehyde</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ortho-phthalaldehyde (Cidex OPA®)</td>
<td></td>
</tr>
<tr>
<td>Bag Sterilization</td>
<td>Ethylene Oxide</td>
<td>Chemical Carcinogen requiring IACUC approval prior to use</td>
</tr>
<tr>
<td>Peracetic Acid</td>
<td>Perasafe®</td>
<td>Compatible with most material. May adversely affect anodized aluminum. Needs only 10 min contact time. Must be made freshly.</td>
</tr>
</tbody>
</table>

* The use of brand names as examples does not indicate a product endorsement. Always follow manufacturer’s instructions.
Instruments must be rinsed thoroughly with sterile water or saline to remove chemical sterilants before use.

This method should only be used between animals with instruments previously sterilized with another method.

### Table 3: Skin Disinfectants

<table>
<thead>
<tr>
<th>AGENTS</th>
<th>EXAMPLES</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohols</td>
<td>70% ethyl alcohol</td>
<td><strong>NOT ADEQUATE ALONE FOR SKIN PREPARATION!</strong> Not a high level disinfectant. Not a sterilant. Flammable.</td>
</tr>
<tr>
<td></td>
<td>70-99% isopropyl alcohol</td>
<td></td>
</tr>
<tr>
<td>Chlorhexidine</td>
<td>Nolvasan®, Hibiclens®</td>
<td>Rapidly bactericidal and persistent. Effective against many viruses. Excellent for skin. Presence of blood does not interfere with activity.</td>
</tr>
</tbody>
</table>

The use of common brand names as examples does not indicate a product endorsement.

**NOTE:** Alternating disinfectants is more effective than using a single agent.

### Table 4: Suture Selection

<table>
<thead>
<tr>
<th>SUTURE</th>
<th>CHARACTERISTICS AND FREQUENT USES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vicryl®, Dexon®</td>
<td>Absorbable; 60-90 days. Braided. Inert. Nonantigenic. Noncollagenous. Excellent knot security. Ligate or suture tissues where an absorbable suture is desirable.</td>
</tr>
<tr>
<td>PDS® or Maxon®</td>
<td>Absorbable; 6 months. Monofilament. Ligate or suture tissues especially where an absorbable suture and extended wound support is desirable</td>
</tr>
<tr>
<td>Silk</td>
<td>Nonabsorbable. Silk is very easy to use and to knot. Ideal for microvascular surgical applications. Caution: Tissue reactive and may wick organisms into the wound. The use of silk must be justified and approved by the IACUC.</td>
</tr>
<tr>
<td>Stainless Steel Wound Clips, Staples</td>
<td>Nonabsorbable. Requires instrument for removal from skin.</td>
</tr>
</tbody>
</table>

The use of common brand names as examples does not indicate a product endorsement.

**NOTE:** Braided sutures have a wicking effect and are not recommended for closure of skin incisions.
Appendix B:

Surgery Post Op cage cards for documentation by Research Personnel.

Post Surgical Animals

DATE OF SURGERY: