

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 compliance with all relevant IACUC SOPs and policies regarding animal anesthesia, surgery, analgesia and euthanasia.
 - c. Ensure appropriate training of personnel and documentation of procedures in accordance with this and other relevant IACUC SOPs and policies.
 - d. Ensure that all anesthesia, analgesia and surgical procedures are followed and documented per approved protocols.
 - e. Assure animals receive adequate post-surgical/procedural care.
 - f. Assuring that any anesthetic, sedative or analgesic drug administered to animals is within the manufacturer provided expiration date.
 - g. Contact the IACUC whenever you see unexpected complications associated with the procedure that have not been identified in the protocol.

- h. Contact a veterinarian if there are post procedural/ post-surgical health related concerns, or if pain is not alleviated by IACUC approved analgesic regimens.
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.
 - e. Check that analgesic, sedative, and analgesic drugs are administered to animals as described in the IACUC approved protocol and are within the manufacturer provided expiration date.
 - f. Post approval review of anesthetic and surgical procedures
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
 - c. Coordinate communication between research personnel, CM and Training Coordinator to assure training requirements are met in accordance to IACUC Policy.
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.
 - d. Verify proficiency of personnel in approved experimental procedures as determined by the IACUC.
 - e. 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 contaminants in the area. Surgical suites and procedure rooms in animal facilities will be kept sanitary by Comparative Medicine (CM) staff according to relevant SOPs.

3. Sterilization of surgical instruments

NOTE: Alcohol is NOT a sterilant and will not be approved as such by the IACUC

- a. All surgical instruments must be sterile.
- b. Sterilization of the instruments can be achieved in a number of ways. Heat sterilization via steam (autoclave) is 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.
- d. Sterilized instruments stored in sterile unopened packages must be labeled with the date of sterilization and have 6-month shelf life if stored properly.
- e. In rodents, a sterile surgical pack can be used consecutively in a group of animals (maximum of 5 rodents) provided a hot bead sterilizer is used to

sterilize the cutting end/tips of the instruments prior to use between animals. Care must be taken to remove blood and debris before re-sterilizing using this method. Caution should be taken when using the hot bead sterilizer. Instruments should be left in for a minimum of 10 seconds. Ensure adequate cooling time to prevent tissue damage. After 10 seconds, instruments can be placed back on sterile field.

- f. All materials to be implanted into animals must be sterile and biocompatible. When available, industry made implants should be used.

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 cannot be withheld unless required by the protocol. It needs to be described in the IACUC protocol.
- c. Weigh animals pre-operatively and record weight on surgical log.

5. Preparation of the animal

- a. The surgical prep area must be distinctly separate from the surgical area.
- b. Clip or shave the surgical site with a large enough border area to prevent hair from contaminating the incision site. This can also be done the day before surgery.
- c. Apply an ophthalmic lubricating ointment (i.e. artificial tears) to the eyes to prevent corneal drying during the procedure.
- d. Cleanse/disinfect the surgical site with a germicidal scrub (see Appendix A: Table 3). Start the scrub from the center of the surgical site (where the incision will be located) and extend in an outward circular motion toward the periphery. This is followed by a scrub using an alcohol, dilute disinfectant or sterile saline solution again beginning in the center and extending outward in a circular pattern. 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.
- e. 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 skin disinfectant to the surgical site.
- f. 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.

- g. Lidocaine or lidocaine/bupivacaine blocks should be performed by subcutaneous injection at the incision site prior to the second complete scrub to allow time for absorption.

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. (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 exam 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, chemical heating pads or infrared heating device (e.g. PhysioSuite) can be safely used to provide supplemental heat. However, heat lamps and commercial house-hold heating pads are not permitted.
- c. During long surgeries, warmed (at least 25°C-27°C, but better normal body temperature at 37°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 vivaria. 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 should 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. It is acceptable to place the cage ½ on ½ off of a commercial heating blanket during recovery. Recover animals in a clean cage to minimize contamination of the incision site. A pinch of dirty bedding or transfer of nesting material from the original cage might aide to provide olfactory cues for the animal and reduce stress when waking up in the new clean cage.
- b. Observe all animals until completely recovered from anesthesia. Complete recovery is defined as the ability of the animal to maintain sternal recumbency and make purposeful movements. Do not leave animals unattended until they are completely recovered and returned to general housing. Rotate the animals a couple of times until they can maintain sternal recumbency and are capable of purposeful movement.
- c. Once the animals are returned to the housing room, place a Surgery Post-OP card (see Appendix B) on the cage clearly indicating the date of surgery. Keep the Post-Surgical Animal(s) card on the cage until sutures are removed if applicable or at least for 7 days post-operatively.
- d. Monitor the animal for signs 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.
- e. Contact a veterinarian if you see post-operative complications.
- f. Animals need to be provided with supportive care for at least 2-5 days post-operatively, which might include but is not limited to palatable feed and feed supplements, a clean and quiet environment, thermal support, and proper hydration.
- g. Animals undergoing any surgical procedure must receive subcutaneous fluids for maintenance of hydration.
- h. Assess animals for pain at least once daily even when analgesics are administered during the immediate post-operative period and a minimum of two (2) days after completing the analgesic regimen as approved in the IACUC protocol. This means animals undergoing minor surgical procedures

will need to be monitored for three (3) days and those undergoing major surgical procedures will need to be monitored for five (5) days.

10. Provision of Analgesics

- a. Pre-emptive or intra-operative/procedural analgesia and most often post-operative analgesia are REQUIRED whenever pain is expected. All drugs administered must be within manufacturer provided expiration date.
- b. Any exception to use of analgesics must be scientifically justified in the protocol and approved by the IACUC.
- c. Additional/supplementary analgesics have to be provided if an animal is still showing pain either during or beyond the regular analgesic regimen as described in the IACUC protocol on an as needed basis. The AV or designee should be consulted.
- d. Multi-modal analgesic regimens are recommended.

11. Documentation requirements

- a. Documentation must be maintained by the research staff and kept in the animal housing room until the end of the post-operative period (i.e. when sutures are removed) but no less than 7 days after surgery. Clip boards/binders will be provided by CM.,
- b. Maintain records until the end of the study with all other study related documents in the research lab.
- c. Documentation must 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 and any complications encountered during or after the procedure. Rodent Surgery and Post-Procedural Record Forms are available at <http://www.fau.edu/research/research-integrity/iacuc-policies-and-procedures.php>. Group Rodent Surgery and Post-Procedural Forms are only allowed for minor surgical procedures. Any rodent receiving a major survival surgery must be monitored individually, and Individual Rodent Surgery and Post-Procedural Record Forms used. Records can be adjusted to the needs of an individual study in collaboration with the veterinarian.
- d. All records must be available on demand from the IACUC, outside agencies (e.g. AAALAC, NIH OLAW), and the veterinary staff.
- e. Flag the cage of the animal(s) that underwent surgery with a red cage card indicating the date of surgery (See Appendix B) and keep this with the cage 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. Anesthetic drugs administered must be within manufacturer provided expiration date.
3. A lab coat and gloves are required for non-survival surgeries/procedures.
4. Short non-survival surgical procedures such as transcatheter 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/research-integrity/iacuc-policies-and-procedures.php>).

Appendix A:

| Table 1: Hard Surface Disinfectants | | |
|--------------------------------------------|------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| NAME | EXAMPLES* | COMMENTS |
| Quaternary Ammonium | Roccal®, Cetylcode® | Rapidly inactivated by organic matter. Compounds may support growth of gram-negative bacteria. |
| Chlorine | Sodium hypochlorite (Clorox® 10% solution) Chlorine dioxide (Clidox®, Alcide®, Vimoba®) | Corrosive depending on the product. Presence of organic matter reduces activity. Chlorine dioxide must be fresh; kills vegetative organisms within 3 minutes of contact. |
| Aldehydes | Glutaraldehyde (Cidex®, Cide Wipes®) | Rapidly disinfects surfaces. Toxic. OSHA has set exposure limits. |
| Phenolics | Lysol®, TBQ® | Less affected by organic material than other disinfectants. |
| Chlorhexidine | Nolvasan®, Hibiclens® | Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses. |

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

| AGENTS | EXAMPLES * | COMMENTS |
|--------------------------------------------|----------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Physical: Steam sterilization (moist heat) | Autoclave | Effectiveness dependent upon temperature, pressure and time (e.g., 121°C for 15 min. vs. 131°C for 3 min). |
| Physical : Dry Heat | Hot Bead Sterilizer ² | Make sure instruments are clean and free of debris before using this method for effective sterilization |
| Ionizing radiation | Gamma Radiation | Requires special equipment. |
| Hydrogen Peroxide | Sterad® | Not useful for delicate items. |
| Chlorine ¹ | Chlorine Dioxide (Clidox®, Alcide®) | A minimum of 6 hours required for sterilization. Presence of organic matter reduces activity. Must be freshly made. |
| Aldehydes ¹ | Formaldehyde (6% sol.), Glutaraldehyde Dialdehyde Ortho-phthalaldehyde (Cidex OPA®) | Many hours required for sterilization. Corrosive and irritating. Consult safety representative on proper use. Only 12 min contact time required. Compatible with most materials. |
| Bag Sterilization | Ethylene Oxide | Chemical Carcinogen requiring IACUC approval prior to use |
| Peracetic Acid | Perasafe® | Compatible with most material. May adversely affect anodized aluminum. Needs only 10 min contact time. Must be made fresh. |

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

¹ Instruments must be rinsed thoroughly with sterile water or saline to remove chemical sterilants before use.

² This method should only be used between animals with instruments previously sterilized with another method.

| AGENTS | EXAMPLES | COMMENTS |
|---------------|---------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Alcohols | 70% ethyl alcohol 70-99% isopropyl alcohol | NOT ADEQUATE ALONE FOR SKIN PREPARATION! Not a high level disinfectant. Not a sterilant. Flammable. |
| Iodophors | Betadine®, Prepodyne®, Wescodyne® | Reduced activity in presence of organic matter. Wide range of microbe killing action. Works best in pH 6-7. |
| Chlorhexidine | Nolvasan®, Hibiclens® | Rapidly bactericidal and persistent. Effective against many viruses. Excellent for skin. Presence of blood does not interfere with activity. Do not use near the eye. |

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 | |
|-----------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| SUTURE | CHARACTERISTICS AND FREQUENT USES |
| Vicryl®, Dexon® | Absorbable; 60-90 days. Braided. Inert. Nonantigenic. Non-collagenous. Excellent knot security. Ligate or suture tissues where an absorbable suture is desirable. |
| Dexon™ ^S | Absorbable. Monofilament. Inert. Nonantigenic. Non-collagenous. Excellent knot security. Ligate or suture tissues where an absorbable suture is desirable. |
| PDS® or Maxon® | Absorbable; 6 months. Monofilament. Ligate or suture tissues especially where an absorbable suture and extended wound support is desirable |
| Silk | 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. |
| Stainless Steel Wound Clips, Staples | Nonabsorbable. Requires instrument for removal from skin. |
| Prolene® | Nonabsorbable. Inert. Monofilament. |
| Nylon | Nonabsorbable. Inert. Monofilament. General skin closure. |

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: