OFFICE OF RESEARCH COMPLIANCE
CENTRAL MICHIGAN UNIVERSITY

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I. Purpose

These guidelines have been approved by the Institutional Animal Use and Care Committee (IACUC) and apply to all survival surgical procedures performed on rodents at CMU. These guidelines provide information on rodent survival surgery requirements, aseptic technique, operative techniques, post-operative care, and record keeping.

II. Background

The Animal Welfare Act (AWA), Public Health Service (PHS) Policy on Human Care and Use of Laboratory Animals, and The Guide for the Care and Use of Laboratory Animals (Guide) addresses specific requirements concerning aseptic technique for rodent survival surgery, pre-surgical planning, training and qualifications, surgical monitoring, and assessment of outcomes. These guidelines are intended to address these types of requirements. Any exceptions must be approved by the IACUC.

III. Definitions

Analgesia	The relief of pain without loss of consciousness.
Antiseptics	Chemical agents that either kill pathogenic microorganisms or inhibit their growth as long as the agent and microbe remain in contact.
Asepsis	The prevention of contact with microorganisms.
Aseptic Surgical Procedures	Surgery performed using procedures that limit microbial contamination so that significant infection does not occur.
Disinfectant	Kills 100% of vegetative bacteria (of certain species) under conditions specified by the Environmental Protection Agency, but are not efficacious against fungi, viruses, Mycobacterium tuberculosis or bacterial spores. These agents are only effective if used according to the manufacturers instruction and may be inactivated by organic matter such as blood.
Disinfection	The chemical or physical process that involves the destruction of pathogenic organisms. All disinfectants are effective against vegetative forms of organisms, but not necessarily spores.
Hemostasis	To stop bleeding.

Hypothermia	A body temperature below the average normal temperature.
Major Surgery	Any surgical intervention that penetrates and exposes a body cavity; any procedure that has the potential for producing permanent physical or physiological impairment (laparotomy, thoracotomy, craniotomy); and /or any procedure associated with orthopedics or extensive tissue dissection or transection.
Minor Surgery	Any surgical intervention that neither penetrates and exposes a body cavity, nor produces permanent impairment of physical or physiologic function (e.g., wound suturing, superficial vascular cutdowns, and percutaneous biopsy).
Sanitize	To make sanitary by cleaning (remove gross debris first).
Sterilant	Essentially the same as sporicides. They kill all microorganisms including bacterial endospores. A sporicidal product kills all microorganisms including bacterial endospores.
Sterile Zone	Area in front of the body, between the shoulder and the waist.
Sterilization	The process whereby all viable microorganisms are eliminated or destroyed. Sterilants are essentially the same as sporicides. They kill all microorganisms including bacterial endospores. The criterion of sterilization is the failure of organisms to grow if a growth supporting median is supplied.
Surgical Drape	Cloth or material used to cover parts of the body other than those to be operated on.

IV. Guidelines

A. General Information

1. Aseptic technique, a key factor in successful experimental surgery, is addressed below. There is a common perception that rodents are resistant to post-operative infections. This notion is erroneous. Relatively low-level bacterial contamination associated with surgical procedures may alter rodent physiology metabolism, immune function, and behavior, potentially confounding experimental results. These efforts can alter experimental results even though no overt clinical signs of sepsis are evident.
2. All surgical procedures and post-operative care must follow the process outlined in an approved protocol. Any deviations would require an approved amendment from the IACUC.

B. Training

1. Investigators and other personnel shall be appropriately qualified and experienced with conducting procedures on living animals.
2. Adequate arrangements shall be made for their in- service training, including the proper and humane care and use of laboratory animals.
3. For more information regarding required training, refer to the IACUC Policy on Training for Research and Animal Care Personnel.

C. Surgical Areas

1. A rodent surgical room or surgical area can be any room or portion of a room that is uncluttered and easily sanitized.

2. Access should be limited to the people directly involved in the procedure(s). This includes those performing the procedure as well as those observing or participating in the procedure for teaching or health and safety purposes.
3. No other activities should occur in the area when surgery is in progress.
4. Additional space considerations:
    a. There should be a quiet place for cages of rodents awaiting surgery.
    b. A separate animal preparation room or area, for hair removal and initial skin preparation, should be available. The surgical area cannot be utilized for these procedures.
    c. A quiet area must be utilized for surgical recovery.

D. Surgical Instruments
1. Instruments, implantable devices (e.g., catheters, trocars, osmotic pumps, telemetry) supplies and wound closure material(s) must be sterilized prior to surgery. The method of sterilization selected will depend upon the composition of the material.
2. For major survival surgeries, instruments must be properly packaged and sterilized.
    a. Use a new sterile pack for each cage of animals.
    b. Utilize bead sterilization between animals.
3. For minor recovery procedures, the instruments must be wiped clean with sterile gauze to remove blood/tissue, then disinfected and rinsed with sterile water or saline before use on another animal.

E. Animal Preparation
1. Prior to surgery, animals must be properly identified noting age, weight, and sex.
2. Fasting is not required in rodents due to high metabolic rate, unless specifically mandated by the protocol.
3. For some procedures it may be beneficial to administer preoperative fluids.
4. When indicated, administer preoperative antibiotic and analgesics.
5. Initiate anesthetic induction as indicated in the approved IACUC protocol.
6. Following anesthetic induction, apply ophthalmic ointment to the eyes to prevent cornea drying. Additional applications will be needed for longer procedures (e.g., procedures lasting longer than an hour).
7. Anesthesia alters thermoregulation and slows metabolism which may prolong recovery and increase the risk of complications.
    a. Prevent heat loss during the surgical procedure by utilizing circulating water heating blankets, warmed fluid bags, warming blankets, or warming discs.
    b. Heat loss occurs from the tail, ears, open body cavities, and evaporation of body fluids.
    c. Monitor animals closely for changes in respiratory rate, heart rate, and body temperature. Make necessary adjustments to maintain a surgical plan of anesthesia and a normothermic animal.

F. Initial Skin Preparation
   1. Skin preparation of all animals must take place at a separate location or bench away from the surgical area.
      a. Enough hair should be removed from the area to ensure a clear view of the and sutures or staples.
         i. Sutures or staples should not be contaminated by the fur.
         ii. No fur should be incorporated in the wound closure.
      b. Hair can be removed with surgical hair clippers or a depilatory agent.
      c. Using a #40 blade on hair clippers, carefully remove hair. Avoid skin abrasion or thermal injury.
      d. If depilatories are used, avoid contact with eyes. Wipe all depilator off skin prior to surgical preparation of the skin.
   2. The animal can be transferred to the surgical area at this point for surgical site preparation.

G. Preparation of the Surgical Site
   1. The surgical site is prepared by alternating wipes with disinfectant and alcohol to clean the site.
   2. A sterile gauze sponge or Q-tip can be used for cleaning the surgical site.
   3. Begin cleaning with a swipe along the incision line and extend outward in a circular pattern. Never move from the outer area (dirty) toward the center (clean).
   4. This sequence should be performed three times.
   5. Use a separate gauze or Q-tip for each round of skin preparation.

H. Draping
   1. Once the site has been properly cleaned, the animal/surgical site could be draped to reduce contamination of the surgical site. The decision to drape depends on the procedures being performed.
   2. Minor Surgical Intervention
      a. Optional to drape.
      b. Tips of instruments must stay within the sterile field.
      c. “Press and Seal” plastic wrap is a good option for these procedures.
   3. Major Surgical Intervention
      a. When feasible the surgical site should be draped.
      b. Towels, stockinet, drapes, gauze, plastic wrap, or “Press and Seal” plastic wrap are acceptable ways to drape.
      c. Drapes should cover the surgical field. Do not completely enclose the animal to prevent overheating.
      d. The surgeon must utilize sterile techniques throughout the procedure.

I. Maintaining Aseptic Technique (sterile field)
   1. Always be aware of instrument and hand position.
   2. If an instrument or glove touches something outside the sterile field, the instrument or glove should be replaced immediately.
   3. All instruments, suture, and implantable devices must be maintained on a sterile field during surgery. An additional sterile drape may be necessary.

4. If using a "tip-only" technique, the sterility of the instrument's tips must be maintained throughout the procedure. If performing multiple surgeries, make sure gloves and instruments are kept clean and disinfected between animals.

J. Perioperative Care and Monitoring (during surgical procedures)
   1. Monitor animals, respiratory rate and effort, movement, and mucous membrane color (should remain pink).
   2. Monitor animal's temperature.
   3. Reduce fluid loss by:
      a. Irrigating the operative field with warm 0.9% saline. Do not wet the drapes.
      b. Pre and/or post-procedure subcutaneous (SC) or intraperitoneal (IP) fluids can be used to support hydration, such as Lactated Ringers Solution and 0.9% saline.

K. Postoperative Care
   1. House rodents individually until they are fully ambulatory, to prevent cannibalism or suffocation.
      a. Place animals in a clean and dry recovery cage with absorbent toweling.
      b. DO NOT PUT ANIMALS IN A CAGE WITH BEDDING.
      c. Provide warmth with a circulating water blanket, warm water bottle, blankets, blue diaper pads, heat discs, or other methods. Body temperature must be maintained to minimize hypothermia. Provisions must be made so that an awake animal can move away from the heat source. (e.g. do not place recovery cage completely on a heating source). Place the heat source under half the recovery cage so the awake animal can move away from the heat if necessary.
   2. The period of postoperative monitoring must be specified in an approved protocol.
   3. Investigators may create and utilize their own forms for monitoring. See appendix for sample form.
   4. Monitor the animal regularly (at least every 10- 15 minutes) until the animal is fully ambulatory.
      a. Observe breathing, activity and mucous membrane color (should remain pink).
      b. Make sure the animal's head/nose is clear of any obstructions like toweling or bedding.
      c. Turn animals from one side to the other every 10-15 minutes until they are able to maintain sternal recumbency.
   5. Do not return the animal(s) to the vivarium until they are stable and fully awake.
   6. Provide analgesics as described in the approved IACUC protocol.
   7. Postoperative fluid and nutritional support will be provided as needed in accordance with the approved protocol.
   8. Post-surgical animals must be observed at least daily until sutures or staples are removed. Observations are to be made by a member of the Principal Investigator's staff or other trained individuals to ensure that there are no

complications.

9. Contact the facility coordinator and research staff immediately if the animal appears ill, painful, or the surgical site appears abnormal. Contact the veterinarian or facility manager to assist with determining the cause of pain/distress and for recommendations for supportive care.

L. Postoperative Records

1. Although individual records are desirable, a composite postoperative record may be used for a group of rodents.
2. A postoperative record must be maintained in the animal housing room with appropriate operative and post-operative information for the entire post-operative period. The period of postoperative monitoring (7, 10, 14 days) may be specified in the IACUC approved protocol.
3. Postoperative records are important because:
   a. Complete records document the animal's condition to the animal care staff.
   b. Following the NIH body scoring system is helpful to demonstrate the condition of the observed animal(s).
   c. It assures animal care staff and inspectors that the animal is receiving appropriate postoperative care.
   d. It assists the animal care staff with decision making for the animals under their care. Do they need to contact the PI? Is the PI monitoring these changes as well?
4. Postoperative cage cards must be in place for the duration of the postoperative monitoring period. The cards must be filled out completely, including the date for suture or staple removal. The card is removed once the staples/sutures are removed, and the animal has completed the postoperative monitoring.