Rodent Surgery Guidelines

In accordance with PHS Policy, the 'Guide' and best aseptic practice standards, the following guidelines must be followed for rodent surgeries at the University of Maryland, Baltimore. Any exceptions from these guidelines must be specifically justified and approved by the IACUC.

BACKGROUND: Though rodents are hardy and seldom show clinical signs of infection post-surgery when aseptic techniques are not applied, they often experience subclinical infections that can affect immune responses, metabolism, and hormonal parameters during post op periods. Please provide descriptions for the following aspects of planned surgeries (*stated chronologically as ordered below*) to show use of aseptic efforts to minimize such effects.

1. **SURGICAL AREA PREP**: The area where surgery will be performed must be clean, uncluttered and in an area away from high foot traffic. The surface should be disinfected with a 10% solution of household bleach in water or similar disinfectant spray (i.e., Quaternary ammonium or chlorine dioxide spray) and wiped dry with clean paper towels. Alcohol is not acceptable. Once dry, a clean or sterile pad/drape is placed on the surgical surface.

2. **SURGICAL INSTRUMENT PREP:**
   a. **SURVIVAL SURGERY:** Prior to **first use** on any day, instruments are to be sterilized by: autoclaving at 121 °C for 30 minutes minimum or by use of ethylene oxide gas (12 hrs. heated cycle or 24 hrs. cold cycle) or by use of FDA approved liquid sterilant solution following required contact times for sterilization (6-10 hrs. contact dependent on sterilant). "High Level Disinfection" contact times are not adequate/acceptable. Autoclave performance and sterility of surgical packs should be evaluated regularly through the use of indicators that demonstrate steam penetration of packs and supplies. Liquid sterilants must be rinsed off instruments with sterile water prior to use on animals.
   NOTE: Alcohol is **NOT** considered a sterilant.
   NOTE: If liquid sterilants are used, please consult EHS for current disposal requirements.

   REPEAT INSTRUMENT USE BETWEEN ANIMALS: For surgical instrument re-use after the first surgery on any day (*as in serial rodent surgeries*), a glass bead sterilizer (230 °C) may be used between animals. Use water (+/- soap if needed) to remove organic debris then place the distal 1/3 of the instrument (that will contact the animal) in the glass bead unit for 10 seconds (or longer dependent on manufacturer requirements). After removal, the instrument tips should be placed on a sterile field (i.e., rolled sterilized gauze pads) to air cool 5 minutes minimum prior to use on the next animal or may be rinsed with sterile water or saline to cool the tips if immediate re-use is required.

   b. **NON-SURVIVAL SURGERY:** For non-survival surgeries where euthanasia is performed less than 6 hours after the start of surgery, instruments must be clean (use of soap/water rinse) but do not need to be sterile. If used for serial non-survival surgeries, the instruments must be cleaned with soap and water rinse between animals. NOTE: *Non-survival surgeries lasting > 6 hours requires instruments to be sterilized as for Survival Surgery (detailed above).*

3. **THERMAL SUPPORT:** Thermal support must be provided for any anesthesia lasting longer than 3-5 minutes as rodents have a very high surface to mass ratio and thus lose body heat very quickly. Provide support from the time of induction of anesthesia continuously through recovery from anesthesia until the animal is ambulating normally. Thermal support units are to be of scientific or human medical grade and thermostatically controlled. Options include: warm water recirculating pump/ pad(s), Braintree Scientific Deltaphase Isothermal Pad, or forced warm air

blankets, Harvard Apparatus units with rectal probe, servo controlled etc. Home style electric heating pads **are NOT** permitted. A thick towel or piece of cardboard under heating pads should be used to insulate pad from table surfaces.

4. **ANESTHESIA:** Select the anesthetic agent(s) that provide duration of anesthesia appropriate to the procedures to be performed. Anesthetics with long duration are not appropriate for very short procedures. For example – inhalant anesthesia is more appropriate for short procedures such as tail tipping than would be an injectable anesthetic lasting 20-30 minutes. Recommended Anesthetic Agents *(by species)* can be located on the OAWA Website. Please consult with a Veterinary Resources Veterinarian if you need guidance.

5. **ANIMAL PREP:** Evaluate the animal for alertness, hydration status, and verify /record body weight prior to anesthetizing. A surgical record for each animal is required. Records should minimally include pre-operative weight, description of anesthesia used (*including drug doses, times administered and routes of administration*) and periodic evaluation of anesthetic depth (*at least every 15 minutes*). Surgery start and stop times are to be recorded. Batch records (multiple animals on a single record) for rodents are acceptable but the above information for each animal must be contained on the record.
    a. **HAIR/FUR REMOVAL:** In a location separate from the designated surgery site (more than 1 foot away), remove fur/hair over the intended surgical incision site to provide at least 1 cm margins around planned incision lines(s) unless anatomy prevents such margin. Use an electrical clipper *(# 40 or 50 blade*) or a chemical depilatory (*e.g., Nair*). Post removal of clipped/loose hairs, the animal is then to be transferred to the surgery site/table.
    NOTE: Apply depilatory cream in a circular pattern, with a cotton Q-tip applicator, until observing loose hair then remove with mild soap and water ensuring that all depilatory cream is removed from the site and surrounding hair. If a chemical depilatory is left on too long, a severe chemical burn will result.
    NOTE: Use of a razor to shave the area requires scientific justification due to increased post-operative infection with razor use.

    b. **OCULAR PROTECTANT**: After removal of hair, apply ocular protectant to both corneas to reduce chance of desiccation during anesthesia due to loss of blink response. Use petrolatum based ocular protectants without antibiotics (*such as Para-lube or Lacri-lube*) and re-apply every 20-30 minutes during anesthesia. Indicate reapplications on the anesthesia record.

    c. **PRE-EMPTIVE ANALGESIC**: Unless scientifically justified in the protocol, analgesics must be used and administered at least 10 minutes prior to initial incision – ideally at the time of anesthetic induction. Use is to be continued as described in the approved IACUC protocol to provide a duration of analgesic coverage post operatively appropriate to the type of surgery performed. Recommended Analgesic Agents *(by species)* can be located on the OAWA Website. Please consult with a Veterinary Resources Veterinarian if you need guidance.

    d. **SURGICAL SITE SCRUB**: The use of surgical scrub soap will remove organic debris, skin oils and waxes, facilitating the full antimicrobial effects of the antiseptic liquids. Use Betadine, Chlorhexidine or other surgical scrub soap diluted 3 to 10 fold with water then apply using clean cotton gauze or clean cotton tipped applicators. Rinse with 70% alcohol. Repeat both steps two additional times.
    Alternatively, if betadine or chlorhexidine solution is used to prepare surgical site, organic debris, skin oils and waxes can be removed with a diluted antibacterial soap and rinsed with 70% alcohol before applying disinfectant solution. Apply Chlorhexidine or Betadine solution and rinse with 70% alcohol and repeat both steps one more time. After final alcohol application has evaporated, a sterile surgical drape should be employed to conserve body heat and prevent contamination in survival surgeries. For rodents, good options include a sterile 4x4 cotton gauze sponge unfolded or an appropriately sized sterile piece of paper drape material with a hole cut to expose the planned incision site.

    e. **ANESTHETIC MONITORING**: Animals should be maintained under a surgical plane of anesthesia during the operative procedure. Anesthetic monitoring of small rodents includes testing for lack of rear foot reflexes via 'deep toe pinch' before any incision is made, and continual observation of respiratory pattern and responsiveness to manipulations throughout the procedure. Monitor the rodent continually, documenting findings every 15 minutes and note the following:

i. Toe pinch method: The toe pinch method to evaluate depth of anesthesia is useful, but not enough in itself. One must use two fingers and give the toe/foot a good squeeze. If there is no withdrawal reaction, the animal is judged deep enough to commence surgery. Remember that after this has been done, the fingers are not sterile anymore. A sterile gauze pad may be used to protect the sterile gloves. Alternatively, a hemostat may be used to squeeze toe/foot. In this case, one must be careful not to squeeze too hard. Remember that after the hemostat has been used to squeeze toe, it is not sterile anymore and must not be used for surgery.

ii. Respiratory pattern: Surgical plane of anesthesia will cause a distinct regular but slow respiratory rate (RR). The surgeon must evaluate if RR becomes too slow, shallow and/or irregular, the dose of delivered anesthetic needs to be decreased. If the respiration rate increases, there is need for supplemental anesthesia to be administered.

iii. Reaction to surgical manipulations: If the animal makes any kind of move in response to incision or manipulation of organs (*in the absence of response to toe pinch*), surgery must be temporarily stopped, and anesthesia must be supplemented.

6. **SURGEON PREP**: The surgeon will wear a clean lab coat, surgical scrub top and a mask (cap is optional but recommended). The surgeon will perform a hand scrub using antibacterial soap (Chlorhexidine or Betadine preferred) then dry hands with a clean towel. If a sterile gown is to be worn, the hand scrub should occur prior to putting on the gown. After drying hands, sterile gloves are put on. Gloves are to be changed between animals or if contaminated (break in asepsis) or excessively soiled with blood or other fluids intra-operatively.

7. **SURGICAL PROCEDURE DESCRIPTION:** A brief, complete **chronological** description of the surgical procedure(s) to be performed needs to be included in your protocol. Anatomic location and approximate incision length, tissue dissection and manipulation as well as tissue closure procedures should be described.

   a. **TISSUE CLOSURE:** For dorsal or lateral body surfaces, skin may be closed with surgical clips, staples or monofilament sutures: For ventral areas (the animal's underside), skin is to be closed with non-braided (monofilament) skin sutures or with buried SQ suture patterns – no clips or staples. Sterile veterinary skin glue may be used when appropriate. If used, glue application should be limited to margins of the opposed incision edges. The suture gauge used should be proportional to the size of rodent and incision length and skin tension. For mice, suture gauges 4-0, 5-0 or possibly 6-0 suture are generally appropriate. For rats, suture gauge 3-0, 4-0 or 5-0 are generally appropriate. Dermal / Skin sutures require the use of sterile monofilament type suture such as nylon or PDS. For other tissues, synthetics, i.e., Vicryl, Maxon or PDS, are preferred. Chromic gut is not recommended because of excessive inflammatory response during suture absorption. Skin sutures, staples or clips should be removed 7-14 days post-surgery. NOTE: Silk is not a monofilament suture.

8. **ANTIBIOTIC USE:** If the principles of aseptic technique are adhered to there is no need for post-operative prophylactic antibiotic treatment. If the use of antibiotics is requested, the scientific rationale for administering antibiotics must be clearly discussed in the protocol submission.

9. **RECOVERY:** Animals should be placed in a clean cage with fresh bedding. The cage should be placed with ½ of the cage bottom on a thick towel or piece of cardboard and the other ½ of the cage bottom on a thermal supportive device as described above. Animals are to be monitored until able to ambulate normally prior to return to their assigned husbandry rooms.

10. **POST OP MONITORING:** Animals are to be evaluated at least twice daily for the first 72 hours post op to check for signs of incision dehiscence or site infection (i.e., redness, swelling, or discharge). If noted, the PI should request a VR Veterinary Consult unless pre-approved treatments are described in the IACUC approved protocol.

11. **DOCUMENTATION:**

a. **Green Procedural Cage Cards** must be completed and inserted into cage card holder. Green procedural cage cards can be obtained from the animal facility supervisors. Cards must be completed on both sides and include: Name of procedure, date of procedure, responsible contact person with cell and lab phone numbers. Cards are to remain on the cage until all animals in the cage to which the card applies have been euthanized.
b. **PI Research Records** must include surgical and post-operative documentation. Examples of information that should be recorded include anesthetic and analgesic dosage administration, anesthetic monitoring documentation, surgery start and end time, brief description of surgical procedures, time of animal recovery, post-operative observation, and summary of complications. The IACUC recommends that procedural and animal observation records be placed in a centralized location. Surgical and post-operative templates can be obtained from the OAWA Website. These records must be retained for 3 years post protocol expiration.

Please contact a Veterinary Resources Veterinarian (Staff Directory) for questions relative to aseptic technique, anesthesia, or analgesics.