TITLE: Rodent Surgery and Instrument Sterilization

PURPOSE: To standardize approved methods for rodent surgeries, including instrument sterilization.

REVIEW/REVISIONS: The IACUC will review and revise this guidance as needed.

Instrument sterilization for rodent surgeries

1. All instruments used in survival rodent surgeries must be steam or gas sterilized (this can be done by UAC BSS for a fee) prior to each group of surgeries (biological indicator monitoring of sterilizer effectiveness is advisable).
2. Instruments must be kept on sterile non-porous drapes during use.
3. Instruments must be cleaned of blood and debris by brushing or wiping with sterile water or saline and sterile gauze sponges between surgeries. Instruments must be placed in a chemical agent or a glass bead sterilizer for the appropriate period of time for the method used to be effective (or the pack replaced by a new one).
   a. Chemical agents include: Cidex OPA, phenols, 2% glutaradehyde, SporexII, Sterilox, and chlorine dioxide.
      i. Note that glutaradehyde is mutagenic and phenols are corrosive and both require ORCBS approval and special disposal procedures.
      ii. Chlorine dioxide has a short useful life for sterilization (1 day) and is corrosive to metals.
   b. The effectiveness of chemical sterilizing agents is dependent upon following manufacturer’s recommendations.
   c. If a chemical agent is used, instruments must be rinsed with sterile water or saline before being used on the next animal.
4. Surgical gloves and blades should be changed between each animal or after becoming contaminated.
5. Following surgery, all instruments must be thoroughly cleaned and preferably placed in an ultrasonic cleaner and rinsed.

Procedures for rodent surgeries

1. Surgical Area: There must be a dedicated and separate area for surgery (a surgical suite is not required) and for the preparation of the animal for surgery. This will decrease contamination of the surgical area.
   a. Remove all extraneous equipment and other materials.
   b. Disinfect (10% bleach solution, chlorhexidine, Clidox, MB-10) and place clean towel or surgery tray to cover the work surface.
   c. Place a heating source under the work surface if the procedures lasts more than 20 minutes or for procedures which open a body cavity (i.e. thoracotomy, laparotomy). Use the lowest setting to assure the animal is not overheated.
   d. Prepare the area, anesthetic devices, and appropriate equipment before unwrapping instruments and putting on gloves.
   e. Instruments must be kept on sterile non-porous drapes during use.

2. Animal Preparation: Performed in a separate area away from the surgical area. Prep includes anesthetic induction, hair clipping and initial scrub.
   a. Evaluate intended animals to ensure they are apparently healthy.
   b. Do NOT withhold food in rodents before surgery unless specifically mandated by the protocol or the surgical procedure. Water must NOT be withheld unless required by the protocol, and never more than 6 hours, unless warranted by the Veterinarian or with IACUC approval.
   c. Induce anesthesia, per approved IACUC protocol, and assure adequate depth of anesthesia by using the toe
pinch method.
d. Apply a bland sterile ophthalmic ointment to the eyes to prevent drying.
e. Remove hair from the surgical site (Electric clippers or depilatory cream may be used). Shave an area at least 2-3 cm beyond the intended surgical incision site.
f. Put on fresh gloves and scrub the shaved skin with a gauze pad soaked with povidone iodine or betadine solution, starting in the center and working in concentric circles towards the outer edge of the shaved area. Dispose of that gauze pad and repeat concentric circles with a gauze pad soaked with 70% isopropyl alcohol. Repeat this for 2 more 'scrubs', leaving the final (third) gauze pad soaked with povidone or betadine solution covering the intended surgical area.
g. Move the animal to the surgical area.

3. Pre-Surgery/Anesthesia: Place the animal on a clean absorbent pad, over the heating source, if appropriate.
a. Position animal with tape, taking care not to overstretch or bind limbs in a manner that alters circulation.
b. Repeat one final scrubs with the providone or betadine solution followed by 70% isopropyl alcohol.
c. Cover the animal, if appropriate, with a sterile (recommended) drape with a fenestration (opening) over the proposed incision site.
d. Anesthesia must be monitored throughout the surgical procedures (a minimum of every 15 minutes) to assure the appropriate depth of anesthesia is maintained.
   i. Toe Pinch: Using two fingers, give the toe/foot a good squeeze. If there is no withdrawal reaction, the anesthesia is judged deep enough to commence surgery. A sterile gauze pad may be placed on the rodent’s foot to protect sterile gloved hands, if not, gloves must be changed following the toe pinch.
   ii. Respiratory Rate (RR) and Pattern: Anesthesia causes a distinct slowing of the RR. The RR can then be evaluated and if too slow, the anesthesia can be lightened. If the depth of the respiration becomes too shallow or the RR increases, the anesthesia can be supplemented.
   iii. Mucous Membranes (MM): MM is evaluated by the color of gums, the pinnea (ears) and the toes. If these become bluish, this is an emergency indicating the animal does not have enough oxygen. The MM should be pink. Red means the animal is probably too warm, which can occur during recovery if a heating lamp is placed too close.
   iv. Reaction to Surgical Manipulation: If the animal moves in response to the incision or movement of organs or tissues, then the surgery must be stopped temporarily, the anesthesia supplemented, and the animal reevaluated to assure the depth of anesthesia is again achieved.

4. Surgeon: The surgeon must assure proper cleanliness and attire.
a. Clean lab coat or scrub top is recommended. All jewelry is removed (hands and wrists).
b. Wear face mask and hair bonnet or cap for all procedures.
c. Wash and scrub hands with disinfectant soap or surgical scrub bush. Dry hand with a clean towel.
d. Wear sterile gloves. It is acceptable to use clean nitrile gloves disinfected with Clidox and then rinsed thoroughly with sterile wear or sterile saline solution, although sterile surgical gloves are preferred.
e. Change gloves between animals or if they become contaminated during the surgery.

5. Surgical Procedures: Check level/depth of anesthesia frequently (methods given above #3).
a. Assure necessary instrumentation is set up and prepared.
b. Make incision using new clean blade. Perform procedure as approved in the IACUC protocol.
c. If performing more than one surgery:
   i. Use new sterile pack (preferred), OR
   ii. Clean instruments with saline or distilled water and chemically sterilize (see above) or sterilize in a bead sterilizer (preferred method).
iii. Clean gloves with disinfectant or change gloves (preferred).
iv. If a known contamination has occurred, the instrument should not be reused before resterilization and
gloves should be changed.

6. Post Surgical Care: Post operative pain must be addressed, as described in the IACUC approved protocol.
   a. Animals must be recovered in a clean separate cage. The environment should be warmed.
      i. This can be provided by a warming pad under a portion, but not all, of the cage. This allows the animals
to move out of the warmth if needed.
      ii. Other methods of heat include a warm water bottle or saline bag or a heat lamp.
      iii. If a heat lamp is used, avoid direct contact with heating element and use the lowest level of heat that is
          acceptable.
   b. Warmed fluid replacements should be given for prolonged or invasive surgeries.
      i. Warmed balanced electrolyte solution given intraperitoneally (IP) or subcutaneously (SC). 0.5-1.0 ml to
         mice or 3-5ml to rats. Larger rodents may have an indwelling IV catheter placed if necessary.
      ii. Fluids may be given to improve hydration: oral hydration gels are ideal.
   c. Monitor color of pinnae or footpad, respiration.
   d. Administer analgesics or other drugs as stipulated in protocol or approved by Veterinarian.
      i. Repeat as necessary.
      ii. Maintain a record of administration of all drugs, including animal information, dosage, and time.
   e. Monitor daily: appetite, wound healing, energy/normal movement. Repeat Analgesics as necessary.
   f. Consult Veterinarian if complications arise.
   g. Remove skin closure materials 10-14 days post surgery (record removal in lab records).

Records

Records should be kept for each surgical procedure, anesthesia, pre- and post-operative care, route, time, and dosage of
analgesics and antibiotics, and removal of sutures. The animal or cage identifier should be recorded with each entry.
Records of rodent surgery are not required, but are recommended. Record of analgesia given is required. All record
notations should be signed/initialed and dated.

PROVISO:

Exceptions to this policy must be specifically justified as an essential component of the research protocol and
reviewed/approved by the IACUC. Any questions regarding this policy should be directed to University Animal Care
Surgery Section at 626-7304.

JUSTIFICATION:

Both PHS policy and AAALAC require that rodent surgeries are performed using aseptic procedures (aseptic techniques,
gloves, caps, masks and sterile instruments and preferably a scrub top) as well as separate surgical and animal prep
areas. It often is not possible to have a fresh pack of sterile instruments available for repetitive rodent surgeries; thus,
resterilization or decontamination procedures must be available. It should be noted that agents such as zepharin,
Nolvasan® (chlorhexidine) and Betadine (povidone iodine) are disinfectants not sterilants and therefore inappropriate
for solitary use.

Available methods of sterilization include steam, gas, glass bead, and chemical agents. Chemical agents include Cidex
OPA, phenols, 2% glutaradehyde, Sporexll, Sterilox, and chlorine dioxide. Glutaradehyde is mutagenic, phenols are
corrosive and both require special disposal procedures. Chlorine dioxide has a short sterilizing useful life (1 day) and is corrosive to metals. All agents require rinsing with sterile solutions prior to tissue contact. Glass bead sterilizers will sterilize only the portion of the instrument placed in the beads.