POLICY ON ASEPTIC RECOVERY SURGERY ON RODENTS AND BIRDS

Adopted by the University Committee on Animal Resources – Update and Approved 3-27-20

The ILAR Guide for the Care and Use of Laboratory Animals states that "In general, unless an exception is specifically justified as an essential component of the research protocol and approved by the IACUC, aseptic surgery should be conducted in dedicated facilities or spaces. When determining the appropriate site for conducting a surgical procedure (either a dedicated operating room/suite or an area that simply provides separation from other activities), the choice may depend on species, the nature of the procedure (major, minor or emergency), and the potential for physical impairment or postoperative complications, such as infection" (1). As required by the United States Public Health Service (PHS), United States Department of Agriculture (USDA) and the University Committee on Animal Resources (UCAR), all vertebrate animal-use protocols, regardless of the funding source, must comply with the guidelines stated in the Guide and Animal Welfare regulations.

The "tips-only" surgery technique is a modified approach to rodent surgery that is especially useful for multiple-surgery sessions. This technique allows the surgeon to wear non-sterile exam gloves because it relies on the surgeon's ability to only use the sterile tips of the instruments for all surgical manipulations without touching the animal. While the "tips only" technique does not strictly meet the Guide's requirements specific to use of sterile gloves, NIH has supported this approach for rodent aseptic recovery surgery. Investigators must identify the intent to use the "tips only" technique in the protocol. The "tips only" approach requires attention to detail and must fulfill the requirements for this approach below.

For USDA regulated rodents (e.g. hamsters, gerbils, guinea pigs, mole rats and voles), a new set of sterile surgical gloves and sterile instruments must be used for each animal. The "tips-only" surgery technique is not applicable to these species.

Investigators who feel that their vertebrate animal experiments require exceptions to the guidelines should contact UCAR for assistance. Otherwise, investigators will be expected to follow:

1. Surgery must be conducted on a clean, uncluttered lab bench or table surface. Wipe the surface with a disinfectant before and after use and/or cover with a clean drape.

2. Remove hair or feathers from the surgical site with clippers or a medical depilatory or by plucking. Disinfect the surgical site with at least a two-minute total contact time using the following two-step process:
    a. Gross contamination should be removed by using a surgical scrub at the surgical site (chlorhexidine or povidone iodine scrub and solution).
    b. The surgical site should then be treated with 70% ethyl alcohol, povidone iodine solution or chlorhexidine solution (2).

3. Apply bland ophthalmic ointment to eyes to prevent corneal drying.

4. A sterile drape is recommended to avoid sterile instruments, sterile gloves or exposed viscera from coming in contact with unprepped areas. The Press'n Seal Cling film can be used as drape material for up to 28 days after the box has been opened. Before each use, discard the first 25 centimeters of the roll as this is where the greatest contamination may occur (see figure 1.). The drape may then be cut aseptically by the surgeon, or non-aseptically if the edges that were touched with nonsterile gloves are outside the sterile work area. In the reference section, there are YouTube clips demonstrating the draping techniques.

5. The temperature in the surgery room should be increased and/or the animal placed on a covered warming device (e.g. circulating warm water blanket, warm water bottle, slide warmer or chemical hand warmer) to prevent hypothermia. The use of heating pads is prohibited due to the potential for thermal burns.

   All instruments must be sterilized **for both standard and "tips only" aseptic technique**, but the method of choice may vary depending upon the surgical instruments or devices used. Acceptable sterilization techniques include autoclaving using steam under pressure or cold sterilization. Approved cold sterilization methods include: soaking instruments in 2.5-3.5% glutaraldehyde (e.g. Cidex Plus for 10 hrs. at 20-25° C) or 7.5% hydrogen peroxide (e.g. Sporox Sterilizing and Disinfection Solution for 6 hours at 20° C) according to manufacturer's instructions and safe work practices. (3) U.S. Food and Drug Administration, (March 2009) FDA-Cleared Sterilants and High Level Disinfectants with General Claims for Processing Reusable Medical and Dental Devices.
   http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/ReprocessingofReusableMedicalDevices/ucm437347.htm.

6. The surgeon should wash his/her hands with an antiseptic surgical scrub preparation and then aseptically put on sterile gloves. If working alone, the surgeon should have the animal anesthetized and positioned and have the first layer of the double-wrapped instrument pack or any individually wrapped items opened before donning sterile gloves.
    a. Use of the tips only technique (for non USDA regulated rodents) does not require the use of sterile gloves; however, the surgeon should still surgically scrub his or her hands prior to use of exam gloves. The tips only technique allows the surgeon to anesthetize and position the animal between surgeries.

8. The surgeon must wear a face mask, sterile gloves and a clean lab coat. A cap and sterile gown are recommended, but not required as part of the surgeon's attire.
    a. Sterile gloves are not required for the tips only aseptic technique. A sterile field must be prepared on which to place instruments.

9. Surgery performed on multiple rodents and birds in a series presents special challenges. After the first surgery, the sterilized instruments may be kept in a sterile tray containing 70 – 90% ethyl or isopropyl alcohol (4) for (contact time for instruments is 2 minutes and finger tips is 30 seconds) and no more than a total of five rodents (5). The alcohol must be replaced when contaminated with blood or other body fluids. Alternatively, a glass bead sterilizer can be used. It is important to remove any gross debris prior to placement of instruments in the sterilizer as well as allowing the instruments to cool sufficiently prior to reuse.

    Sterile gloves should be changed between surgeries if the surgeon touches nonsterile surfaces; alternatively, surgeons may wipe their sterile gloves for 30 seconds with sterile gauze pads soaked in 70 – 90% ethyl or isopropyl alcohol (4) or nonsterile surfaces may be handled aseptically with sterile gauze pads.

    a. TIPS ONLY (for non USDA regulated rodents) – Only handle instruments by the handles, and do not allow the tips of instruments to touch non-sterile surfaces. Sutures, catheters, and other sterile materials to be used in the surgery must only be handled with the instrument tips. Tissues must only be touched with instrument tips.
        i. Instrument tips must be sterilized between surgeries utilizing the same techniques described in #9 for standard aseptic technique.

10. Monitoring of anesthesia in rodents and birds may be accomplished by observation of color, respiratory rate and pattern, body temperature and observation for the loss of pedal, corneal and pinnal (external ear) reflexes. More sophisticated methods of patient monitoring include EKG and heart rate, pulse oximetry, blood pressure measurements, blood gas measurements, etc.

11. The abdominal or thoracic body wall should be closed with absorbable suture material in a simple interrupted pattern. The skin must be closed with staples or with a nonabsorbable suture material in a simple interrupted pattern or absorbable sutures in a simple interrupted <u>subcuticular</u> pattern. Avoid using braided nonabsorbable material (silk) to close skin or muscle as it has the tendency to wick bacteria into skin and muscle causing an inflammatory response. Absorbable sutures placed in a subcuticular pattern to close the skin need not be removed postoperatively since they are buried under the skin. All other skin sutures or staples must be removed seven to ten days after surgery.

    a. When using the tips only technique, it is important to only handle suture with the tips of the surgical instruments.

12. Rodents and birds should be recovered from anesthesia in a warmed environment. Warm fluids (lactated Ringer's or normal saline solutions) may be administered subcutaneously to improve postoperative hydration and enhance recovery (rats: 5 – 10 mls, mice: 1 – 3 mls and birds: 0.5 ml of 50% PlasmaLyte/50% D_5W given subcutaneously or warm LRS 10-15 ml/kg and up to 25 ml/kg if over a 5-7 minute period, SQ). Antibiotics should not be given routinely after surgery unless justified by the investigator and DCM Veterinary staff.

    <u>Post procedural or anesthetized animals may not be left unattended or returned to housing</u> until their righting reflex has returned and they are sternal with pink mucous membranes and stable respirations.

13. Monitoring of autoclave equipment –-Heat sensitive chemical indicators must be used to verify that surgical instruments and other materials are appropriately sterilized. Investigators must use one autoclave integrator strip

in each pack to be autoclaved. The strip should be placed in a location considered to be the hardest for the steam to reach. Autoclave tape must be placed on the pack surface. Contact DCM for more information about these methods.

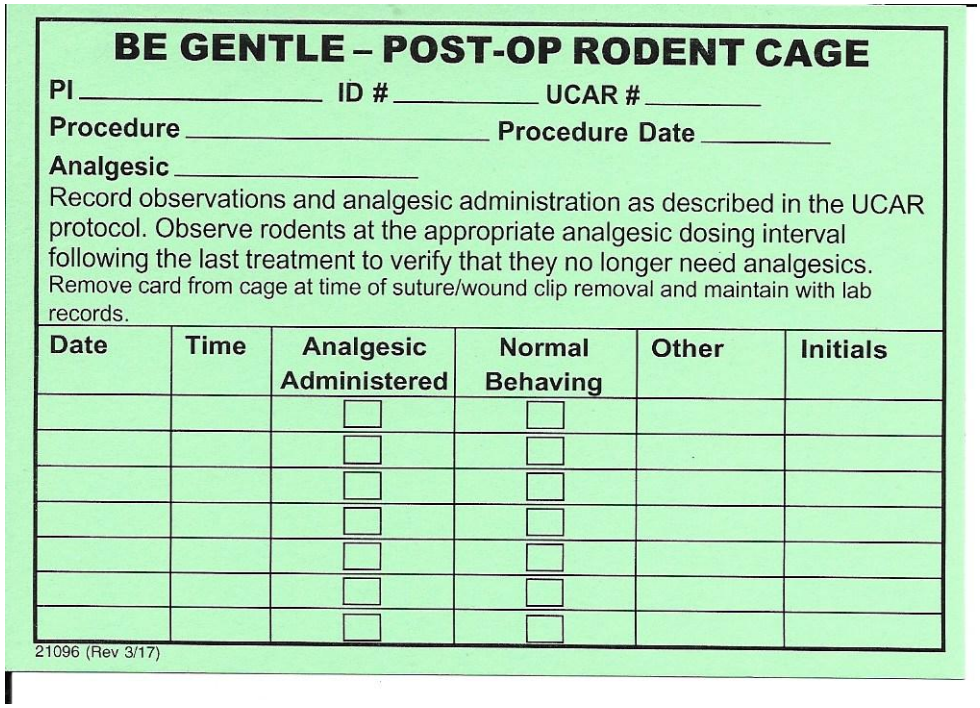
14. Systemic analgesics must be given to all species experiencing recovery surgical procedures as well as those undergoing minor procedures that may result in post-op discomfort. Analgesics must be administered prior to the surgical manipulation and must continue for 72 hours from the first pre-surgical administration of analgesic* for major invasive surgery, or longer if the animal demonstrates continued signs of pain. Analgesics must be given at the dosing frequency stated in the UCAR protocol**. The decision to discontinue analgesic therapy should be made based on the observation that the animal does not appear painful at the end of the previous dosing interval (when the next analgesic treatment is due).

    DCM can with advanced notice administer Buprenorphine SR pre-emptively which lasts for 72 hours. Buprenorphine HCL given pre-emptive and is followed by retreatment at least every 4 hours in mice and every 4 hours in rats (surgery day counts as day zero**.)*

    ***In the event that the analgesic described in the UCAR protocol is unavailable, please contact a DCM veterinarian so they may provide a satisfactory substitute and oversight of the change in analgesic therapy.*

15. All rodents and birds must be observed at least daily for 3 days post-surgery for signs of pain or distress. Surgeons must use the green "Be Gentle-Post-Op "cage cards to identify post-surgical animals in the vivarium. The cards must contain all information (PI name, procedure/date, observations, analgesics etc). Entries should be initialed by the person who made the observation and/or administered the analgesics. Date and time of entry must be recorded chronologically and with a specific time (i.e., 8 am; 12 pm or use military time). If an investigator has scientifically justified that analgesics cannot be used pre and post-operatively, it should be noted on the green post-op cards.

    Remove cards when sutures/wound clips are taken out or when the wound has healed. Keep the completed cards with your lab notes for approximately 1 year.



BE GENTLE – POST-OP RODENT CAGE

PI _____ **ID #** _____ **UCAR #** _____

Procedure _____ **Procedure Date** _____

Analgesic _____

Record observations and analgesic administration as described in the UCAR protocol. Observe rodents at the appropriate analgesic dosing interval following the last treatment to verify that they no longer need analgesics.

Remove card from cage at time of suture/wound clip removal and maintain with lab records.

Date	Time	Analgesic Administered	Normal Behaving	Other	Initials
		☐	☐		
		☐	☐		
		☐	☐		
		☐	☐		
		☐	☐		
		☐	☐		
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Pain in rodents and birds may be identified by observing the animal's reluctance to move about, decreased appetite and/or water consumption, weight loss, listlessness, salivation, hunched posture, favoring of the affected body part,

piloerection (rodents), ruffled feathers (birds), increased respiration, respiratory sounds (chattering in mice), vocalization with handling and/or self-mutilation. Please review the Sedation/Tranquilization, Anesthesia and Analgesia in Laboratory Animals and Veterinarian-Recommended Formularies or contact a DCM veterinarian (X5-2653).

References:

1. U.S. Dept. of Health and Human Services, Public Health Service, National Institutes of Health, (2010) Guide for the Care and Use of Laboratory Animals. Washington D.C.: National Academy Press.

2. AAALAC, From AAALAC's Perspective…Using Alcohol as a Disinfectant. AAALAC Connection Newsletter. 2001 Winter/Spring. http://www.aaalac.org/connection_4wsp2001.htm.

3. U.S. Food and Drug Administration, (March 2009) FDA-Cleared Sterilants and High Level Disinfectants with General Claims for Processing Reusable Medical and Dental Devices. http://www.fda.gov/cdrh/ode/germlab.html

4. Block S.S., (1983) Disinfection, Sterilization and Preservation, 3rd. Ed, Philadelphia: Lea & Febiger.

5. Keen, J., The Efficacy of 70% Isopropyl Alcohol Soaking on Aerobic Bacterial Decontamination of Surgical Instruments and Gloves in Serial Mouse Laparotomies, accepted May 2010 for publication in J Am Assoc Lab Anim Sci

6. National Institutes of Health. **Guidelines for Survival Rodent Surgery.** **http://oacu.od.nih.gov/ARAC/documents/Rodent_Surgery.pdf**

7. AAALAC, Inc. Guidelines for the Assessment and Management of Pain in Rodents and Rabbits. http://www.aclam.org/Content/files/files/Public/Active/position_pain-rodent-rabbit.pdf

8. Emmer, Kathryn M., et al. "Evaluation of the Sterility of Press'n Seal Cling Film for Use in Rodent Surgery." *Journal of the American Association for Laboratory Animal Science* 58.2 (2019): 235-239.

9. http://research.utsa.edu/research-funding/laboratory-animal-resources-center/training/

10. "Draping the Table & Animal for Rodent Surgery with Press'n Seal with Non-Sterile Gloves" https://youtu.be/vgl3nn4DOlE

11. "Removing a Surgical Drape Sheet from a New/Unopened Press'n Seal Box" https://www.youtube.com/watch?v=2jeHX25tegA

12. "Removing a Drape Piece from an Already Opened Press'n Seal Box" https://www.youtube.com/watch?v=3WEgxlfXh74

13. "Cutting Press'n Seal Surgical Drape Over the Animal" https://youtu.be/09nMBxINra4

Figure 1.