Performing Rodent Survival Surgery

Policy: Survival surgical procedures in rodents require aseptic techniques appropriate for the procedure being performed, including an appropriately prepared, dedicated surgical area, sterile instruments, and both animal and surgeon preparation. Research personnel performing surgery must be appropriately trained and qualified. The Comparative Medicine Research Unit (CMRU) veterinary staff offers hands-on rodent surgery courses, which individuals can sign up for through the UCAW website. Anesthesia, analgesia, and perioperative monitoring must be appropriate for the species and procedures performed and must be recorded. All anesthetic, peri-operative, and post-operative records must be retained for 3 years following the procedure and available for review by the UCAW, CMRU veterinary staff, and external regulatory agencies. All anesthetics and analgesics **must** be administered as described in the approved UCAW *Proposal*. Note: all newly received rodent species must be acclimated in accordance with UCAW policy prior to use (*see:* "Acclimation Periods for Newly Received and Transferred Animals").

Rationale: According to the Animal Welfare Act and the *Guide for the Care and Use of Laboratory Animals* ("*Guide"*), a dedicated facility is not required for rodent survival surgery. However, a dedicated space appropriately managed to minimize contamination from other laboratory activities during surgery must be provided. Surgical procedures must be performed using aseptic techniques. Regulations further state that personnel conducting surgical procedures must be appropriately qualified and trained. In addition to outlining the facility, training, and procedural requirements, regulations/policies also provide definitions for major and minor surgical procedures. The *Guide*[1] defines major survival surgery as any surgical intervention that "penetrates or exposes a body cavity or produces substantial impairment of physical or physiological functions (such as laparotomy, thoracotomy, craniotomy, joint replacement, and limb amputation)." The Animal Welfare Act similarly[2] defines a major operative procedure as "any surgical intervention that penetrates and exposes a body cavity or any procedure which produces permanent impairment of physical or physiological functions." Minor surgeries include procedures such as skin biopsies or vascular catheterization/cannulation that do not result in permanent impairment of physical or physiological functions. Laparoscopic procedures may be classified as major or minor depending on their impact on the animal[1]

Procedures, Guidelines, and Exceptions:

[***Note***: "Tables" refers to the document entitled ***Recommended Surgical Disinfectants, Sterilants, and Suture Materials***.]

1. Dedicated Surgical Area

A specific area within the laboratory must be identified as the site in which rodent survival surgery is performed; other laboratory functions may not be conducted in this area during the time of surgery. The surgical area must be impervious such that it can be easily cleaned and sanitized *(see* ***Table 1****)*. The area should be returned to a level of hygiene acceptable for major survival surgery prior to a surgical session. The animal preparation site should be separate from the surgery area to avoid contamination of the surgical field with hair.

2. **Aseptic Techniques** – All tables below reference the UCAW document "**Recommended Disinfectants, Surgical Sterilants, and Suture Materials**."

   a. *Animal Preparation*
   The surgical site and a wide surrounding area should be clipped (#40 blade or smaller for rodents) and prepared for aseptic surgery in standard fashion. To avoid contamination of the surgical area, hair removal should be done at another location, and the loose hair should be vacuumed or otherwise removed from the environment (tape is a practical and effective alternative for small amounts of hair). Depilatory creams are not generally recommended; their use should be described in the UCAW *Proposal*. If used, great care must be taken to ensure complete removal to avoid excessive skin irritation.

   The surgical site should be prepared by swabbing in a circular fashion from the center (incision site) to the periphery without returning the applicator to the center. Nolvasan® (Chlorhexidine) or Betadine® (Povidone iodine) scrub are recommended antimicrobial solutions for surgical skin preparation *(see **Table 2**)*. The scrub solution should then be removed with sterile water, sterile saline, or alcohol. This should be repeated at least three times. While some recommend a final application of an antimicrobial (chlorhexidine or povidone iodine) *solution*, it is important to completely remove all *scrub* (which contains detergents) from the incision site. Use alcohol cautiously; excess volume can reduce body temperature and increase hazards associated with electrocautery.

   A non-medicated ophthalmic ointment should be placed in the anesthetized animal's eyes to prevent drying. In prolonged or very invasive surgeries, warm sterile saline or Lactated Ringers Solution (LRS) should be administered subcutaneously to prevent dehydration (1-2 ml in mice and 5-7 ml in rats).

   b. *Surgeon Preparation*
   A clean laboratory coat or scrubs, surgical mask, and hair bonnet/cap should be donned prior to scrubbing hands with an antimicrobial soap. If surgical procedures are performed within a HEPA-filtered cabinet (*e.g.,* Biological Safety Cabinet), then caps and masks may be exempted. Sterile gloves must be worn during surgery; the use of non-sterile gloves may be acceptable if "tips only" techniques are employed.

   Following preparation, the surgical site should be protected from contamination by the use of sterile surgical drapes with an opening over the proposed incision site. Anesthetized animals should be positioned for surgery such that the surgical site is readily accessible and yet the position does not interfere with the animal's ability to breathe. Please note: Glad Press'n Seal cling wrap is a permissible draping option if used as described in reference 7. If your lab wishes to use any other similar product, please reach out to one of the CMRU faculty veterinarians (https://louisville.edu/research/cmru/contact-us) to discuss its use and to have the sterility of these products evaluated by means similar to those in reference 7. Other cling wrap/food products drapes are not permissible without having sterility testing performed prior to use.

c. *Sterile Instruments and Surgical Packs*
In general, surgical instruments must be clean and sterile *(see **Table 3**)*. Autoclave sterilization of surgical packs, including instruments, drapes, and gauze sponges is preferred (3). Sterilization indicators should be used to identify material that has undergone proper sterilization. Absorbent gauze sponges may be sterilized by autoclave or can be purchased from the CMRU in pre-sterilized packets. Instruments or equipment that cannot be treated by autoclave or chemical agents may be sterilized by Ethylene Oxide (ETO). The Research Resources Center, Clinical Translational Research, and Baxter II Vivaria are equipped to perform ETO sterilization. Additional methods of sterilization include the use of dry heat or radiation.

Clean instruments may also be sterilized by a number of chemical agents (*e.g.,* Clidox®, Sterad®), which may be available from the CMRU. Sterilization by chemical means often takes several hours of exposure to the sterilant, so care must be taken to read the label to ensure proper use of the particular agent. When instruments sterilized by chemical agents are used to perform surgery, care must be taken to rinse instruments thoroughly with sterile saline or water to prevent chemical-tissue interaction, since many agents are toxic to animal tissues. *Alcohol is not a sterilant or a disinfectant and may not be used to disinfect instruments or gloves without specific UCAW approval (4).*

d. *"Tips-Only" Techniques*
Some surgical procedures require very small incisions and little tissue handling. In these cases, when only the tips of instruments will touch the surgical site, "tips only" technique may be appropriate. Surgical skin preparation and clear delineation of the sterile field, e.g., with sterile surgical drapes, are paramount to success using this technique. With attention to detail and handling of the instrument tips, the use of sterile gloves is unnecessary. It may also be acceptable to sterilize only the sections of the disinfected surgical instruments *(see **Table 4**)* that will touch the animal or animal's tissue. In these cases, glass bead sterilizers are particularly useful. Glass bead sterilizers have been found to sterilize instruments in 10 seconds (5) and can also be used to help maintain instrument sterility when performing repetitive surgical procedures in rodents. Allow the instrument to cool after using a glass bead sterilizer prior to its use in animals. Instruments must be autoclaved prior to tips-only technique.

When using "tips only" techniques, surgical instruments must be touched only on the handles. The sterile tips must not be allowed to touch anything that is not sterile, including non-sterile drapes, non-sterile gloves, or animal skin that is not surgically prepared. Conversely, the animal skin that has been surgically prepared must not be touched with non-sterile gloves or instrument sections. Similarly, sutures, catheters, and other sterilized materials must only be handled via the sterilized instrument tips.

e. *Maintaining the Surgical Field*
The surgical field, gloves and instruments must be protected from contamination during surgery. Any break in aseptic technique should be immediately rectified. Corrective measures include: 1) the exchange of soiled instruments, gloves, *etc*. for those which are

sterile, 2) the flushing of contaminated tissue with sterile physiologic saline, and/or 3) the use of antibiotics or other prophylactic methods. In general, *antibiotics should not be required for procedures of short duration with proper tissue handling and proper attention to aseptic technique.* Should antibiotic use be indicated, consultation with CMRU veterinarians is encouraged.

f. *Repetitive Surgical Procedures*
Performing the same procedure on a number of animals presents special challenges to maintaining sterile instruments and aseptic procedures. As mentioned above, glass bead sterilizers can be used to rapidly sterilize the ends of instruments between animals as long as the instruments are first autoclaved and all visible organic matter is removed prior to glass bead sterilization. **Glass bead sterilizers must not be used for more than 5animals, and only once if intra-gastric or bowel procedures are performed.** Segregating instruments during use can also help minimize infection. Instruments can be segregated based on use on a disinfected surface, such as the skin, or use in a sterile area, such as instruments used within internal body cavities (6). Performing serial procedures requires greater attention to the sterile field. Sterilized instruments or gloves must only touch sterile drapes or incision sites prepared for surgery. Touching with non-sterile instruments or gloves renders an area or site non-sterile. When in doubt, re-sterilize.

g. *Suture Materials/Methods*
Sterile suture materials must be used for rodent survival surgery *(see* ***Table 4****)*. Prepackaged sterile sutures with needles attached are advisable and may be available from the CMRU. Technical assistance with the selection of suture type, size, and placement method is also available. Skin incisions should be closed with well-placed clips/staples or monofilament sutures in a simple interrupted pattern; suture glue may also be used. Incisions associated with the implantation of cranial electrodes should be additionally coated with an antibiotic ointment and may require daily debridement. *Skin sutures or clips/staples must be removed when the wounds have healed*. Usually, this is between 7-10 days, although up to 14 days may be necessary for some procedures. The Project Director is responsible for removing skin suture/staples (*i.e.,* not the CMRU staff).

3. Anesthesia and Anesthetic Monitoring

Anesthesia & anesthetic monitoring and recordkeeping requirements can be found in the UCAW Policy "Rodent Anesthesia." Broadly, an appropriate anesthetic plane must be achieved, maintained, and documented throughout surgery. For information on the dosing and usage of specific anesthetics and analgesics, please see ***Recommended Rodent Anesthetics and Analgesics***. CMRU veterinarians should be consulted for questions regarding rodent anesthesia. Thermal support should be provided to rodents anesthetized for surgery.

4. Postoperative Treatment and Care

The Project Director or a trained member of his/her research staff has the primary responsibility for monitoring animals during the immediate post-operative period. Animals must be closely observed while recovering from anesthesia, and recovery should occur in a warm cage. Recovery is generally defined as the ability to ambulate and/or maintain normal posture. Following recovery, animals may be returned to regular housing. A plan for post-operative treatment must be stated in the approved UCAW *Proposal.* Post-operative analgesia, as well as any study-specific post-operative regimens, such as specific wound care and indwelling catheter maintenance, must also be described in the approved UCAW *Proposal* and include drug(s) to be used as well as dose and frequency of administration (*see:* UCAW Policy, "Use of Postoperative Analgesics" and ***Recommended Rodent Anesthetics and Analgesics***). The individual(s) responsible for the administration of post-operative therapy and recordkeeping must also be stated and provisions for the use of analgesics must be included in all treatment plans.

5. **Peri-operative Recordkeeping**

To assist in the oversight process, *the date(s) of surgical procedures must be indicated on rodent cage cards*. This will not only serve to remind research staff when suture/clip removal is necessary but also allow monitoring and reporting by the CMRU Animal Husbandry Staff. Cage card notation may simply be an inscription such as "Sx – XX/XX/XX," or may be an indication of the procedure itself (*e.g*., "Appendectomy – XX/XX/XX"). Research personnel must also place a yellow cellophane card over the rodent cage card following surgery and should remove the yellow cards 10-14 days after surgery or when sutures are removed. This marking serves to remind research staff that suture/clip removal may be necessary and also improves health monitoring by CMRU husbandry and veterinary staff.

Survival surgery on all species requires that written documentation exists to verify that the procedures described in the animal use *Proposal* are in use, including anesthetic monitoring and analgesic administration. Dates and times (including AM/PM or military time) of all time-sensitive observations or treatments (post-operative evaluations, pain medication) must be recorded. Such documentation may be recorded in individual animal records or a laboratory notebook. If the notebook is not maintained with the animals, then it must be readily available for review by the UCAW, CMRU veterinary staff, and regulatory agencies. At a minimum, records must include notation of all surgical procedures; surgeon and/or anesthetist names; animal identification; date of surgery; UCAW *Proposal* number and Project Director name; medications administered, including agent, dose, route, and date/time of administration; dates and times of all time-sensitive observations and treatments; notations of any variations from the normal and expected events, including any actions taken and time performed, the animal's response, and the name of the person; all anesthetic record requirements (see UCAW Policy, "Rodent Anesthesia"); and other peri-operative care procedures. All anesthetic, peri-operative, and post-operative records must be retained for 3 years following the procedure and be readily available for review. The UCAW has created ***Rodent Surgery & Anesthesia Record*** templates that can be used or modified as appropriate.

6. **Analgesia and Recognition of Pain** (see UCAW Policy, "Use of Postoperative Analgesia")

Recognition of pain in animals is usually subjective. Knowledge of the signs of distress and pain in individual species is an important part of conducting research, particularly in studies requiring surgery. Surgical procedures that are known to be associated with pain in humans must also be assumed to cause pain in non-human animals. The proper selection and use of analgesics are an important part of postoperative care. Information on pain recognition and products that can be used to alleviate pain is available from the CMRU.

Examples of signs of pain in the mouse are reflex withdrawal, biting response to handling, piloerection, hunched back, sunken eyes and abdomen, dehydration, and weight loss. Key signs of pain in the rat are vocalization, struggling, licking/guarding, weight loss, piloerection, hunched position, and hypothermia. Key signs of pain in the guinea pig are struggling, withdrawal, vocalization, easy restraint or capture, and unresponsiveness. ***Note, however, that masking signs of pain, distress, and clinical illness is one of the most conserved instincts in these "prey species"; therefore, it is paramount to proactively treat post-operative pain*** *(see:* ***Recommended Rodent Anesthetics and Analgesics****)*.

Special Notes:

1) Injectables used during surgical procedures must not be contaminated. Maintaining needles, with or without syringes attached, in rubber stoppered vials is not acceptable. The use of sterile needles and syringes for the parenteral administration of drugs is mandatory. Needles should not be recapped prior to disposal in an approved sharps container.
2) All drugs, fluids, suture material, *etc*. used *or on-hand* for use, must be within the use period allowed by the expiration date indicated on the box or label. ***Note:*** *When combined into a single formulation, the commonly-employed anesthetic combination of Ketamine HC1 (37.5 mg/ml) and Xylazine (5 mg/ml) should be used* ***within 30 days*** *of preparation.*
3) Rodents do not normally actively regurgitate, and hence, preoperative fasting food is not mandatory. If, however, food is withheld, fasting should not exceed 12 hours. Water should not be withheld.
4) Please note that UCAW Policy "Use of Postoperative Analgesia" requires the use of postoperative analgesia for *at least* 48 hours following survival surgery unless specifically exempted within an approved *Proposal*. All analgesics **must** be administered as described in the approved UCAW *Proposal*.
5) A ***Rodent Survival Surgery Checklist*** has been assembled to assist the laboratory in ensuring appropriate aseptic technique and perioperative care.

References:

1) National Research Council, *Guide for the Care and Use of Laboratory Animals*, 8th Ed., National Academy Press, Revised 2011.
2) Animal Welfare Act; 9CFR, Subchapter A, Parts 1,2,3.
3) Bloch, SS, *Disinfection, Sterilization and Preservation*, 3rd Ed. Lea and Febiger, Philadelphia 1983.
4) Rutala, W.A., APIC guidelines for selection and use of disinfectants, *Am. J. Inf. Control* 18(2): 99-117, 1990.
5) Callahan, BM, *et al*. A comparison of four methods for sterilizing surgical instruments for rodent surgery, *Contemp. Top. Lab Anim. Sci*. 34(2): 57-60, 1995.
6) Cunliffe-Beamer, TL, Applying Principles of Aseptic Surgery to Rodents, *Anim. Welfare Info. Ctr. Newsletter*, 4(2): 3-6, 1993.
7) Emmer, KM, et al. Evaluation of the sterility of Press'n Seal cling film for use in rodent surgery, J Am Assoc Lab Anim Sci 58(2):235-239, 2019. doi: 10.30802/AALAS-JAALAS-18-000096.
8) Holdridge, JA, et al. The effectiveness of hot bead sterilization in maintaining sterile surgical instrument tips across sequential mouse surgeries, J AM Assoc Lab Anim Sci 60(6):1-9, 2021. doi: 10.30802/AALAS-JAALAS-21-000047.