Purpose
This is a general Standard Operating Procedure for Aseptic Surgical Technique. The Institutional Animal Care and Use Committee (IACUC) maintains oversight review for federally mandated rules and regulations with regard to animal research and has endorsed this SOP as a summary of survival surgery requirements and procedures at the University of Colorado Boulder. This policy applies to all personnel approved to conduct research utilizing vertebrate animals. References: Animal Welfare Act, PHS Policy on Humane Care & Use of Laboratory Animals, Guide for the Care & Use of Laboratory Animals (8th Edition), and the NIH Guidelines for Rodent Survival Surgery (Version 9/12/12).

Aseptic technique is required by the IACUC for all survival surgeries, unless scientific justification is provided. Aseptic technique is used to reduce microbial contamination to the lowest possible practical level. No procedure, piece of equipment, or germicide alone can achieve that objective. The contribution and importance of each of these techniques will vary with the surgical procedure. The species of animal may also influence the selection of appropriate aseptic techniques used to prevent infection.

General: The following is based on principles described in the Guide for the Care and Use of Laboratory Animals and the NIH Guidelines for Survival Rodent Surgery.

- Appropriate pre-operative and post-operative care of animals in accordance with established veterinary medical and nursing practices are required.
- A dedicated rodent surgical facility is NOT required. However, a designated animal procedure space IS required and at the time of use the aseptic surgery should be conducted in an area which is dedicated to surgery and related activities, and at all times during the surgery managed to minimize contamination. **A dedicated survival surgery location is required for survival surgery on all USDA covered species.**
- All survival surgery will be performed by using aseptic procedures, including masks, sterile gloves (if applicable), sterile instruments, and aseptic techniques.
- The Guide states that it is important for research personnel to be appropriately qualified and trained in all procedures to ensure that good surgical technique is practiced. Good technique includes:
  - Asepsis,
  - Gentle tissue handling,
  - Minimal dissection of tissue,
  - Appropriate use of instruments,
  - Effective hemostasis, and
  - Use of suture materials and patterns or other wound closure techniques that minimize trauma and remain intact.
- Analgesia, preservation of corneal integrity, nutritional support and maintenance of body temperature and hydration should be considered in the surgical plan. The surgical plan should also give consideration to the availability of personnel to provide anesthetic induction and post-operative care appropriate to the surgical procedure.

Procedures: The following is based on a guidance document promoted by the NIH called Guidelines for Survival Rodent Surgery

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A. Personal Protective Equipment
Personnel are to wear the following while conducting rodent survival surgery, to protect both themselves and the rodents.

- Clean lab coat, disposable surgical gown, etc.
  - Note: If you are utilizing the “tips-only” surgical technique described below, it is permissible to wear short sleeve scrubs or a surgical gown during surgery, but ONLY IF you are willing to thoroughly scrub down your arms, wrists, and hands prior to beginning surgery. For sterile surgery technique, you must wear a long sleeve gown or scrubs.
- Mask
- Hair cover
- Gloves (either sterile surgical gloves OR clean exam gloves, see below)
  - Sterile surgical gloves. Using sterile surgical gloves allows you to touch all areas of the sterile surgical field and surgical instruments with your gloved hand.
  - Clean exam gloves. Using clean exam gloves and a “tips-only” technique restricts you to using only the sterile working ends of the surgical instruments to manipulate the surgical field. The gloved, but not sterile, hand must never touch the working end of the instruments, the suture, suture needle, or any part of the surgical field. This technique is useful when working alone and manipulation of non-sterile objects (e.g., anesthesia machines, microscopes, lighting) is required.

B. Pre-Operative:
- Surgery should be conducted in a disinfected, uncluttered area that promotes asepsis during surgery. Disinfect the surgical surface areas prior to beginning surgery.
- Prepare the animal by removing hair from the surgical site. Whenever possible, perform this procedure in an area separate from where the surgery is to be conducted.
- Administer analgesics (preemptive analgesia) as appropriate and approved in your Animal Study Proposal.
- Protect the corneas from drying out by applying an ophthalmic ointment, if the surgery will last longer than five minutes.
- Use surgical techniques to reduce the likelihood of infection. This includes the use of sterile drapes.
- Prepare the surgical site(s) with an appropriate skin disinfectant.
- Surgeons should wash and dry their hands before aseptically donning sterile surgical gloves or clean exam gloves (for tips only technique).

C. Operative:
- The animal must be maintained in a surgical plane of anesthesia throughout the procedure.
  - If using the pedal withdrawal reflex to test depth of anesthesia, the rear paw has been shown to be more reliable than the forepaw.
  - If neuromuscular blocking agents (e.g. pancuronium, succinyl choline) are used, monitoring of autonomic nervous system responses (e.g. heart rate, blood pressure) should be used to monitor anesthetic depth.
- Begin surgery with sterile instruments and handle instruments aseptically.
- When using “tips-only” technique, the sterility of the instrument tips must be maintained throughout the procedure. If using clean exam gloves (not sterile) and performing the “tips-only” technique, you must not touch the surgical field/open surgical site with your glove. If you are touching the animal in a location on the body where you are not
performing surgery (manipulating the animal during surgery), clean exam gloves are sufficient (no need for sterile gloves for this purpose).

- Instruments and gloves may be used for a series of similar surgeries in the same session, provided they are maintained clean and disinfected between animals. For example, instruments can be washed at a sink and then disinfected in a hot bead sterilizer prior to a second surgery (maximum of 5 surgeries, see the next point).
- With Rodent Survival Surgery, it is acceptable to use instruments on successive animals up to five (5) times, provided that the tips of the instruments are sterilized in between animals appropriately. An example of an appropriate method is a bead sterilizer. After use of five different animals, instruments must be FULLY sterilized once again with an autoclave or by some other standard method approved by the IACUC.
- Monitor and maintain the animal's vital signs and hydration.
- Close surgical wounds using appropriate techniques and materials.

D. Post-Operative:
- Move the animal to a warm, dry area and monitor until recovered. Return the animal to its routine housing only after it has recovered from anesthesia (i.e., the animal can maintain itself in sternal recumbency).
- Provide analgesics as appropriate and approved in your Animal Protocol.
- If appropriate, consider giving fluids and/or nutritional support.
- Generally, remove skin closures 7 to 14 days post-operatively after verifying that the wound has healed unless stated otherwise in the protocol and approved by the IACUC.
- Maintain a written surgical record with important operative and post-operative information (e.g., annotate cage card with procedure and date, body weight on the day of surgery, analgesic administration, wound closure removal, etc.). Researchers should maintain a record that suits their needs as well as documents that an animal was observed during recovery on a particular date and time, and notes any variations from normal recovery behavior.
- Continue daily monitoring of the animal until it is stable (e.g., body weight, body condition, activity, etc.).

Modifications in standard aseptic techniques above may be approved by the IACUC if scientifically justified by the investigator in a protocol or addendum. Each Animal Use Protocol proposing the use of non-standard aseptic techniques will be considered and reviewed by the IACUC on a case-by-case basis.

Per regulatory requirements, failure to comply with this policy may result in notification of your funding agency (e.g. NIH) and regulatory agencies (e.g. OLAW, USDA) that your research has violated federal and/or local policies regarding the humane use of animals. This notification may affect continuous funding of your animal-related research. Further, depending on the violation, you may be required to take additional training and/or your privilege to conduct animal research at UC Boulder might be temporarily suspended or even completely revoked.