

SURGERY GUIDELINES – RODENTS

IACUC Guideline

Effective Date: May 2024

Program Scope:

These guidelines apply to all surgical procedures performed on rodents.

Section 1 - Survival Surgery in Rodents

Section 2 - Non-Survival (Terminal/Acute) Surgery in Rodents

Section 1 - SURVIVAL SURGERY

Any surgery conducted on animals that are expected to recover from anesthesia is considered survival surgery.

Refer to the [IACUC Rodent Anesthesia Guidelines](#) for more details about rodent anesthesia. Survival surgery on rodents should be performed using sterile instruments and sutures, clean or sterile surgical gloves, and aseptic technique to reduce the potential for microbial contamination of exposed tissues to the lowest practical level.

Expired anesthetics, analgesics and replacement fluids must never be used. See [IACUC Expired Drugs, Medical Materials & Devices Policy](#).

Pharmaceutical-grade agents (USP) must be used unless an exemption is approved by IACUC. See [IACUC Non-Pharmaceutical Grade Compounds Policy](#).

PROCEDURES:

Pre-Operative:

1. Surgeons must wear a clean lab coat, gown, or scrub top and a mask. A surgical bonnet is also recommended. Sanitize hands before gloving.

Note on gloves: Sterile gloves are required if you will manually manipulate animal tissues, sterile implants, the tips of your sterile instruments, and/or disinfected areas with your hands. If you will only touch the handles of your instruments and practice “tips-only” technique, then clean, non-sterile gloves are acceptable.

2. Surgery should be conducted in an uncluttered, disinfected area. Sanitize the work surface/laboratory bench with sodium hypochlorite (Bleach), chlorhexidine, Clidox, or Vimoba / MB-10 solution before surgery. Alcohol is not a sterilant nor a high-level disinfectant therefore the use of alcohol is inappropriate. Use sterile drapes or clean absorbent pads or towels. Replace these materials after each surgery session or when soiled.

All instruments and implants must be cleaned and sterilized prior to use. Before sterilization, any organic material must be removed from instruments and implants. Preferred sterilization methods are steam autoclave, glass bead sterilizer, ethylene oxide gas, or hydrogen peroxide vapor sterilization. Autoclaved packages should be labeled with date of sterilization and initials and stored in clean dry location to preserve package

integrity to avoid need for re-sterilization. Packs should be visually inspected prior to use to ensure they are intact.

Chemical sterilization may also be used. For the different methods available, consider contact time, chemical sterilant used, and items being sterilized. These agents must be used with appropriate safety precautions, and the items to be sterilized must be compatible with the sterilant. Further, chemically sterilized items must be rinsed with sterile water or saline before any contact with animals.

Examples of common commercial sterilants:

- Cidex: glutaraldehyde (2.4% to 3.4%)
 - Active for 14 to 28 days, depending on formulation, once prepared
 - 10 hours of exposure at 25°C required for sterilization
- Spor-Klenz Ready-To-Use (RTU): peracetic acid/hydrogen peroxide blend
 - Active for up to 14 days.
 - 5.5 hours of exposure at 20°C required for sterilization
- Sporicidin: glutaraldehyde (1.12%) and phenol/phenate (1.93%)
 - Active for up to 14 days once prepared
 - 12 hours of exposure at 25°C required for sterilization

Store sterilized instruments and implants appropriately in sealed packs or similar coverings and label with the date of sterilization. If surgical packs are compromised the contents should be re-sterilized before use. For additional information regarding autoclave-based sterilization, see [FACT SHEET - Non-LARC \(Researcher-owned\) Autoclave Quality Control Measures](#)

3. After the animal is anesthetized, shave/remove fur from the surgical/incision site. You must shave/remove the minimum amount of fur necessary to prevent instruments and other sterile implements from coming into contact with non-disinfected areas. Perform this procedure in a location separate from the surgery area (i.e., a completely separate procedure bench or a segregated area on the same procedure bench).
4. Administer any pre-operative analgesics per the IACUC-approved protocol.
5. Apply non-medicated ophthalmic ointment (eye lubricant) to the animal's eyes to prevent corneal damage.
6. Disinfect the surgical/incision site with dilute chlorhexidine or betadine scrub (avoid solutions). For cranial surgeries, betadine is preferred over chlorhexidine to reduce potential inadvertent chlorhexidine eye exposure and corneal damage. Three alternating scrubs each (six in total) of disinfectant and alcohol (or sterile saline) wash are required. Use a clean applicator for each swab. Alcohol alone is not an appropriate skin disinfectant for surgery.
7. If animal will be relocated, maintain sterility during transport.
8. Place a sterile drape over the non-sterile part of the animal to prevent contamination of instruments and implements from exposed fur and other non-sterile surfaces.

Operative:

1. Provide the animal with a heat source to maintain normal body temperature for the duration of the procedure. Circulating warm water blankets and chemical or specific

temperature-controlled heating platforms are preferred over heating lamps and pads. Always use a towel or drape between the heat source and the animal.

2. Test the depth of anesthesia by performing a paw pinch on both hindlimbs (avoid the toes). If either paw pinch elicits a response, adjust your anesthesia accordingly, and re-test before making your incision.
3. Monitor the animal's vital signs, such as respiratory pattern and skin/mucous membrane color, to assess depth of anesthesia. If the animal responds to stimuli during surgery, stop the procedure until additional anesthetic is administered and a paw pinch test elicits no response.
4. Handle sterile instruments aseptically to minimize contamination. Instrument tips should not touch non-sterile surfaces. A glass bead sterilizer may be used mid-procedure if an instrument becomes contaminated. Clean organic material off of the instruments and then re-sterilize per manufacturer's instructions. Make sure instruments are cool to the touch by rinsing or dipping into a cool sterile water or saline prior to any contact with the animal. Hot instruments are extremely damaging to tissue.
5. Instruments may be used for a series of similar surgeries provided they are cleaned and re-sterilized using a glass bead sterilizer between animals. Gloves should be changed if soiled. A new set/pack of autoclaved sterilized instruments should be used for each cage or up to five animals in the same experimental cohort. Keep sterile instruments on a sterile field when not in use.
6. Close incisions using appropriate techniques and materials. If more than one anatomic layer was incised (e.g., both skin and thoracic/abdominal tissues were cut), a two-layer closure must be performed. The usage of monofilament suture is recommended, absorbable suture for the muscle layer and non-absorbable for the skin suture. Note: multifilament suture (e.g., silk) is not recommended as it elicits more intense tissue inflammatory response. Consult LARC veterinarians regarding the use of surgical glue and appropriate application: it must be applied at the apposing wound edges with a $\leq 27g$ needle and never placed inside the incision/wound.

Post-Operative:

1. Recover the animal according to the [IACUC Rodent Anesthesia Guidelines](#).
2. Place Surgery Green Tag/Card on cage. Enter dates and initial when performing rechecks on post-operative days 1 and 2. Remove green card after 7-14 days.
3. The day surgery is performed is Day 0. On Day 0, monitor until animal recovers fully from anesthesia. Monitor animal again, later in Day 0 (at the interval described in the approved IACUC protocol). Monitor animal post-operatively at least once daily for a minimum of 2 days post Day 0 (surgery) including weekends and holidays. Observe animal for signs of distress or discomfort, such as: abnormal posture or movement, weight loss, increased attention to surgical site. If problems arise, document and consult LARC. Perform any additional monitoring per the IACUC-approved protocol.
4. Administer post-operative analgesics per the IACUC-approved protocol.
5. Clean and dry all surgical instruments and re-assemble them to be sterilized again. Dispose of all soiled drapes, pads, towels, etc. Place all sharps in sharps disposal container; do not recap needles!

6. Ensure that surgical records are complete and contain the minimum required documentation: animal/cage ID, surgeon name, date of surgery, anesthetic and analgesic agents, experimental agents, doses and routes of administration, surgical procedure performed, and any surgical or anesthetic complications. If the protocol describes other parameters that will be recorded, these must be documented in addition to the above records. Refer to the [Mouse Survival Surgical Record Template](#) and/or [Rat Survival Surgical Record Template](#)
7. Remove wound clips or skin sutures 10-14 days post operatively. Female mice that undergo surgery for in utero procedures may have clips or sutures left in until their pups have been weaned. Any other exceptions must be described in the IACUC-approved protocol.
8. Dehiscence repair must be approved in your IACUC protocol prior to performing the procedure. If approved, use the standard procedure: [Repair of Incisional Dehiscence in Mice and Rats](#).

In case of emergency, contact the LARC on-call veterinarian at 415- 502-8687.

Section 2 - NON-SURVIVAL (TERMINAL/ACUTE) SURGERY

Any surgery conducted on animals that are not allowed to regain consciousness is considered non-survival surgery. This includes terminal vascular perfusion.

- Non-survival surgeries require neither aseptic technique nor dedicated facilities, provided that animals are not anesthetized long enough to develop infections and study data are not impacted by non-aseptic procedures. Fur removal and supportive care should be performed.
- Non-survival surgeries must be performed in a clean area, free of clutter. Personnel present in the area must observe reasonable cleanliness practices for both themselves and the animals, including the use of personal protective equipment (PPE) and other engineered controls to prevent contamination.
- The method of euthanasia following surgery should be consistent with the most current AVMA Guidelines on Euthanasia and must be listed in the approved IACUC protocol.