

Institutional Animal Care Program		
Title: RODENT SURVIVAL SURGERY		
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A) PURPOSE

In accordance with the Guide for the Care and Use of Laboratory Animals, all survival surgical procedures on rodents must be performed using aseptic procedures. This includes the use of sterile instruments, and the aseptic preparation of the surgical site in order to prevent postoperative infections.

B) FACILITY

- 1) A dedicated facility for rodent surgery is not required. A rodent surgical area can be a room or portion of a room that is easily sanitized. Such an area must be approved by the University Veterinarian or designee prior to any surgery. The immediate surgical area must not be used for other purposes during the time of surgery.
- 2) Surgery must be conducted on a clean, uncluttered lab bench or table. The surface of the lab bench or table must be impervious to liquids. The work surface must be wiped with disinfectant before and after use or covered with a clean drape.
- 3) Surgery area should be separate from area where hair is removed from the animal.

C) PROCEDURES

1) Preparation of Animal

- a) Anesthesia must be administered followed immediately by an application of an ophthalmic lubricant to the eyes to prevent corneal desiccation. Non-ophthalmic lubricants should not be used on the eye.
- b) Hair must be removed in an area separate from the surgical site.
- c) The skin must be thoroughly scrubbed with a surgical detergent. The surgical detergent of choice is chlorhexidine scrub. **Povidone iodine** (e.g., Betadine)

has been shown to be inferior to chlorhexidine and **is therefore no longer recommended**. The scrub (note that scrub is not the same as solution) must be applied at least three times alternating each scrub with 70% (isopropyl or ethyl) alcohol, sterile water or saline. After the third alcohol, sterile water or saline is applied, chlorhexidine **solution** is applied and allowed to dry on the skin before the incision is made. This drying activity is a crucial step to adequately kill surface microorganisms.

- d) Unless justified in the protocol, the surgical site must be covered with a sterile drape after the surgeon has donned sterile gloves.
- e) An external heat source must be used to prevent hypothermia and complications. The use of a water-circulating blanket is an ideal source of heat. Electrical blankets and heat lamps must be used with great caution as they can induce severe thermal damage to animals. Heating blankets should be covered to prevent overheating the animal. The use of homeothermic blankets are ideal as they adjust their temperature according to the animal's core temperature.

2) Preparation of Surgeon

- a) Hands should be washed with an antiseptic soap or a surgical scrub and sterile surgical gloves (not exam gloves) must be worn.
- b) Wear gown, face mask and hair bonnet to prevent contamination of the surgical field.
- c) Sterile gowns are recommended. The sleeves of garments must not be allowed to come in contact with sterile surfaces (e.g., gloves, the animal, instruments, etc.).
- d) A new pair of sterile surgical gloves must be used for each animal.
- e) If working alone, the surgeon should have the animal anesthetized and positioned prior to gloving.
- f) If the instruments are in a sterile pack, the first layer of the double-wrapped instrument pack should be opened before gloving. Otherwise, place the instruments onto a sterile drape.

3) Instruments

a) **Instrument Preparation:** All instruments must be cleaned and sterilized prior to use. First, all instruments must be cleaned of any debris by hand washing or by mechanical washer/sterilizer. Then, prior to surgery, the instruments must be sterilized using one of the methods listed below. The method of choice may be determined by the procedure, or the delicacy of the surgical instruments or the devices being used. Steam autoclaving is the preferred method.

(1) Heat Sterilization:

- a) **Steam Autoclave:** The instruments must be enclosed in a material which steam can penetrate. This should be secured with a thermo-sensitive tape. Use of such tape provides some indication that the autoclave procedure has been effective. Instruments should be autoclaved at 121^o C for 21 minutes in a vacuum autoclave. Autoclaved items should have a standard indicator to prove complete sterilization. Wrapped autoclaved items should be clearly labeled with an expiration date (expiration is one year from date of sterilization as long as the wrapping is not torn or becomes wet). Note that steam is unsuitable for plastic with a low melting point, powders or anhydrous solutions.
- b) **Flash Steam:** Used to sterilize articles intended to be used immediately. The temperature must reach 132^o C for three to five minutes.
- c) **Sterile (Hot or Glass) Bead Sterilizer:** These are handy accessories that will sterilize the tips of metal instruments in 60 seconds. This type of sterilization is ideal for multiple surgeries (see ‘Multiple Surgeries’ below).

(2) **Cold (Chemical) Sterilization:** Chemical sterilants (not disinfectants) have finite shelf lives and must be used, depending on the agent, within one to four weeks (follow label directions). The solutions must be protected from contamination. Effective cold sterilization requires thorough cleaning of instruments prior to processing because blood and organic

debris may inactivate chemical germicides and/or shield microorganisms from the sterilization process. Clean Rubbermaid-type containers with secure lids or stainless steel instrument trays and lids can be used for cold sterilization procedures and instrument storage. Sterile water or saline must be used to rinse the instruments, implants and tubing (inside and outside) prior to use to avoid tissue damage to the animals. The most commonly used chemical sterilant is glutaraldehyde, 2.5% . Exposure time should be followed according to manufacturer's instructions.

Ethylene Oxide Gas can also be used to sterilize delicate items but UTSA does not currently have an ethylene oxide sterilizer. Local hospitals may provide this service.

- b) **Multiple Surgeries:** If multiple surgeries are to be done on different animals, then previously sterilized instrument tips can be placed in a glass bead sterilizer for at least 60 sec at 250°C. Note that it may take up to 30 minutes to reach this sterilizing temperature. Only the instrument tips are placed in the glass bead sterilizer. Before placing instrument tips into the sterilizer, the instruments must be cleaned with alcohol to remove excess debris from the tips. No more than five successive surgeries are allowed to be conducted per instrument pack. If used immediately, hot instruments may be cooled down in a container with 70% alcohol or sterile saline to avoid burning both to the operator and the animal.
- 4) **Intraoperative Monitoring**
- a) The animal must be monitored carefully during the surgical procedure: i.e., the animal's respiratory rate and characteristic, response to noxious stimuli (e.g., tail pinch, rear toe pinch), and when possible, oxygen saturation, end tidal CO₂, heart rate and body temperature.
 - b) The animal must not be left unattended at any time during surgery.
- 5) **Post-Surgical Care**
- a) Post-surgical care must include observing the animal to promote uneventful recovery from anesthesia and surgery, administering analgesics, providing

adequate care to surgical incisions and maintaining appropriate medical records.

- b) Administration of analgesia is required as the default and preemptively (before the start of the surgical procedure) is generally preferred.
- c) To prevent hypothermia, place the animal(s) in a warm room or cage. If necessary, the cage may be placed on a bedded or padded surface and supplied with extra bedding or supplemental heat as required. Be cautious with supplemental heat sources; they can cause thermal burns and hyperthermia if used inappropriately. A way to escape excessive heat should be provided to the animals, for example, when recovering the animal in the cage, only place half of the cage on a heating pad to allow the animal to move to the cooler portion of the cage if necessary.
- d) Dehydration can be ameliorated by the administration of appropriate fluid therapy. Initially this may be done by administering warm (approximately 37-38 °C) sterile fluids such as 0.9% NaCl or Lactated Ringers Solution (1 to 2 ml for an adult mouse and 5-10 ml for an adult rat) by subcutaneous or intraperitoneal injection. If significant blood loss is anticipated or it is a long procedure, fluids can be administered pre-op. If unexpected blood loss occurs during the surgical procedure or if the animal is slow to recover from the anesthetic, additional fluids may be indicated.
- e) During the anesthetic recovery process, animals must be monitored continually until they gain the righting reflex. Do NOT return unconscious animals back to the animal holding room.
- f) To prevent cannibalism or suffocation, rodents may be housed individually until they are ambulatory.
- g) When returned to the recovery cage and the animal holding room, rodents must be housed in clean/fresh bedding. Sterilized bedding is recommended and a sterile pad for the next 24 hours instead of bedding is preferred.
- h) If recovery from anesthetic will be prolonged (i.e., over one hour), the animal must be rotated from side to side every 30 minutes to minimize atelectasis

(collapse) of the lungs. This practice must be continued until the animal regains the righting reflex.

- i) Post-op animals must be evaluated daily for at least five days by a member of the principal investigator's staff or other individuals to whom post-operative care has been delegated. Animals must be monitored for evidence of excessive inflammation of the incision site, suture dehiscence (incision line failure or separation), infection, behavioral abnormalities indicative of illness or pain (anorexia, listlessness, lethargy, dehydration, ruffled coating, lack of movement, hunched back, weight loss greater than 10%). If evidence of wound infection or illness is noted, LARC vet services must be contacted for evaluation and treatment or the animal should be euthanized as soon as possible.
 - j) External sutures, staples, and wound clips generally must be removed 10-14 days after surgery.
 - k) Non-pharmacologic control of pain should be considered as an element of post-procedural or perioperative care. Appropriate nursing support may include a quiet, darkened recovery or resting place, timely wound or bandage maintenance, increased ambient warmth and a soft resting surface, rehydration with oral or parenteral fluids, additional nesting material and a return to normal feeding through the use of highly palatable foods or treats.
- 6) **Surgical Records**
- a) A "Surgical Record" must be completed per cage immediately after the surgical procedure. Records may be somewhat abbreviated and in composite format and can be included as part of research data collected, but should also be available for review by regulatory bodies including the IACUC. The records should be maintained in the laboratory.
 - b) Records must consist of the following:
 - (1) *PI records*: Pre- and intra-operative records are maintained by the PI and should contain IACUC-approved protocol number, animal ID, procedure performed, anesthetics/medications/analgesics (including dose and route), complications, date of surgery.

tasks in a humane and scientifically acceptable manner. The LARC veterinary staff will provide basic training to all researchers who will conduct animal surgical procedures, covering aseptic and surgical techniques, and the proper administration of anesthesia, analgesia and euthanasia.

8) **Exceptions**

Any deviations from this policy must be approved by the IACUC.