

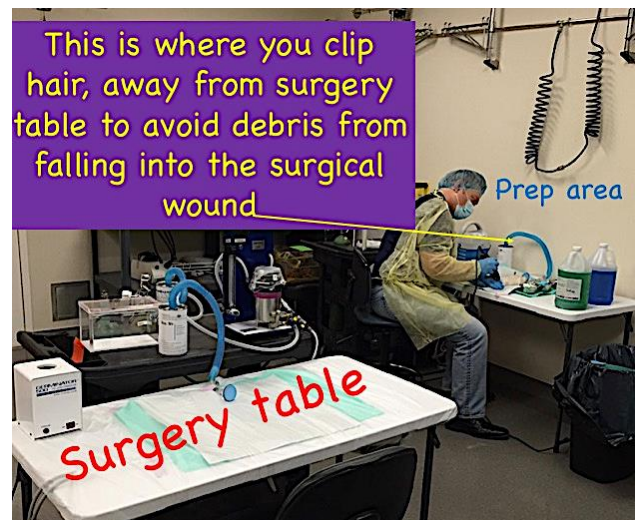
Institutional Animal Care Program		UTSA
Title: RODENT SURVIVAL SURGERY		
Policy#: IACP 004	Date in Effect: 12/14/07	
Rev #: 01	Rev Date: 12/14/12	
Rev #: 02	Rev Date: 03/08/13	
Rev #: 03	Rev Date: 12/13/13	
Rev #: 04	Rev Date: 11/20/15	
Rev #: 05	Rev Date: 09/14/18	
Rev #: 06	Rev Date: 11/12/21	
Rev #: 07	Rev Date: 02/03/23	
In Effect <input checked="" type="checkbox"/> Rescinded <input type="checkbox"/>	Date Rescinded: NA	

A) PURPOSE

In accordance with the Guide for the Care and Use of Laboratory Animals, 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. Infections can be observable (clinical) or hidden (sub-clinical) and both can impact animal welfare and data.

B) FACILITY

- 1) Aseptic rodent surgery ought to be conducted in dedicated facilities or spaces (dedicated at the time of the surgical procedure). Traffic and activities ought to be minimized in the room or area at the time of the procedure.
- 2) Conduct surgery on a clean, uncluttered lab bench or table that is impervious to liquids.



- 3) Mist/wipe the work surface wiped with disinfectant before conducting surgery.
- 4) Ensure surgery table/area is separate from where hair is removed from the animal.

C) PROCEDURES

1) Instrument Preparation

- a. Clean surgical instruments prior to sterilization. Lubrication with a surgical grade lubricant extends the life of the instrument.

Instrument Cleaning & Lubrication



Open ~~**Closed**~~

Cleaning steps:

1. Soak in enzymatic sol (ultrasound if available)
2. Brush/clean
3. Rinse
4. Lubricate & dry on

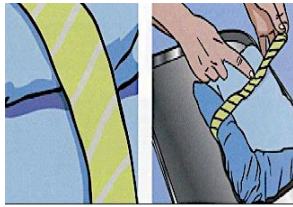
ENZYMATIC INSTRUMENT CLEANER
LIQUID CONCENTRATE
CATALOG NO. 100-1000
Enzymatic instrument cleaner to neutralize and eliminate strongly alkaline residues of surgical instruments, mechanical and glassware. Biodegradable acid neutralizer.
8 FL. OZ. (236.6 mL)

LUBRISPRAY
SURGICAL INSTRUMENT LUBRICANT
CATALOG NO. 100-1000
Acid neutralizer. No corrosion. Resistant to cleaning. High pressure cleaning. Sterilization. Lubricates all metal parts.
8 FL. OZ.

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- b. Wrap or encase instruments prior to sterilization. Following sterilization, label with an expiration date (not to exceed one year from sterilization date).

- c. Use a method to verify sterilization such as a steam chemical indicator or autoclave tape.



Non Sterile Sterile
Autoclave tape



Steam chemical indicator



Autoclave pouch

- d. Autoclaving is the sterilization method of choice.
- e. Delicate materials may be cold sterilized with chemicals such as glutaraldehyde but be sure to follow label directions for preparation and exposure times. Although alcohol may be acceptable in selected cases, it is neither a sterilant nor a high-level disinfectant.

2) Preparation of Animal

- a. Soon after anesthesia induction:
 - a. Apply ophthalmic ointment to eyes. Non-ophthalmic lubricants such as mineral oil, Vaseline, etc. are not appropriate.




- b. Administer analgesics (local and systemic) & antibiotics if indicated in protocol.
- c. Administer SQ or IP fluids (saline or lactated Ringer's solution)
 1. Adult rat: 5-10 ml.

2. Adult mouse: 0.5-1 ml.

- b. Remove hair from the surgical site with:
 - a. Hair clippers (#40 blade) – method of choice.
 - b. Depilatory creams are less preferred and can be used in selected cases such as when clipping may interfere with imaging procedures. Remove depilatory cream within 45-60 sec with alcohol to prevent skin irritation.
- c. Prep the surgical site with surgical prep chemicals in a concentric pattern starting at the estimated center of the incision site and gradually moving outwardly.
- d. Prep the skin with chlorhexidine (e.g., Nolvasan) or povidone iodine (e.g., Betadine) **SCRUB (soap)**, alcohol, & lastly with chlorhexidine or povidone iodine **SOLUTION**. Leaving the solution in place until dry allows for extended intra-surgical protection. For a demonstration on how to prep the skin [click here](#).



Skin Disinfection



1. Remove hair & clean surgical area with 70% alcohol
2. Apply chlorhexidine or povidone iodine SCRUB (NOT solution) followed by 70% alcohol
3. Repeat previous SCRUB & alcohol step 2 more times
4. After last alcohol, paint area with chlorhexidine or povidone iodine SOLUTION (NOT scrub)
5. Allow SOLUTION to dry on

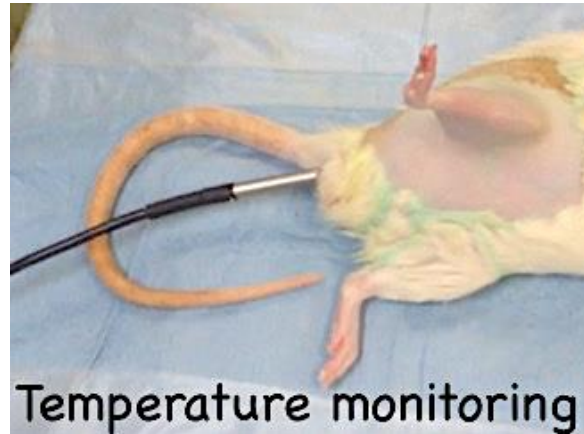
- e. Don sterile surgical gloves. For a demonstration on donning surgical gloves [click here](#).
- f. Drape skin with sterile drape. For a demonstration on placing a sterile Press'n Seal drape [click here](#), [here](#) and [here](#).



- g. Hypothermia has profound implications on the recovery of the animal, and it starts immediately upon anesthesia induction. The following points will help in minimization of hypothermia:
 - i. Provide an external heat source. Homeothermic blankets are preferred as they both monitor and maintain body temperature evenly by way of a feedback mechanism.
 - ii. Warm up the chamber before anesthesia induction.
 - iii. Incorporate the tail within heating pad if possible (the tail is a major thermoregulatory organ).
 - iv. Warm up fluids and skin prep chemicals.
 - v. Monitor temperature (how else will you know the animal's normal temperature is maintained?).

Rat: 99.4 °F or 37.5 °C.

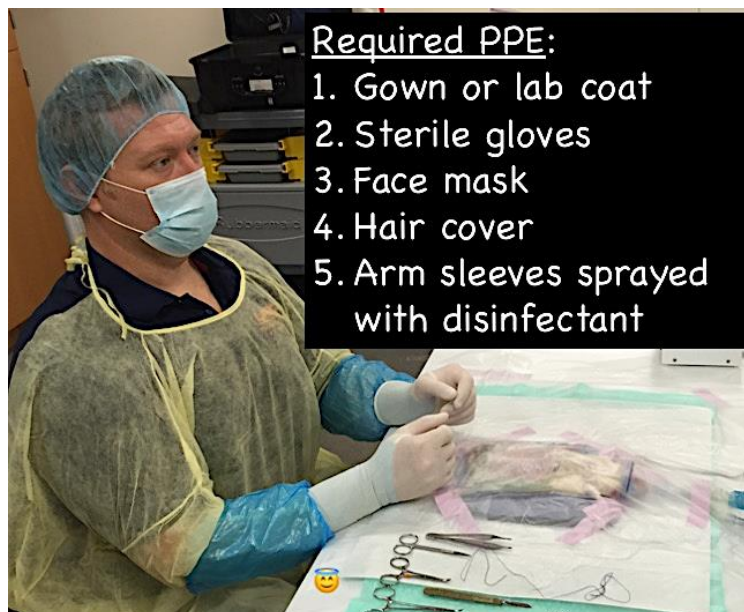
Mouse: 98.8-99.3 °F or 37.0-37.2 °C.



A word of caution: Hyperthermia can also have deleterious effects and some, in their eagerness to prevent hypothermia, end up overheating the animal. A homeothermic pad provides the solution by maintaining the animal at normal set temperatures.

3) Surgeon Preparation

- a) Wash hands with an antiseptic or surgical soap.
- b) Don surgical personal protective equipment (PPE). The following image illustrates the required surgeon PPE.



- c) A new pair of sterile surgical gloves must be used for each animal.

d) Arm sleeves must be misted with a high-level disinfectant.



4) Prevention of Cross-Contamination

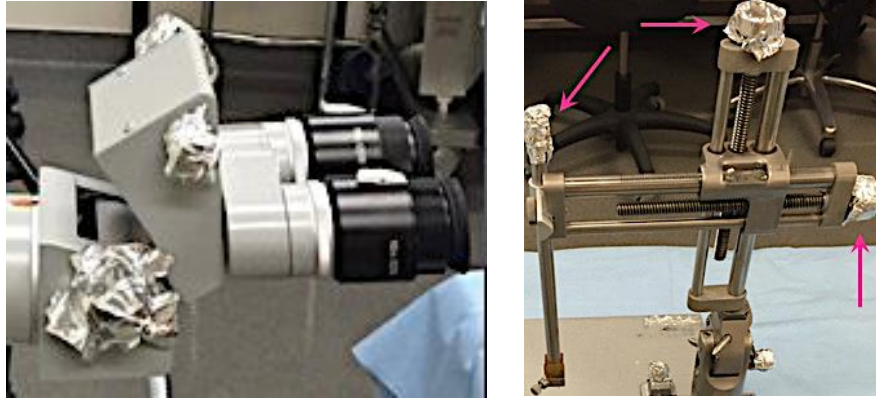
a. Prepare an extensive surgical field with a sterile drape to minimize cross-contamination. For a demonstration [click here](#).



b. Asepsis must be maintained during surgery. Non-sterile items that will be touched with sterile gloves such as stereotaxic device parts, light handles, microscope knobs and anesthesia machine



dials must be wiped with a high-level disinfectant or preferably covered with a sterile material (e.g., Press'n Seal or autoclaved aluminum foil).



aluminum foil).

5) Oxygen Supplementation

- a. Previous studies suggest that mice anesthetized with both inhalant and injectable anesthetics, without supplemental oxygen, may be profoundly hypoxic, leading to rough recoveries and preventable deaths.
- b. Delivery of anesthesia with room air is not acceptable.
- c. All rodents undergoing anesthetic episodes greater than 15 min must have high concentration of oxygen supplementation, although we still recommend supplementation for shorter anesthetic periods.

Note: The 15 min period is the time from induction or injection of anesthesia to the time when animals recover the righting reflex. Most injectable episodes will last greater than 15 min

- d. Two examples on how to deliver supplemental oxygen when using injectable anesthesia are:

1. Use a gas anesthesia/vaporizer system. Turn the oxygen **ON** at 0.5 L/min, maintain the vaporizer in the **OFF**



position. O₂ can be delivered via mask

2. An oxygen concentrator with the flow as close as possible to 0.5 L/min. If this is not possible (as some systems deliver no less than 1 or 2 L/min), some of the oxygen may be diverted into the environment to avoid excessive cooling of the animal. O₂ can be delivered via mask or tubing next to the nose.
3. Endotracheal intubation may be considered.
4. In cases that investigators can justify not supplementing O₂, the IACUC recommends that at the conclusion of the procedure, during the recovery period, animals be placed on supplemental O₂ for as long as possible.

6) Intraoperative Monitoring

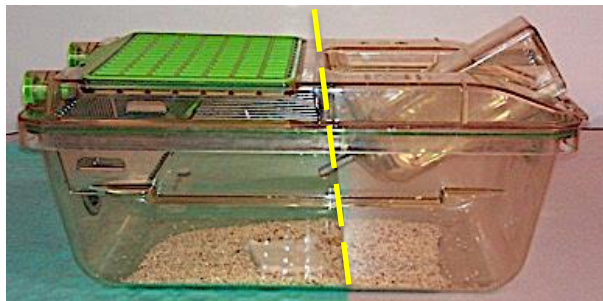
- a. The depth of monitoring increases with the duration and invasiveness of the procedure. Here are some ways to monitor the animal:
 1. A vigorous **rear** toe pinch and/or tail pinch. ALWAYS DO THIS before making the incision. Absence to a response means the animal is in a surgical plane of anesthesia.

2. Respiratory rate and/or character.
3. Overall color of the animal.
4. Oxygen saturation.
5. Heart rate.
6. Electrocardiogram.
7. End tidal CO₂.
8. Body temperature.

b. NEVER LEAVE THE ANIMAL UNATTENDED DURING SURGERY.

7) Post-Surgical Care

- a. Monitor animals continually until they regain the righting reflex.
- b. Place animals in a clean cage.
- c. Place clean cage on half of a heating pad so that the animal can escape to a cooler area of the cage if necessary.



Warm side

Cool side

- d. Post-op care must be done and documented daily for at least five days.
- e. If sutures or clips are in place, monitoring and documentation must continue until they are removed.
- f. Parameters to monitor:
 1. Incision: Redness, inflammation, discharge, suture dehiscence.
 2. Behavioral abnormalities such as pain, anorexia, listlessness, lethargy, dehydration, ruffled coating, lack of movement, hunched back, weight loss and excessive

attention to the wound site. If evidence of wound infection or illness is noted, contact LARC veterinary services x6692; LARC@utsa.edu .

3. Post op scoring sheets can be used but they need to be described in the protocol.
- g. Remove external sutures, staples, and wound clips 10-14 days after surgery.
- h. Non-pharmacologic pain control should be considered as an element of the post-procedural care. Examples of non-pharmacologic methods of pain control are:
 1. Energy-dense highly palatable nutritional supplementation (e.g., gel cups and Bacon Softies).
 2. Fluids.
 3. Minimize light intensity.
 4. Provide a quiet environment.
 5. Additional nesting material and huts.
 6. Thermal support.
 7. Social housing is the default post-op housing. It decreases morbidity and mortality.
 8. Refer to: LARC [Guidelines for Evaluating and Treating Surgical Pain in Mice & Rats.](#)

8) Surgical Records

- a. There are two records to be completed post-operatively:
 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, data collected, date of surgery, etc.

Records should be available for review by AAALAC International and the IACUC.

2. *LARC Green surgery card* (post-operative record): For each survival surgery cage, the LARC CDLARC003 Green 3" x 5" POST-SURGICAL CARE CARD must be filled out and kept current for five post-surgical days (longer if complications arise). The first day is considered the day of surgery. If external sutures, staples or clips were applied to the skin, they must be removed 10-14 days post-op and such removal must be recorded on the POST-SURGICAL CARE CARD. Documentation should include any treatments given, incision and patient assessment.

Note: If surgical procedures are performed on animals or groups within the same cage on different days, then a POST-SURGICAL CARE CARD must be filled out per group or day.

LARC RODENT POST-SURGICAL CARE CARD (CDLARC003) must remain with the LARC. PI may copy these cards as needed.

FRONT		BACK																															
LARC Post-Surgical Care Card		Post-operative monitoring/notes must be continued for a minimum of 5 days after surgery																															
Surgery Date:	_____	Date	Drugs (dose, route), incision, pain, behavior, suture removal, etc.																														
Suture/Clip Removal Date:	_____		Initials																														
PI:	_____	<table border="1" style="width: 100%; border-collapse: collapse;"><tbody><tr><td style="width: 20%; height: 15px;"></td><td style="width: 80%;"></td></tr><tr><td style="height: 15px;"></td><td></td></tr><tr><td style="height: 15px;"></td><td></td></tr><tr><td style="height: 15px;"></td><td></td></tr><tr><td style="height: 15px;"></td><td></td></tr><tr><td style="height: 15px;"></td><td></td></tr><tr><td style="height: 15px;"></td><td></td></tr><tr><td style="height: 15px;"></td><td></td></tr><tr><td style="height: 15px;"></td><td></td></tr><tr><td style="height: 15px;"></td><td></td></tr><tr><td style="height: 15px;"></td><td></td></tr><tr><td style="height: 15px;"></td><td></td></tr><tr><td style="height: 15px;"></td><td></td></tr><tr><td style="height: 15px;"></td><td></td></tr><tr><td style="height: 15px;"></td><td></td></tr></tbody></table>																															
Protocol #:	_____																																
Room/Cage Card #:	_____																																
Contact Phone:	_____																																
Surgeon's Name	_____																																
Surgery Site (e.g. head, hind leg, abdomen)	_____																																
Date	Drugs (dose, route), incision, pain, behavior, suture removal, etc.	Initials																															

9) **Surgery-Related Tips**

- a. If multiple surgeries are to be done on different animals, the first surgery must begin with a sterilized instrument pack.
- b. For subsequent surgeries instruments tips can be placed in a glass bead sterilizer for at least 60 secs at 250°C.
 - 1. Pre-heat the glass bead sterilizer prior to surgery. It may take up 30 minutes for it to reach sterilization temperature.
 - 2. Before placing instrument tips into the sterilizer, clean instruments tips, grooves and hinges with a brush and alcohol to remove excess debris. No more than five successive surgeries are allowed to be conducted per instrument pack.

3. Do not overcrowd the glass bead sterilizer. Too many instruments will cool down the beads to non-sterilizing



temperatures.

- c. NEW STERILE GLOVES AND STERILE DRAPE MUST BE USED BETWEEN ANIMALS.

10) Non-Survival Surgery

For non-survival (terminal) surgery, when animals are anesthetized for period less than six hours, aseptic technique is encouraged, but not required. For non-survival surgeries lasting longer than six hours, the stipulations described in this policy are in effect.

11) Training

Professional and technical personnel and students who want to perform anesthesia, analgesia and surgery must be trained to accomplish these tasks in a humane and scientifically acceptable manner. The LARC veterinary staff provides basic aseptic surgical training to all researchers who will conduct animal surgical procedures. For illustrated rodent surgery, anesthesia and other topics training [click here](#).

12) Exceptions

Any deviations from this policy must be detailed in the protocol or an amendment and approved by the IACUC.

The following checklist will help you prepare for aseptic rodent surgery (add or remove to fit your individual needs):

- Dedicate the room/area to the surgical procedure, remove clutter and disinfect surfaces
- Skin prep area separated from the location where the surgery will be conducted
- Have instruments been pre-cleaned & lubricated prior to sterilization?
- Are instruments and implants sterilized? Did I make sure the expiration of the sterilized materials does not exceed one year?
- Ensure proper sterilization did occur (e.g., use of autoclave tape)
- If instruments or implants were cold sterilized, follow recommended contact times
- Don surgical gloves without contaminating them
- Prevent cross contamination of non-sterile parts that will be manipulated with sterile gloves during surgery. For example, disinfection or covering with Press'n Seal or autoclaved aluminum foil
- Apply ophthalmic ointment upon induction
- Administer analgesics, fluids (0.5-1 ml mice; 5-10 ml rats) & other required medications before surgery
- Remove hair from surgical site
- If I used depilatory cream, did I remove it within 45-60 secs of application?
- Prep the surgical site 3 times with surgical soap & alcohol, followed by a last application of surgical solution
- Maintain normothermia:
 - ✓ Provide an external heat source
 - ✓ Warm up fluids and skin prep solutions
 - ✓ Incorporate the tail over or within heating source
 - ✓ Monitor temperature

- Wash hands before donning sterile gloves
- Surgeon PPE:
 - ✓ Gown or lab coat
 - ✓ Hair cover
 - ✓ Sterile gloves
 - ✓ Face mask
 - ✓ Hair cover
 - ✓ Arm sleeves misted with disinfectant
- Disinfect table and cover extensively with sterile drape
- Test that the rear toe pinch reflex is absent prior to making the incision
- Monitor the animal during surgery
- Immediate post op recovery
 - ✓ Place half of a clean recovery cage on a heating pad
 - ✓ Provide fluids and a highly palatable/energy dense supplement
 - ✓ Complete post-op records

Common Violations in Aseptic Technique

Examples of Violations of Sterile Technique

- 1) No hair cover
- 2) Touching contaminated object (glasses) with gloves
- 3) Using non-sterile (nitrile) gloves
- 4) Syringe outer package on sterile field
- 5) Tube of ophthalmic ointment on sterile field
- 6) Suture outer package on sterile field
- 7) Anesthesia hoses & animal not covered with sterile drape
- 8) Sterile glove outer packaging on sterile field
- 9) Red top blood tube on sterile field
- 10) Disinfectant bottle on sterile field



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