| Institutional Animal Care & Use Program - UTEP | |
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| **Title**: Rodent Survival Surgery | |
| **Policy#**: 004 | **Date in Effect**: 24 October 2014 |
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| **In Effect** ☒ **Rescinded** ☐ | **Date Rescinded**: |

1) RESPONSIBILITIES

It is the responsibility of all personnel using animals at The University of Texas at El Paso (UTEP) to abide by this policy.

2) APPLICATION

This policy applies to all rodents used in research and teaching at UTEP.

3) PURPOSE

The purpose of this policy is to clarify the requirements of all personnel performing survival surgery on rodents. Surgeries involving United States Department of Agriculture (USDA) covered rodents (e.g., guinea pigs, hamsters, voles, etc.) follow the same procedures as for mice and rats with specific requirements noted below each section. All personnel performing survival on rodent species must adhere to the minimum standards addressed in this policy. Alternatives can be approved by the IACUC based upon specific requests.

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.

4) DEFINITIONS

1) Major survival surgery penetrates and exposes a body cavity, produces substantial impairment of physical or physiological functions, or involves extensive tissue dissection or transection (e.g., laparotomy, thoracotomy, limb amputation).
2) Minor survival surgery does not expose a body cavity and causes little or no physical impairment (e.g., wound suturing, peripheral vessel cannulation, percutaneous biopsy).
3) Sterile field includes the portion of the animal and the area immediately around the animal that has been aseptically prepared for the surgical procedure, considered to be free of microorganisms, and will be maintained in a sterile manner.

4) Sterilization is the process whereby all viable microorganisms, including spores, are eliminated or destroyed. The criterion of sterilization is the failure of organisms to grow if growth supporting medium is supplied.

5) 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 disinfected. Such an area must be approved by the Attending Veterinarian or designee prior to any surgery. The immediate surgical area must not be used for other purposes during the time of surgery and personnel traffic must be minimized.

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 the area where hair is removed from the animal.

6) PROCEDURES

**1) Surgeon Preparation**

a) Surgeons and surgical assistants must wash their hands with antibacterial soap prior to initiating the surgical procedure and after preparation of the animal. To avoid contamination with aerosols released during the scrubbing, this surgeon prep area should be separated from the operating area.

b) All personnel taking part in the surgery must:

(1) Wear clean lab coat, scrubs, or appropriate disposable gown

(2) Wear appropriate face mask and head covering

(3) Wear sterile gloves when contacting tissues or instruments within the sterile field. Gloves must be replaced if aseptic technique is disrupted, e.g., touching the isoflurane vaporizer with the sterile gloves, moving the animal with the sterile gloves, or between animals.

(4) Sterile gowns are recommended but not required. The sleeves of garments must not be allowed to come in contact with sterile surfaces (e.g., gloves, the animal, instruments, etc.).

(5) In order to maintain sterility while handling equipment during a procedure, portions of "Press 'n' Seal" wrap can be placed on the equipment aseptically to allow intraoperative manipulation of items.

**2) Instruments**

a) All instruments must be cleaned and sterilized prior to use. First, all instruments must be cleaned of any debris by hand washing or by ultrasonic cleaner. 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 $^{0}$C for 21 minutes in a vacuum autoclave. Higher temperatures/shorter times may also be used. Autoclaved items should have a standard indicator to verify effectiveness of the sterilization process. 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).

b) Flash Steam: Used to sterilize articles intended to be used immediately. The temperature must reach 132 $^{0}$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 according to 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. Examples of common commercial sterilants include:

a) Cidex or glutaraldehyde (2.4% to 3.4%)
   - Active for 14-28 days, once prepared
   - Requires 10 hours of exposure at 25°C for sterilization

b) Spor-Klenz Ready-To-Use (RTU) or peracetic acid/hydrogen peroxide blend
   - Active for up to 14 days
   - Requires 5.5 hours of exposure at 20°C for sterilization

c) Sporicidin or glutaraldehyde (1.12%) and phenol/phenate (1.93%) blend
   - Active for up to 14 days
   - Requires 12 hours of exposure at 25°C for sterilization

b) Multiple Surgeries: If multiple surgeries are to be done on different animals, then previously sterilized instruments tips can be placed in a glass bead sterilizer for at least 60 sec at 250 ºC. 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 (5) successive surgeries are allowed to be conducted per (sterilized) instrument pack. If used immediately, hot instruments may be cooled down in a container with 70% alcohol or sterile saline to avoid burning both the operator and the animal.

**3) Preparation of Animals**

a) Anesthesia must be administered, followed immediately by an application of an ophthalmic lubricant to the eyes to prevent corneal desiccation. Non-ophthalmic lubricants such as petroleum-based agents should not be used on the eye.

b) Hair must be removed in an area separate from the surgical site. Remove the hair using clippers or depilatory cream in a wide area around the intended surgical site.
   - If using depilatory cream, it should not remain on the skin for more than 60 seconds to prevent chemical burns
   - The skin should be rinsed following depilatory cream application with several rounds of sterile saline.

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 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 last alcohol, sterile water or saline is applied, chlorhexidine **solution** (not scrub) 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 homeothermic blanket is an ideal source of heat, however, a water-circulating blanket is also adequate. 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 the animal from overheating. The advantage of homeothermic blankets is that they adjust the temperature according to the animal's core temperature.

f) When used for post-surgical monitoring (or whenever an animal is unable to ambulate normally due to anesthesia, restraint or other factors), heat lamps must be described and approved in the protocol with the following plan in place:

- The heat lamp must be positioned such that the half of the cage where the animal is not recovering is excluded from the heat lamp field.
- Animals must be monitored continuously until they become ambulatory to ensure they will be able to escape the heat field or until the heat lamp is removed.
- A timer must be in place.- the suggested time is no more than 15 minutes.

**4) Intraoperative Monitoring**

a) The animal must be monitored carefully during the surgical procedure: The following methods of intraoperative monitoring can be used, depending on extent of the procedure and equipment available:

- Respiratory rate and characteristics
- Response to noxious stimuli (e.g., tail pinch, vigorous rear toe pinch,

response to surgical pain)

- Oxygen saturation
- End tidal $CO_2$
- Heart rate
- Body temperature
- Animal's color (white animals or areas of light skin)

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, administrating 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.

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.

c) Dehydration can be ameliorated by the administration of appropriate fluid therapy. Initially this may be done by administering warm (approximately 37-38 $^0$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. Administration of these fluids pre-operatively has been demonstrated to improve the overall post operative recovery while minimizing potential deaths during surgery and the immediate post-op period. If significant blood loss occurs or if it is a long procedure, fluid therapy can be repeated.

d) During the anesthetic recovery process, animals must be monitored continually until they regain the righting reflex (ability to stand on their four feet when

place on lateral or dorsal recumbency). Do NOT return unconscious animals back to the animal holding room.

e) To prevent cannibalism, rodents should not be housed with fully alert rodents until they are ambulatory.

f) 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.

g) 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.

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. If evidence of wound infection or illness is noted, Laboratory Animal Resource Center (LARC) must be contacted for evaluation and treatment, or the animal should be euthanized as soon as possible.

h) External sutures, staples, and wound clips generally must be removed 10-14 days after surgery.

i) Non-pharmacologic methods of pain control should be considered as an element of post-procedural or perioperative care. Appropriate methods 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, hiding huts and a return to normal feeding through the use of highly palatable foods or treats.

**6) Surgical Records**

a) A Surgical Record (*CDVS001-00-Treatment/Surgery Card*) must be completed per cage immediately after the surgical procedure. Records must be included as part of research data collected, but should also be available for review by Veterinary Services and regulatory bodies including the IACUC.

b) Records must consist of the following:

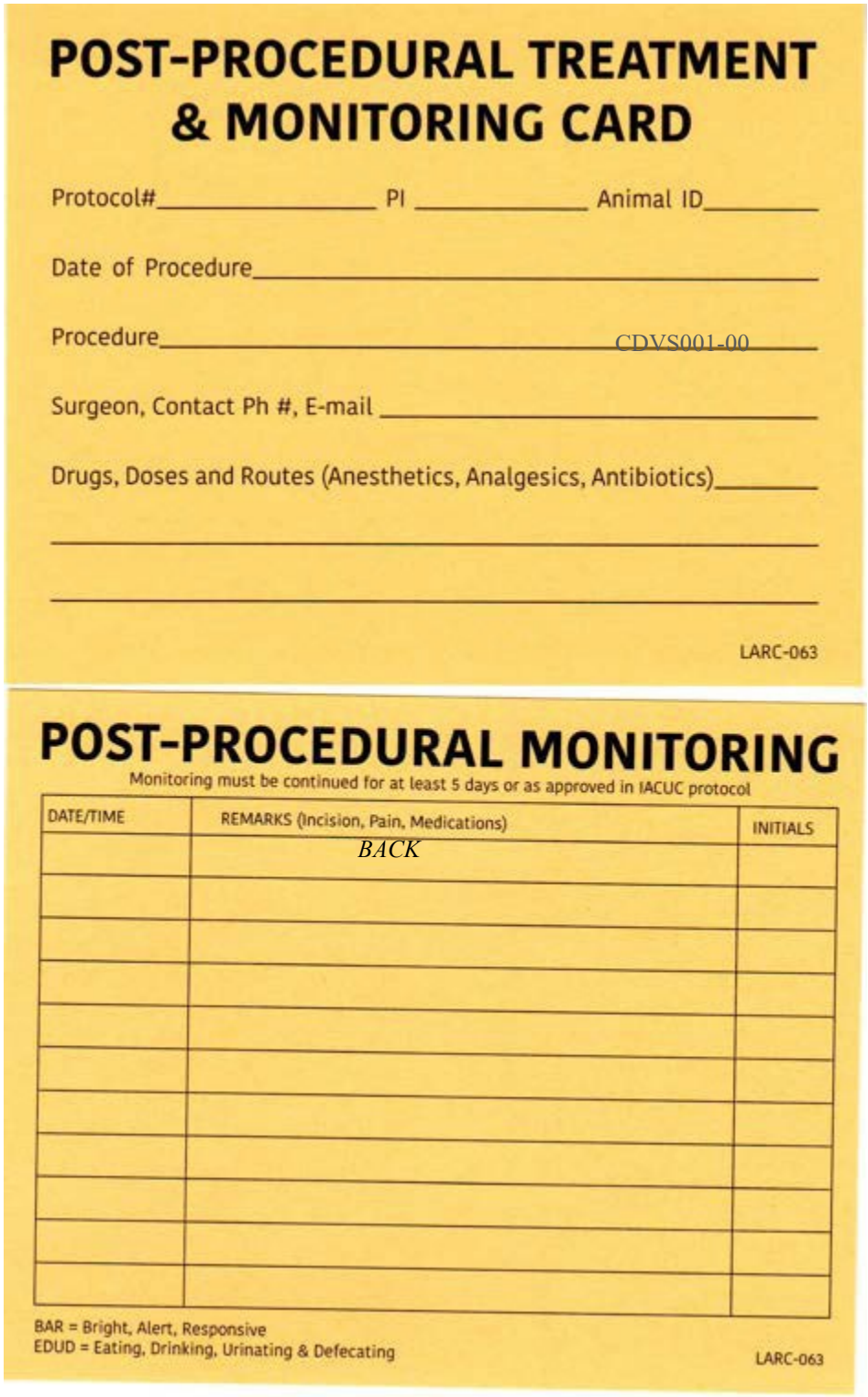
i. *PI records*: Pre- and intra-operative records are maintained by the PI and should contain IACUC-approved protocol number, animal ID, procedure performed, anesthetics/medications/analgesics (including dose and route), complications, date of surgery.

ii. *Treatment/Surgery Card* (post-operative record) (Figure 1): For each survival surgery cage, the LARC yellow 3" x 5" *CDVS001-00- Treatment/Surgery Card* must be filled out both front and back 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-operation and such removal must be recorded on the POST-SURGICAL CARE CARD. LARC keeps the original of these cards. PI may copy these cards as needed.

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.

**7) Training**

Professional and technical personnel and students who want to perform anesthesia, analgesia, surgery, and euthanasia must be trained to accomplish these tasks in a humane and scientifically acceptable manner. LARC 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.



POST-PROCEDURAL TREATMENT & MONITORING CARD

Protocol#_____ PI _____ Animal ID_____

Date of Procedure_____

Procedure_____ CDVS001-00

Surgeon, Contact Ph #, E-mail _____

Drugs, Doses and Routes (Anesthetics, Analgesics, Antibiotics)_____

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LARC-063

POST-PROCEDURAL MONITORING

Monitoring must be continued for at least 5 days or as approved in IACUC protocol

| DATE/TIME | REMARKS (Incision, Pain, Medications) | INITIALS |
|---|---|---|
| | *BACK* | |
| | | |
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| | | |
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| | | |
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BAR = Bright, Alert, Responsive
EDUD = Eating, Drinking, Urinating & Defecating

LARC-063

Figure 1