**UNIVERSITY OF DELAWARE**

**OFFICE OF LABORATORY ANIMAL MEDICINE**

**Guidelines for Survival Rodent Surgery SOP PRO#016**

**Scope**: These guidelines apply to all surgical procedures performed on rodents at the University of Delaware in which the animals are expected to recover from anesthesia. Prior to performing any survival surgery techniques on rodents, an approved Animal Study Proposal must be in place with descriptions of the surgical procedures to be performed and personnel must be appropriately trained. Specific procedures to accomplish these guidelines can be obtained from your veterinarian.

**General:** It is important to note that rodents do not vomit, so it is not necessary to fast them prior to surgery (Horn et al. 2013). The following principles described in the Guide for the Care and Use of Laboratory Animals apply to rodent surgery.

• Appropriate pre-operative and post-operative care of animals in accordance with established veterinary medical and nursing practices are required.

• A designated animal procedure space for rodent surgeries is required; for example, a location within a procedure room or laboratory space free from clutter and easily disinfected prior to the surgical

procedure such that cleanliness is ensured and contamination is minimized at the time of use.

• All survival surgery will be performed by using aseptic procedures, including masks, sterile gloves, sterile instruments, and aseptic techniques. Additionally, sterile gloves are preferred for ‘tips only’ technique. 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, with maintaining tissue moisture at all times

• Minimal dissection of tissue, and minimal time incision is open

• Appropriate use of instruments,

• Effective hemostasis, and

• Use of suture materials and patterns or other wound closure techniques that minimize trauma and ensure incision remains 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 consider the availability of personnel to provide anesthetic induction, aseptic preparation of the surgical site, and post-operative care appropriate to the surgical procedure. Investigators must assure that the challenges of consecutive surgeries within one work session are adequately addressed.

**Procedures:**

**Personal Protective Equipment:**

• Clean jumpsuit or lab coat

• Mask

• Gloves

o Using sterile surgical gloves allows you to touch all areas of the sterile surgical field and surgical instruments with your gloved hand.

o 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.

• Hair cover

**Pre-Operative**:

Allow a minimum of a 3-day acclimation to the new environment to overcome the stress of transportation. Animals should be free of clinical signs of disease

•. Surgery should be conducted in a disinfected, uncluttered area that promotes asepsis during surgery (see Table 1 below). Animal prep should be away from the surgical area to prevent contamination. Avoid areas directly under supply ducts or in high traffic areas.

• If limbs must be positioned for control of the surgical field, avoid placing excessive tension on the limbs, which may cause neural damage and shut off circulation and in some cases, respiratory compromise. Never use the anesthetized animal’s body as a table. Do not rest your hands or your instruments on the chest or abdomen. External pressure interferes with respiration and blood circulation.

• After anesthetizing the animal, remove the hair from the surgical site by either clipping, plucking, or the use of depilatories. If a depilatory is used, thoroughly rinse the chemical from the rodent’s skin or apply a neutralizing agent.

• Administer analgesics (preemptive analgesia) as appropriate and approved in your Animal Study Proposal.

• Protect the corneas from drying out by applying an ophthalmic ointment since anesthesia abolishes the blink reflex.

• Prepare the surgical site(s) with an appropriate skin disinfectant (see Table 2). If using a stereotaxic frame, the rodent should be placed in the frame before the skin disinfectant is applied. The use of alcohol alone is generally not considered adequate. Standard surgical prep consists of three alternating scrubs of a chlorhexidine scrub and 70% alcohol. Using a gauze sponge or cotton tipped applicator, cleansing should be done in a circular motion. Begin at the center of the hairless area and work toward the periphery. Never go back to the center with the same sponge.

• Surgeons should wash and dry their hands before aseptically donning sterile gloves.

• Nitrile examination gloves can be either autoclaved or gas sterilized as an economical alternative to pre-packaged sterile surgical gloves (LeMoine et al. 2015). Multiple pairs of gloves can be autoclaved in the same pack, but care must be used to avoid contamination of the gloves during donning.

• The same gloves can be worn between surgeries under the following circumstances:

o The surgeon’s gloves have not become contaminated during respective surgeries or

o The “tips-only” technique is used. Examples of ways to prevent glove contamination are to have another person assist the surgeon by recovering and prepping subsequent animals for surgery, have the surgeon anesthetize and prep all animals having surgery before donning the gloves that s/he will wear during the procedure, etc.

• When feasible, the incision site should be draped aseptically with sterile material prior to making an incision to create a sterile surgical field. Draping is especially important when suture material will be used. Glad’s Press’n Seal provides a sterile, inexpensive and effective method to cover the surgical field. Although this is a food/grocery item, it has been tested 100% negative for the presence of any microorganisms and organic material. The sticky part is placed on the animal, which allows easy monitoring due to the see-through nature of this material. Make sure the nose is not covered to avoid suffocation if a gas mask is not used. This type of covering may also be used to cover areas outside the surgical field that may need to be manipulated by the surgeon (e.g., gas anesthesia dials, knobs of the microscope or stereotaxic apparatus) and the surgical table.

• Instruments, suture material, suture needle, etc. must never touch outside of the sterile surgical field. • When working alone and manipulation of non-sterile objects (e.g. anesthesia machines, microscopes, lighting, etc.) is required, it may be helpful to use sterile aluminum foil or sterile plastic covers to manipulate the objects.

• Consult with your IC’s Animal Program Director to ensure that your surgery practices meet the standards of aseptic surgery.

**Operative:**

• The animal must be maintained in a surgical plane of anesthesia throughout the procedure.

o If using the pedal withdrawal reflex to test depth of anesthesia, the rear paw has been shown to be more reliable than the forepaw.

o If neuromuscular blocking agents (e.g. pancuronium, succinyl choline) are administered then alternative indicators of anesthetic depth must be monitored. Contact your veterinarian for equipment recommendations and information on how to interpret monitoring results. Animals on neuromuscular blockers must be mechanically ventilated.

• Provide an external heat source (preferably a feedback-controlled, infrared, warm water or air circulating heating device) throughout anesthesia and surgery; contact your veterinarian for information about alternative thermal support devices. Electric heating pads and heat lamps are not recommended because of their potential to cause burns. Hypothermia is a common cause of mortality in rodents undergoing a surgical procedure due to their high surface area to body mass ratio.

• Begin surgery with sterile instruments and handle instruments aseptically (see Table 3).

• When using “tips-only” technique, the sterility of the instrument tips must be maintained throughout the procedure.

• Monitor and maintain the animal's vital signs and hydration.

• Close surgical wounds using appropriate techniques and materials (see Table 4).

**Post-Operative:**

• Move the animal to a warm, dry area and monitor during recovery. Recovery cages should be clean and have supplemental heat. Frequently cages are placed with a heating device under half of the cage. Return the animal to its routine housing only after it has recovered from anesthesia. (i.e. ambulating purposefully in the cage).

• Continue to provide analgesics as appropriate and approved in your Animal Study Proposal.

• If appropriate, consider giving warm fluids and/or nutritional support.

• Generally, remove skin closures 7 to 14 days post-operatively after verifying that the wound has healed.

• Maintain a 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.).

• Continue frequent monitoring of the animal until it is stable (e.g., body weight, body condition, cage activity, etc.)

Adapted from:

NIH Animal Research Advisory Committee Guidelines: Guidelines for Survival Rodent Surgery

<https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/b6-survival_rodent_surgery.pdf>

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**Table 1. Recommended Hard Surface Disinfectants** (e.g., table tops, non-surgical equipment) Note: Always follow manufacturer's instructions for dilution and expiration periods

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| AGENT | EXAMPLES | COMMENTS |
| Alcohols | 70% ethyl alcohol 85% isopropyl alcohol | Contact time required is 15 minutes. Contaminated surfaces take longer to disinfect. Remove gross contamination before using. Inexpensive. |
| Quaternary Ammonium | Roccal®, Quatricide® | Rapidly inactivated by organic matter. Compounds may support growth of gram negative bacteria |
| Chlorine | Glutaraldehydes (Cidex® Cetylcide®, Cide Wipes®) | Rapidly disinfects surfaces |
| Phenolics | Lysol®, TBQ® | Less affected by organic material than other disinfectants. |
| Chlorhexidine | Nolvasan® , Hibiclens® | Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses. |
| Hydrogen peroxide  Peracetic Acid | Spor Klenz | Contact time 10 minutes |

**Table 2. Skin Disinfectants**

Note: Alternating disinfectants is more effective than using a single agent. For example, an iodophor scrub can be alternated three times with 70% alcohol, followed by a final soaking with a disinfectant solution. Alcohol, by itself, is not an adequate skin disinfectant. The evaporation of alcohol can induce hypothermia in small animals.

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| AGENT | EXAMPLES | COMMENTS |
| Iodophors | Betadine®, Prepodyne®, Wescodyne® | Reduced activity in presence of organic matter. Wide range of microbicidal action. Works best in pH 6-7. |
| Chlorhexidine | Nolvasan®, Hibiclens® | Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses. Excellent for use on skin |

**Table 3. Recommended Sterilants for Surgical Instruments & Equipment** (i.e. implants and catheters) Note: Always follow manufacturer's instructions for dilution, exposure times and expiration periods

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| Steam Sterilization (recommended) | Autoclave | Effectiveness dependent upon temperature, pressure and time, e.g. 121°C for 15 min vs 131°C for 3 min. Autoclave bags, wrapping in surgical drape or hard autoclavabe container recommended, with expiration dates of 1 year. |
| Dry Heat | Hot Bead Sterilizer Dry Chamber (useful to sterilize instruments between surgeries) | Fast. Instruments must be cooled before contacting tissue. Only tips of instruments are sterilized with hot beads. |
| Chlorine | Sterilant Levels of Chlorine dioxide (Clidox®, Alcide®) Sodium hypochlorite (Clorox® 10% solution) | Corrosive to instruments. Items must be clean and free of organic material. Instruments must be rinsed with sterile saline or sterile water before use |
| Gas Sterilization | Ethylene Oxide | Requires 30% or greater relative humidity for effectiveness against spores. Gas is irritating to tissue; all materials require safe airing time. Appropriate sterilization indicators should be used to ensure sterility |
| Glutaraldehydes | Glutaraldehyde (Cidex®, Cetylcide®, Metricide®) | Several hours required for sterilization. Corrosive and irritating. Instruments must be rinsed with sterile saline or sterile water before use. Product expiration dates must be adhered to as per manufacturer’s instructions. |
| Hydrogen peroxide  Acetic acid | Actril®, Spor-Klenz® | Several hours required for sterilization. Corrosive and irritating. Instruments must be rinsed with sterile saline or sterile water before use |

**Table 4. Wound Closure Selection**

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| MATERIAL | CHARACTERISTICS and FREQUENT USES |
| Polyglactin 910 (Vicryl®), Polyglycolic acid (Dexon®) | Multifilament, Absorbable in 60-90 days; 25-50% loss of tensile strength in 14-21 days. Ligate or suture subcutaneous tissues where an absorbable suture is desirable. Not routinely recommended for skin closure due to high capillarity. |
| Polydiaxanone (PDS®) or, Polyglyconate (Maxon®) | Monofilament, Absorbable in 6 months; 40% loss of tensile strength in 30-42 days. Ligate or suture tissues especially where an absorbable suture and extended wound support is desirable. |
| Polypropylene (Prolene®) | Non-absorbable. Inert |
| Nylon (Ethilon ®) | Non-absorbable. Inert. General closure |
| Silk | Non-absorbable. (Caution: Tissue reactive and may wick microorganisms into the wound, so silk is not recommended for skin closure). Excellent handling. Preferred for cardiovascular procedures |
| Stainless Steel Suture/Wound Clips/Wound Staples | Non-absorbable. Requires instrument for removal. |
| Cyanoacrylate (Vetbond®, Nexaband®, Tissue Mend®) | Skin glue. For non-tension bearing wounds. |

**Suture gauge selection**: Use the smallest gauge suture material that will perform adequately.

**Cutting and reverse cutting needles:** Provide edges that will cut through dense, difficult to penetrate tissue, such as skin.

**Non-cutting, taper point or round needles**: Have no edges to cut through tissue; used primarily for suturing easily torn tissues such as peritoneum or intestine.

NIH Animal Research Advisory Committee Guidelines: Guidelines for Survival Rodent Surgery

<https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/b6-survival_rodent_surgery.pdf>