

## Guidelines for Survival Rodent Surgery

**Scope:** These guidelines apply to all surgical procedures performed on rodents at the NIH in which the animals are expected to recover from anesthesia. Prior to performing any survival surgery techniques on rodents, an approved Animal Study Proposal (ASP) must be in place with descriptions of the surgical procedures to be performed and the anesthesia/analgesia<sup>1,2</sup> that will be used pre-emptively, during and post-procedure. Personnel must also be appropriately trained. Specific procedures to accomplish these guidelines can be obtained from your veterinarian.<sup>3,4</sup>

**General:** It is important to note that rodents do not vomit, so it is not necessary to fast them prior to surgery.<sup>5</sup> The following principles described in the *Guide for the Care and Use of Laboratory Animals* apply to rodent surgery.<sup>6</sup>

- 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 may be used. During the surgery period, the area should be dedicated to rodent surgery 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, clean gloves may be used for 'tips only' technique but sterile gloves are preferred.

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:

- Aseptic technique
- Gentle tissue handling
- Minimal dissection of tissue
- Maintain tissue hydration
- Appropriate use of instruments
- Effective hemostasis
- Use of suture materials and patterns or other wound closure techniques that minimize trauma and remain intact.

In addition to clinically sound techniques, a 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.<sup>7</sup> Investigators must assure that the challenges of consecutive surgeries within one work session are adequately addressed. A surgical plan should include:

- Analgesia
- Preservation of corneal integrity/hydration
- Nutritional support
- Maintenance of body temperature
- Hydration

### Definitions:

- **Aseptic Technique:** is used to reduce microbial contamination to the lowest possible practical level.
- **Major Surgery:** Major survival surgery penetrates and exposes a body cavity, produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transaction (e.g., laparotomy, thoracotomy, joint replacement, craniotomy, sciatic

- nerve cuff, and limb amputation).
- **Minor Surgery:** 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, and most procedures routinely done on an “outpatient” basis in veterinary clinical practice). Animals recovering from these minor procedures typically do not show significant signs of post-operative pain, have minimal complications, and return to normal function in a relatively short time.
  - **Sterilization:** The process whereby all microorganisms including spores are eliminated or destroyed. The criterion of sterilization is the failure of organisms to grow if a growth supporting medium is supplied.
  - **Disinfection:** The chemical or physical process that involves the destruction of pathogenic organisms. All disinfectants are effective against vegetative forms of organisms, but not necessarily spores.

## **Procedures:**

### **Personal Protective Equipment:**

- Clean covering such as a lab coat worn over work clothes
- Mask
- Gloves
  - Using sterile surgical gloves allows you to touch all areas of the sterile surgical field and surgical instruments with your gloved hand.
  - 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:**

- Surgery should be conducted in a disinfected, uncluttered area that promotes asepsis during surgery. Hard surfaces such as tabletops and non-surgical equipment should be disinfected prior to setting up surgical area (see Table 1).<sup>8</sup>
- After anesthetizing the animal, remove the hair from the surgical site by either clipping, plucking, or using a depilatory. 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 ASP.
- Protect eyes from corneal desiccation by applying sterile ophthalmic ointment since anesthesia abolishes the blink reflex.<sup>9</sup>
- Take measures to minimize hypothermia by providing heat.<sup>9</sup>
- Prepare the surgical site(s) with an appropriate skin disinfectant (see Table 2).<sup>10</sup> If using a stereotaxic frame, the rodent should be placed in the frame *before* the skin disinfectant is applied.
  - Alternating disinfectants is more effective than using a single agent. For example, an iodophor scrub can be alternated three times with 70% alcohol or sterile saline, followed by a final soaking with a disinfectant solution. Alcohol, by itself, is not an adequate skin disinfectant.<sup>10-12</sup>
  - Aseptic scrub skin preparation may contribute to hypothermia. Alternating with alcohol reduces body temperature but results in a rebound phase in which body temperature returns to baseline within a few minutes after application.<sup>11-13</sup>
- Surgeons should wash and dry their hands before aseptically donning sterile gloves.<sup>14</sup>
- Nitrile examination gloves can be either autoclaved or gas sterilized as an economical alternative to pre-packaged sterile surgical gloves.<sup>15</sup> 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:
  - The surgeon's gloves have not become contaminated during respective surgeries or
  - 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.<sup>16</sup>
- When feasible, the surgical 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.<sup>17</sup>
- 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.<sup>18</sup>
  - If using the pedal withdrawal reflex to test depth of anesthesia, the rear paw has been shown to be more reliable than the forepaw.
  - If neuromuscular blocking agents (e.g. pancuronium, succinylcholine) 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. Hypothermia is a common cause of mortality in rodents undergoing a surgical procedure due to their high surface area to body mass ratio. 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.
- Begin surgery with sterile instruments and equipment (e.g. implants and catheters). Handle instruments and equipment aseptically (see Table 3).
- When using "tips-only" technique, the sterility of the instrument tips must be maintained throughout the procedure.
- Consider monitoring the animal's vital signs (e.g. respiratory rate, heart rate, body temperature) and tissue hydration.
- Ensure hemostasis and minimize blood loss.
- Close surgical wounds using appropriate techniques and materials (see Table 4).
  - 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.
- When surgical procedures are performed in series, utilized instruments, suture material and sterile gloves for multiple animals may be considered. Individual IC ACUC should base the number of animals undergoing a surgical procedure with the same sterile instrument pack, gloves, and suture package on performance standards to ensure animal welfare. In general, the recommendations are to:
  - Instruments:
    - Begin with sterile instruments and utilize a "tips-only" technique to sterilize the tips of the instruments between each procedure using a hot bead sterilizer
    - Begin with sterile instruments and utilize a modified aseptic technique such as soaking instruments in 70% isopropyl alcohol between surgeries for two minutes. A new set of

instruments should be used after 5 animals.<sup>19</sup>

- Clean instrument of blood and organic material prior to bead sterilizer or alcohol soak.
- Assure instruments are cooled after bead sterilizing, before touching tissue (sitting at room temperature for several minutes or dipping in sterile saline). Rinse alcohol after soaking in sterile saline or sterile water.
- Gloves
  - Begin with clean or sterile gloves and utilize a tips only surgery technique to prevent cross-contamination
  - Begin with sterile gloves and dip the fingers of the gloves in alcohol for 30 seconds between surgeries to sanitize them<sup>19</sup>
  - Consider changing gloves after every 5 animals or every cage of animals
- Suture
  - When using the same suture pack across serial surgeries to close muscle or skin the animal should be draped in and suture material must remain in the sterile field for a maximum of 5 animals.

**Post-Operative:**

- Move the animal to a warm, dry area and monitor during recovery. 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 ASP.
- If appropriate, consider giving warm fluids and/or nutritional support.
- Animals must be monitored and evaluated post-operatively. Some examples of monitoring parameters which may be employed include: body weight, grimace scale, nesting behavior, or hydration status. Or refer to the [ARAC Guideline Pain and Distress in Laboratory Animals: Responsibilities, Recognition and Alleviation](#). Frequency and duration of post-operative evaluation are established in consultation with veterinary staff.
- Generally, remove skin closures 7 to 14 days post-operatively after verifying that the wound has healed.

**Surgical Record:**

- Creating and maintaining 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.) is required.
- Continue frequent monitoring of the animal post-surgery until stable (e.g., body weight, body condition, cage activities)

**Table 1. Recommended Hard Surface Disinfectants**

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.
Quaternary Ammonium	Roccal®, Quatricide®	Rapidly inactivated by organic matter. Compounds may support growth of gram negative bacteria.
Chlorine	Sodium hypochlorite (Clorox® 10% solution) Chlorine	Corrosive. Presence of organic matter reduces activity. Chlorine dioxide must be fresh; kills vegetative organisms within 3 minutes of contact.
Glutaraldehydes	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 Acetic acid	Spor Klenz	Contact time 10 minutes.
*The use of common brand names as examples does not indicate a product endorsement ** Always follow manufacturer's instructions for dilution and expiration periods		

**Table 2. Skin Disinfectants**

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.
*The use of common brand names as examples does not indicate a product endorsement.		

**Table 3. Recommended Sterilant For Surgical Instruments & Equipment**

AGENT	*EXAMPLES	COMMENTS
Steam Sterilization (moist heat)	Autoclave	Effectiveness dependent upon temperature, pressure and time, e.g. 121°C for 15 min vs 131°C for 3 min. Appropriate sterilization indicators should be used to ensure sterility.
Dry Heat	Hot Bead Sterilizer Dry Chamber	Fast Instruments must be cooled before contacting tissue. Only tips of instruments are sterilized with hot beads.
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.
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.

Glutaraldehydes	Glutaraldehyde (Cidex®, Cetylcode®, 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.
Alcohol	Ethanol or Isopropanol	Alcohol is neither a sterilant or high-level disinfectant. May be acceptable for some procedures, if prolonged contact time are used. <sup>19,20</sup>
*The use of common brand names as examples does not indicate a product endorsement. Note: Always follow manufacturer's instructions for dilution, exposure times and expiration periods.		

**Table 4. Wound Closure Selection**

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.
Polydioxanone (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®)	Monofilament, Non-absorbable. Inert.
Nylon (Ethilon ®)	Monofilament, Non-absorbable. Inert. General closure.
Silk	Multifilament, 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.
*The use of common brand names as examples does not indicate a product endorsement.	

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