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.[1] 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: 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 dedicated rodent surgical facility is not required. However, a designated animal procedure space is required and at the time of use the aseptic surgery should be conducted in an area which is dedicated to surgery and related activities, and at all times during the surgery managed to minimize contamination.
- All survival surgery will be performed by using aseptic procedures, including masks, sterile gloves, sterile instruments, and aseptic techniques.

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,
- Minimal dissection of tissue,
- Appropriate use of instruments,
- Effective hemostasis, and
- Use of suture materials and patterns or other wound closure techniques that minimize trauma and remain 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 give consideration to the availability of personnel to provide anesthetic induction and post-operative care appropriate to the surgical procedure.

Investigators should work closely with their veterinarian to assure that the challenges of consecutive surgeries within one work session are adequately addressed.

Procedures:

A. Personal Protective Equipment:

1. Clean jumpsuit or lab coat
2. Mask[2]
3. Gloves
   a) Sterile surgical gloves. Using sterile surgical gloves allows you to touch all areas of the sterile surgical field and surgical instruments with your gloved hand.
   b) Clean exam gloves. 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

[1] A compact disc with depictions and expanded explanations of the methods recommended in these guidelines is available by sending a request to rodentcd@od.nih.gov .

[2] Because of the necessity of mouth pipetting, masks are not worn during embryo transfer surgeries.

instruments, the suture, suture needle, or any part of the surgical field. This technique is useful when working alone and manipulation of non-sterile objects (e.g., anesthesia machines, microscopes, lighting) is required (see Brown, PA & Hoogstraten-Miller, S).

4. Hair cover

B. Pre-Operative:

1. Surgery should be conducted in a disinfected, uncluttered area that promotes asepsis during surgery (see Table 1 below).
2. Prepare the animal by removing hair from the surgical site. Whenever possible, perform this procedure in an area separate from where the surgery is to be conducted.
3. Administer analgesics (preemptive analgesia) as appropriate and approved in your Animal Study Proposal.
4. Protect the corneas from drying out by applying an ophthalmic ointment.
5. Prepare the surgical site(s) with an appropriate skin disinfectant (see Table 2).
6. Surgeons should wash and dry their hands before aseptically donning sterile surgical gloves.

C. Operative:

1. The animal must be maintained in a surgical plane of anesthesia throughout the procedure.
   a.) If using the pedal withdrawal reflex to test depth of anesthesia, the rear paw has been shown to be more reliable than the forepaw.
   b.) If neuromuscular blocking agents (e.g. pancuronium, succinyl choline) are used, monitoring of autonomic nervous system responses (e.g. heart rate, blood pressure) should be used to monitor anesthetic depth.
2. Begin surgery with sterile instruments and handle instruments aseptically (see Table 3).
3. When using "tips-only" technique, the sterility of the instrument tips must be maintained throughout the procedure.
4. Instruments and gloves may be used for a series of similar surgeries in the same session, provided they are maintained clean and disinfected between animals (see Table 4).
5. Monitor and maintain the animal's vital signs and hydration.
6. Close surgical wounds using appropriate techniques and materials (see Table 5).

D. Post-Operative:

1. Move the animal to a warm, dry area and monitor it during recovery. Return the animal to its routine housing only after it has recovered from anesthesia (i.e., the animal can maintain itself in sternal recumbency).
2. Provide analgesics as appropriate and approved in your Animal Study Proposal.
3. If appropriate, consider giving fluids and/or nutritional support.
4. Generally, remove skin closures 7 to 14 days post-operatively after verifying that the wound has healed.
5. 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.).
6. Continue daily monitoring of the animal until it is stable (e.g., body weight, body condition, activity, etc.).

References:

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APPENDIX - Guidelines for Survival Rodent Surgery

This appendix includes definitions, tables of information, and references as a resource for investigators.

DEFINITIONS:

ASEPTIC SURGICAL PROCEDURES: Surgery performed using procedures that limit microbial contamination so that significant infection or suppuration does not occur.

MAJOR SURGERY: Major survival surgery (e.g., laparotomy, thoracotomy, joint replacement, and limb amputation) penetrates and exposes a body cavity, produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transaction.

MINOR SURGERY: Minor survival surgery does not expose a body cavity and causes little or no physical impairment; this category includes 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 viable microorganisms 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.

Table 1. RECOMMENDED HARD SURFACE DISINFECTANTS (e.g., table tops, equipment)
Always follow manufacturer's instructions for dilution and expiration periods.

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	Sodium hypochlorite (Clorox ® 10% solution) Chlorine dioxide (Clidox®, Alcide®, MB-10®)	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.
Chlorhexidin	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.

Table 2. SKIN DISINFECTANTS

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.

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.
Cholorhexidine	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 INSTRUMENT STERILANTS

Always follow manufacturer's instructions for dilution, exposure times and expiration periods.

AGENT	EXAMPLES *	COMMENTS
Steam sterilization (moist heat)	Autoclave	Effectiveness dependent upon temperature, pressure and time (e.g., 121^{O}C for 15 min. vs 131^{O}C for 3 min).
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.
Chlorine	Chlorine Dioxide	Corrosive to instruments. Instruments must be rinsed with sterile saline or sterile water before use.
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.
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.

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

Table 4. RECOMMENDED INSTRUMENT DISINFECTANTS

Always follow manufacturer's instructions for dilution, exposure times and expiration periods.

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.
Chlorine	Sodium hypochlorite (Clorox ® 10% solution) Chlorine dioxide (Clidox®,	Corrosive. Presence of organic matter reduces activity. Chlorine dioxide must be fresh. Kills vegetative organisms within 3 min. Corrosive to instruments. Instruments must be rinsed with sterile saline or sterile water before use.
Chlorhexidine	Nolvasan® , Hibiclens®	Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses. Instruments must be rinsed with sterile saline or sterile water before use.

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

Table 5. WOUND CLOSURE SELECTION

MATERIAL*	CHARACTERISTICS AND FREQUENT USES
Polyglactin 910 (Vicryl®), Polyglycolic acid (Dexon®)	Absorbable; 60-90 days. Ligate or suture tissues where an absorbable suture is desirable.
Polydiaxanone (PDS®) or, Polyglyconate (Maxon®)	Absorbable; 6 months. Ligate or suture tissues especially where an absorbable suture and extended wound support is desirable
Polypropylene (Prolene®)	Nonabsorbable. Inert.
Nylon (Ethilon®)	Nonabsorbable. Inert. General closure.
Silk	Nonabsorbable. (Caution: Tissue reactive and may wick microorganisms into the wound, *so silk is not recommended for skin closure*). Excellent handling. Preferred for cardiovascular procedures.
Chromic Gut	Absorbable. Versatile material.
Stainless Steel Suture/Wound Clips/Wound Staples	Nonabsorbable. 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.

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.