

SURGICAL RESEARCH GUIDELINES

Guidelines for Rodent Survival Surgery

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The Academy of Surgical Research (ASR) is an international organization that promotes the advancement of professional and academic standards, education, and research in the arts and sciences of experimental surgery. The Academy interfaces with medical and scientific organizations and governmental agencies in establishing and reviewing ethics, theories, practices, and research pertaining to surgical research and promotion of the results for clinical application. The mission when ASR was founded in 1982 was to encourage, foster, promote, and advance professional and academic standards, education and research. In keeping with the Academy's mission, "Guidelines for Rodent Survival Surgery" were established to outline standards that could be used by institutions to establish local practices and procedures.

The United States Department of Agriculture's (USDA) *Animal Welfare Act (AWA)* regulations, the Public Health Service's (PHS) *Guide for the Care and Use of Laboratory Animals (Guide)*, and Canadian Council on Animal Care's (CCAC) *Guide to the Care and Use of Experimental Animals* require aseptic technique be used for survival surgical procedures performed on animals. Scientific and ethical responsibility also dictates that

the biomedical research community utilizes the highest standard of care to assure humane outcomes and reliable data. The following guidelines were developed by a subcommittee of the Academy of Surgical Research* and subsequently approved by the Board as the standards under which rodent survival surgical procedures should be performed. The following Guidelines apply to major and minor surgical procedures performed on rodents, with the exception of nonsurvival procedures.

DEFINITIONS

Aseptic Surgical Procedures: Surgery performed using procedures that limit microbial contamination (i.e., aseptic technique) so that infection or suppuration does not occur.

Sanitation: The establishment of conditions favorable to good health, especially with respect to infectious diseases or to make physically clean and to remove, to a practical minimum, agents injurious to health. This encompasses general cleaning to make the process of disinfection more effective.

Disinfection: The chemical or physical process that involves the destruction of pathogenic organisms to ineffectual levels. Disinfectants may be effective against vegetative forms of organisms, spores, and viruses depending on the classification.

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Sterilization: The process whereby all viable microorganisms and their spores are eliminated or destroyed. The criterion for sterilization is the failure of organisms to grow if a growth-supporting medium is provided. Sterilization indicators should be used to confirm that materials have undergone proper sterilization.

Major Surgery: Any surgical intervention that penetrates and exposes a body cavity; any procedure that has the potential for producing permanent physical or physiological impairment.

Minor Surgery: Any surgical intervention that neither penetrates and exposes a body cavity nor produces permanent impairment of physical or physiological function.

Survival Surgery: Any surgical intervention performed on a live animal under general anesthesia from which the animal is expected to recover from anesthesia.

Non-survival or terminal surgery: Any surgical intervention where the animal is euthanized before recovery from anesthesia.

GENERAL CONSIDERATIONS IN RODENT SURGERY

1. Rats and mice have a high surface area to body volume ratio and rapid metabolism. Pharmacological doses are usually higher than in larger species.
2. Dehydration can occur faster per unit of time than in larger species.
3. Rats and mice lose body heat rapidly through hairless areas and hypothermia during surgery is a frequent cause of intra-operative mortality.
4. Rodents have limited fat storage and energy reserves, which may contribute to hypothermia.
5. Changes in protein metabolism can occur postoperatively leading to negative nitrogen balance lasting for several days. Even minor surgical procedures can produce prolonged effects.
6. Minimizing tissue trauma, preventing infection, controlling post-surgical pain and discomfort, and supporting animals' nutritional needs will reduce the magnitude of metabolic response to surgery.

Aseptic Technique

According to the AWA and the *Guide*, survival surgery on rodents must be performed using aseptic procedures. Aseptic technique is a set of specific practices and procedures performed under carefully controlled conditions with the goal of minimizing contamination by pathogens. This includes preparation of the patient, preparation of the surgeon, sterilization of instruments,

supplies, and implanted materials, and the use of other operative techniques to reduce the likelihood of infection. All individuals performing surgery must be appropriately trained in aseptic techniques to ensure that good surgical technique is practiced.

Surgical Facilities

1. Rodent surgical procedures do not require a dedicated facility, but the area in which surgery is conducted should be free of clutter and disinfected (Table 1) prior to the beginning of the surgical session. This area should be located away from air supply ducts or other drafts to minimize hypothermia of the animal and limit accumulation of dirt and dust contamination on surfaces.
2. Access to this area by persons not directly involved in the activities should be limited during the surgical procedures. For most rodent surgery, the facility may be small and simple, such as a dedicated space in a laboratory appropriately managed to minimize contamination from other activities in the room during surgery. The area should not be used for any other activities during the surgical procedures.
3. A clean towel, drape, or diaper can be placed beneath the animal to provide a warm, soft, absorptive surface.
4. Rodent surgical facilities can also include other components such as holding and recovery areas located in a quiet area where the animals can be observed.
5. Draping accessory and support equipment (e.g., lights, microscopes, monitoring equipment/leads, anesthesia machine, cautery equipment, etc.) can allow the surgeon to maintain a sterile field if adjustments in equipment are needed during the procedure.

Pre-Operative Preparation

1. *Patient Preparation*
 - a. Fasting of rodents is unnecessary and due to the high metabolic rate may deplete energy reserves quickly. Rodents can be acclimated to postoperative diet supplementation (e.g., gels or liquids intended to deliver analgesics) several days prior to surgery to help prevent neophobia.
 - b. A presurgical evaluation of the animal's health (physical examination) done prior to surgery may help identify animals that have underlying disease conditions and may not be suitable for surgery.
 - c. The surgical site(s) must be prepared by closely clipping and removing the hair. This should be done in an area separate from where the surgical procedures are to be performed. Avoid skin

Table 1. Recommended hard surface disinfectants (e.g., table tops, equipment) (follow manufacturer’s recommended concentration and contact times for all disinfecting agents)

Agent	Examples	Comments
Hydrogen Peroxide (H ₂ O ₂) & Peroxyacetic Acid	Sporklenz [®]	Broad-spectrum disinfectant; requires >10 min contact time
Chlorhexidine	Nolvasan [®] Solution Hibiclens [®]	Presence of blood does not interfere with activity; rapidly bactericidal and has residual effect; effective against many viruses
Phenolics	LpH [®] , Bruphene 256 [®] Lysol [®]	Less affected by organic contamination than other disinfectants
Glutaraldehydes	Cidex [®] , Omnicide [®] , Banacide [®]	High-level disinfectants with 5–45 min contact time; can be used as a sterilant with multiple hours contact time; exposure time limits set by Occupational Safety and Health Administration (OSHA)
Alcohols	70% ethyl or isopropyl	Contact time required is 15 min. Contaminated surfaces take longer to disinfect. Remove gross contamination before using
Chlorine	Sodium hypochlorite (Clorox 10% solution), Chlorine dioxide (Clidox [®] , Alcide [®])	Corrosive. Presence of organic matter reduces activity. Chlorine dioxide must be fresh (<14 days old); kills vegetative organisms within 3 min of contact
Quaternary ammonia	Coverage [®] , TBQ [®] , Roccal [®]	Rapidly inactivated by organic matter. Compounds may support growth of gram negative bacteria

abrasions and thermal injuries during clipping and avoid clipping excess hair as this may exacerbate hypothermia. This is followed by an application of a skin disinfectant solution (Table 2).

- d. If limbs must be positioned for control of the surgical field, avoid placing excessive tension on the limbs and avoid excessive stretching which may traumatize joints and impair breathing.
- e. Surgical site preparation should include use of an appropriate skin disinfectant (Table 2) applied

in three alternating cycles of scrubbing with a surgical soap and rinsing with sterile water or 70% isopropyl alcohol. Using a disinfectant scrub solution and cotton swabs or gauze, disinfection should begin along the incision line and extend outward in a circular pattern. Careful application should be done so as to not wet large areas of the animal as this may exacerbate hypothermia. This is followed by an application of a skin disinfectant solution. Keeping these fluids warmed also helps to reduce hypothermia.

Table 2. Recommended skin disinfectants (alcohol may be used alternating with the agents listed below. Care should be taken as evaporation of alcohol can contribute to hypothermia in small animals)

Agent	Examples	Comments
Chlorhexidine	Nolvasan [®] Scrub, Hibiclens [®]	Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses. Excellent for use on skin
Iodophores	Betadine [®] Scrub, Prepodyne [®] , Wescodyne [®] , Duraprep [®]	Reduced activity in presence of organic matter. Wide range of microbicidal action. Works best at pH 6–7

- f. The use of a sterile drape over the animal is recommended to prevent contamination of suture material and to assure a sterile field at the surgical site.
 - g. Sterile, nonmedicated ophthalmic ointment should be applied to the eyes to prevent corneal drying.
 - h. Supplemental heat sources (water circulating heating pads, forced air-warming blankets, heat lamps, etc.) may be used to help maintain normal body temperatures depending on the length of the surgical procedure. Caution must be used with heat lamps to prevent overheating or burning the animal.
2. *Surgeon Preparation*
- a. The surgeon shall don a surgical cap/hat, face-mask, and a clean lab coat, surgical scrubs, or other garments to cover street clothes.
 - b. Surgeons should wash their hands thoroughly and dry them prior to putting on sterile gloves.

Intra-Operative Procedures

1. The animal must be maintained in a surgical plane of anesthesia throughout the procedure and vital signs (breathing, skin color, etc.) should be monitored as appropriate.
 - a. New technologies for vital sign monitoring in rodents are available including pulse oximetry, ECG, invasive and noninvasive blood pressure, and body temperature-regulated surgical warming pads.
 - b. Anesthesia assessment (toe pinch reflex, respiratory rate and quality, corneal reflex, etc.) should be done prior to initiating an incision and routinely thereafter to assure an adequate plane of anesthesia.
2. Instruments and devices that penetrate the skin or that will be implanted (needles, suture material, catheters, trocars, telemeters, etc.) must be sterilized by an approved method (Table 3) prior to the beginning of surgery.

Table 3. Recommended instrument sterilants (instruments should be cleaned of all gross debris prior to sterilization. Follow manufacturer’s recommended concentrations and contact times for all sterilizing agents)

Agent	Examples	Comments
Moist heat (steam)	Autoclave	Effectiveness depend upon temperature, pressure, and time (e.g., 121°C for 15 min vs. 131°C for 3 min)
Dry heat	Hot bead sterilizer, dry chamber	Fast. Instruments must be clean prior to use and must be cooled before contacting tissue. Note that the hot bead sterilizer acts only on the tips of instruments and should therefore be used to resterilize the working end of instruments between surgeries, not as an initial means of sterilization
Ionizing radiation	Gamma radiation	Requires special equipment and training
Gas sterilization	Ethylene Oxide (ETO) plasma vapor	ETO requires 30% or greater relative humidity for effectiveness against spores. ETO gas is irritating to tissue; all materials require safe aeration time
Hydrogen Peroxide (H ₂ O ₂) & Peracetic Acid	Sporklenz [®] (full strength)	Broad-spectrum. Sporicidal-fungicidal sterilants only with contact time of 11 hr or more. Instruments must be rinsed with sterile water or saline prior to tissue contact
Chlorine	Chlorine dioxide (Clidox [®] , Alcide [®])	A minimum of 6 hr is required for sterilization. Presence of organic matter reduces activity. Solutions must be freshly prepared (<14 days). Instruments must be rinsed thoroughly with sterile water or saline prior to tissue contact
Aldehydes	Formaldehyde (6% solution) Glutaraldehyde (Cidex [®])	All aldehydes are sterilants only with many hours of contact. Corrosive and irritating. Consult Occupational Health on proper use. Glutaraldehyde is less irritating and less corrosive than formaldehyde. Instruments must be rinsed thoroughly with sterile water or saline prior to tissue contact

Table 4. Recommended instrument disinfectants (Follow manufacturer’s recommended concentrations and contact times for all disinfecting agents. Note: Trays of solution used to disinfect clean instruments between surgeries should be reserved for this purpose; instruments should not move in and out of these solutions during the surgery, but should be placed in a sterile location in the surgical area or in a separate tray of sterile water or saline. Tissues and fluids should be removed from the instruments prior to the disinfection process)

Agent	Examples	Comments
Dry heat	Hot bead sterilizer	Actually a rapid means to resterilize the ends of instruments (requiring contact time of <10 s) and therefore preferable to the liquid disinfectants listed below. Instruments must be clean prior to use and must be cooled before contacting tissue
Hydrogen Peroxide (H ₂ O ₂) & Peroxyacetic Acid	Sporklenz [®] (full strength)	Broad-spectrum disinfectant. Requires contact time of ≥10 min. Instruments must be rinsed with sterile water or saline prior to tissue contact
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 water or saline prior to tissue contact
Aldehydes	Formaldehyde (6% solution) Glutaraldehyde (Cidex [®])	Corrosive and irritating. Requires contact time of greater than 15 min. Consult Occupational Health on proper use. Glutaraldehyde is less irritating and less corrosive than formaldehyde. Instruments must be rinsed thoroughly with sterile water or saline prior to tissue contact
Chlorine	Sodium hypochlorite (Clorox 10% solution), Chlorine dioxide (Clidox [®] , Alcide [®])	Corrosive. Presence of organic matter reduces activity. Chlorine dioxide must be fresh (<14 days old); kills vegetative organisms with 3 min of contact. Instruments must be rinsed thoroughly with sterile water or saline prior to tissue contact

3. A sterile drape or tray should be used to provide a sterile place to set instruments during the surgery.
4. Instruments may be used for a series of similar surgeries, provided that they are cleaned and disinfected between animals (Table 4). Two sets of sterile instruments would facilitate not having to disinfect instruments between animals. Use of “tips-only” technique requires that the tips of the instruments remain sterile during the procedure.
5. Contaminated gloves should be changed between animals. Alternatively, if precautions are taken to minimize contamination, gloves can be disinfected and rinsed prior to next animal.
6. Fluids given parenterally or used as lavage during surgery must be sterile. It is recommended that the fluids be warmed prior to administration. Rodents are vulnerable to the effects of fluid loss because of their small size and administering warm sterile fluids before surgery (1–2 ml/100 g SQ) is recommended. This can be repeated following surgery, if necessary, to replenish blood or fluid losses.
7. Proper surgical technique must be practiced, i.e., asepsis, gentle tissue handling, minimal dissection of tissue, appropriate use of instruments, and effective hemostasis.
8. Surgical wounds must be closed using appropriate techniques and materials (Table 5). If body cavities are penetrated, closure of these wounds must be done in a minimum of two layers (body wall and skin).
9. Deep tissue layers (subcutaneous tissues, fascia, muscle, etc.) should be closed using absorbable suture materials (Table 5).
10. Non-capillary materials (nylon, polypropylene, polyethylene, stainless steel, surgical staples, wound clips, etc.) should be used for skin closure. Standard rodent housing potentially exposes wounds to contaminated bedding, which can lead to bacterial pathogens wicking into incision sites via braided materials.
11. Interrupted suture patterns should be used to prevent occurrence of wound dehiscence. Rodent skin has a propensity to invert; so everting suture patterns (horizontal mattress, simple interrupted, etc.) should be used.
12. Emergency care

Table 5. Recommended closure materials (Note: Always use the smallest gauge suture material that will perform adequately. Cutting or reverse cutting needles provide edges that will cut through dense, difficult to penetrate tissues, such as skin. Taper point or round needles have no edges to cut through tissues and are used primarily for suturing easily torn tissues, such as peritoneum or intestine)

Material	Characteristics and typical uses
Vicryl [®] , Dexon [®]	Multifilament. Absorbable in 60–90 days. Used for ligation or suturing where absorbable material is desirable
PDS [®] , Maxon [®]	Monofilament. Absorbable in 6 months. Used for ligation or suturing where absorbable material is desirable, yet extended wound support is required
Ethibond [®] , silk	Multifilament. Nonabsorbable. Used for ligation or suturing where nonabsorbable material is desirable
Ethilon [®] , Prolene [®]	Monofilament. Nonabsorbable. Used for general skin closure
Chromic gut	Multifilament. Absorbable. Moderate tissue reactivity, rapid loss of strength
Stainless steel wound clips or staples	Nonabsorbable. Requires special instrument for removal from skin. Must not be clamped too tight
Nexaband [®] and other tissue adhesives	Absorbable and fast. Slightly less tensile strength than standard suture materials

(Adapted from the National Institutes of Health Intramural Research Program Guidelines for Survival Rodent Surgery and Washington University Rodent Survival Surgery Guidelines.)

- a. *Hypoxia* – The airway should be maintained with proper positioning of the animal’s head and neck and supplemental oxygen can be given if necessary via a mask or tube taped onto the surgery table in the vicinity of the animal’s nose. If the respiration rate falls progressively (40–90 breaths/min acceptable), ventilation may be assisted by gentle compression of the chest at a rate of 1 breath/s. In addition, anesthetic antagonist agents (e.g., naloxone, yohimbine, atipamezole, etc.) or respiratory stimulant (doxapram) can be administered following the surgical procedure.
 - c. *Cardiovascular dysfunction*: This is characterized by poor tissue perfusion. The cause of the cardiac impairment should be identified. For anesthetic overdose, use of appropriate antagonist or an anticholinergic agents (e.g., atropine, glycopyrrolate) can reverse the adverse effects. Additional fluid therapy should be used if there is moderate or significant hemorrhage, and warming pads should be used to prevent hypothermia.
2. The animal’s eyes should be lubricated again, if needed, to prevent drying.
 3. If applicable, anesthetic/sedative antagonists can be used to speed anesthesia recovery.
 4. A patent airway should be maintained by swabbing out the mouth and adjusting body positioning in the recovery cage.
 5. Fluids, analgesics, and antibiotics should be provided as described in an institutionally approved animal protocol.
 6. A surgical record should be maintained, including the type of procedure, date performed, surgeon, and any complications.
 7. Monitoring after anesthesia recovery should include attention to basic biological functions of appetite and elimination, attitude, activity, appearance, and behavioral signs of postoperative pain.
 8. Postoperative monitoring should include daily assessment of the wound for postsurgical infections, monitoring of the surgical incision, and timely removal of skin sutures, clips, or staples (usually in 10–14 days).
 9. The level, type, and duration of analgesia will depend upon the procedure performed. Minimally invasive procedures generally need less potent or shorter-acting analgesics and animals may respond to a single dose of analgesics. More invasive procedures (e.g., laparotomy, orthopedic surgery) may require multiple doses of analgesics for up to 72 hr postoperatively. Specific criteria for evaluating postoperative pain and provision of analgesia must

Postoperative Procedures

1. The animal should be placed in a warm, dry environment after surgery and monitored until it has recovered from anesthesia and is ambulatory. If animals are group-housed postoperatively, careful monitoring should be done to prevent trauma by cagemates.

be developed through veterinary consult and described in the institutionally approved animal protocol.

10. Potential signs associated with pain or distress in rodents include the following:
 - Decreased food and water consumption, weight loss.
 - Self-imposed isolation/hiding.
 - Rapid, open mouth breathing.
 - Biting, aggression.
 - Increased/decreased movement.
 - Unkempt appearance, piloerection (rough, dull hair coat).
 - Abnormal posture/positioning (hunched back, head-pressing).
 - Dehydration, skin tenting, sunken eyes.
 - Twitching, trembling.

NON-SURVIVAL RODENT SURGERY

Consideration should be given to applying the above Guidelines when performing prolonged non-survival surgical procedures (6–12 hr), in order to decrease the chance of introducing bacteria that may colonize the surgical site or equipment and produce untoward effects within a short period of time. At a minimum, as per the *Guide*, the surgical site should be clipped, the surgical bench and instruments should be cleaned, and the surgeon should wear gloves.

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