Guidelines for Rodent Survival Surgery

Scope:

These guidelines apply to all surgical procedures performed on rodents at 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(1, 2) that will be used pre-emptively, during, and post-procedure. All personnel must also be appropriately trained. Specific procedures to accomplish these guidelines can be obtained from the IC veterinarian(3, 4).

Definitions:

- **Aseptic Surgical Procedures**: surgery performed using procedures (i.e., aseptic technique) that limits microbial contamination to minimize or prevent infection(5).
- **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 transection. Examples include: 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. 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. Examples include: wound suturing, peripheral vessel cannulation, percutaneous biopsy, subcutaneous implants, and most procedures routinely done on an "outpatient" basis in veterinary clinical practice.
- **Sterilization**: The process whereby all viable microorganisms, including spores, are eliminated or destroyed(5). 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(5).

General:

- Rodents do not vomit, so it is not necessary to fast them prior to surgery(6-8).
- Appropriate pre-operative and post-operative care of animals in accordance with established veterinary medical and nursing practices is required.
- A designated animal procedure space for rodent surgeries is required. This could be a dedicated procedure room, a location within a larger procedure room, a laboratory space free from clutter, or even a space within an animal housing room such as a biosafety cabinet. Regardless of the location, the area should be easily disinfected prior to the surgical procedure. During the surgery period, the area should be dedicated to rodent surgery such that cleanliness is ensured and traffic should be limited as this can negatively impact asepsis.
- A "tips-only" technique requires using only the sterile working ends of the surgical instruments to manipulate the surgical field. The gloved hand must never touch the working end of the instruments, the suture, suture needle, or any part of the surgical field(9).

All survival surgeries will be performed using aseptic procedures, including masks, lab coats, gloves (sterile and non-sterile for “tips-only” technique), 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:

- 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, as described in the ASP, 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(10). Investigators must ensure that the challenges of consecutive surgeries within one work session are adequately addressed. A surgical plan should include:

- Aseptic technique including sterile instruments
- Staff availability and their assigned tasks
- Anesthesia
- Analgesia
- Preservation of corneal integrity/hydration
- Nutritional support
- Maintenance of body temperature
- Hydration

Procedures:

Personal Protective Equipment (PPE):

- Clean surgical attire or covering (e.g., lab coat, coveralls, scrubs)
- Mask
- Gloves
    - Using sterile surgical gloves allows you to touch all areas of the sterile surgical field and surgical instruments with your gloved hand. Sterile surgical gloves are not necessary if using a strict "tips-only" technique but are still strongly recommended to help maintain asepsis during the procedure. Individual ICs may enforce stricter requirements for sterile gloves regardless of whether the surgery is being done “tips-only’ or not.
- 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 3) (11).
- Perform a cage side evaluation of the animals before surgery to ensure that there are no health conditions that would make them unsuitable for surgery.
- Apply sterile ophthalmic ointment to protect eyes from corneal desiccation since anesthesia abolishes the blink reflex(12, 13).
- After anesthetizing the animal, remove the hair from the surgical site by either clipping, plucking, using a depilatory product, or however described in the ASP. This procedure must be done in a

separate area and not at the designated animal surgical space. If a depilatory product 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 the ASP.
- Take measures to minimize hypothermia by providing supplemental heat when appropriate, especially for longer periods of anesthesia(13).
- Prepare the surgical site(s) with an appropriate skin disinfectant (see Table 1)(14). If using a stereotaxic frame, the rodent should be placed in the frame *before* the skin disinfectant is applied.
    - A surgical scrub agent 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(14-16).
    - Aseptic 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(15-17).
- Surgeons should wash and dry their hands before donning gloves. Sterile gloves are required for surgeries unless a tips-only technique is used. When practicing the tips-only technique, sterile gloves are recommended to help maintain asepsis but non-sterile examination gloves can also be used(18).
    - Nitrile examination gloves can be either autoclaved or gas sterilized as an economical alternative to pre-packaged sterile surgical gloves(19). Multiple pairs of nitrile 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 will be worn during the procedure, etc. (20).
- 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(21).
- 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 the IC's Animal Program Director or designee 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(5, 22).
    - If using the pedal withdrawal reflex to test depth of anesthesia, the rear paw has been shown to be more reliable than the forepaw(12, 23).
    - If neuromuscular blocking agents (e.g., pancuronium, succinylcholine) are administered then alternative indicators of anesthetic depth must be monitored(12). Contact an IC 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 an IC 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 devices (e.g., implants and catheters). Handle instruments and devices aseptically (see Table 4).
- 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 2).
    - 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 ACUCs 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:
    - Instruments:
        - Begin with instruments sterilized appropriately. Usually, this is autoclaving, but can also include chemical sterilants, ethylene oxide, or other methods (Table 4).
        - Between animals or groups of animals consider options such as:
            - Utilize a "tips-only" technique to sterilize the tips of the instruments between each animal and/or group of animals using a hot bead sterilizer
            - Utilize a new set of sterilized instruments between cages or groups of animals(9, 23).
            - Note: Clean instrument of blood and organic material prior to bead sterilizer or other sterilizing method.
        - Assure instruments are cooled after bead sterilizing, before touching tissue (sitting at room temperature for several minutes, dipping in sterile saline, or dipping in alcohol).
    - Gloves:
        - Begin with appropriate gloves as described above. If using the “tips-only” surgery technique to prevent cross-contamination, only touch the hand end of instruments and do not touch the tips of the instruments with gloves. The tips must only touch the surgical field.
        - Consider changing gloves between cages 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 in-between cages.
    - Use new suture pack between cages.
    - Suture selection may vary on surgical procedure, location of closure (deep vs superficial skin), and degree of tension. Recommend seeking veterinary guidance on suture selection.

Post-Operative:

- Move the animal to a warm, clean, dry area and continue to monitor during recovery. Return the animal to its routine housing only after it has exhibited the righting reflex.
- Continue to provide analgesics as appropriate and approved in the ASP.
- If appropriate, consider giving warm fluids and/or nutritional support(8).
- 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 for Pain and/or Distress in Laboratory Animals. 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. 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.
Alcohol	70% ethyl alcohol 85% isopropyl alcohol	Alcohol is neither a sterilant nor high level disinfectant[23,24]. It should be used with other skin disinfectants, e.g., alternating step with an iodophor or chlorhexadine.
*The use of common brand names as examples does not indicate a product endorsement.		

Table 2. Wound Closure Selection

MATERIAL*	CHARACTERISTICS AND FREQUENT USES
Polyglactin 910 (Vicryl®), Polyglycolic acid (Dexon®)	Multifilament, Absorbable in 60-90 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®) or Poliglecaprone 25 (Monocryl®)	Monofilament, Absorbable in 3-8 months. Ligate or suture tissues especially where an absorbable suture and extended wound support is desirable.
Polypropylene (Prolene®)	Monofilament, non-absorbable. Inert. General skin closure.
Nylon (Ethilon ®)	Monofilament, non-absorbable. Inert. General skin 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. General skin closure. Requires instrument for removal.
Cyanoacrylate (Vetbond®, Nexaband®, Tissue Mend®)	Tissue Adhesive, for non-tension bearing wounds.
*The use of common brand names as examples does not indicate a product endorsement.	

Table 3. Recommended Non-Porous Disinfectants

AGENT(24)	EXAMPLES*	COMMENTS**
Alcohols	70% ethyl alcohol 85% isopropyl alcohol	Flammable***, Contaminated surfaces take longer to disinfect. Remove gross contamination before using and use alcohol as a second step after use of other disinfectants noted below. Allow to air dry after placement and before use of the surface.
Quaternary Ammonium	Roccal®, Quatricide®	Corrosive***. Rapidly inactivated by organic matter. Compounds may support growth of gram-negative bacteria. 1–10-minute contact time required depending on product****.
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****.
Phenolics	Lysol®, TBQ®	Less affected by organic material than other disinfectants. Contact time 3 minutes****.
Chlorhexidine	Nolvasan® , Hibiclens®	Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses. Contact time 2-5 minutes****.

AGENT(24)	EXAMPLES*	COMMENTS**
3 % or 6% Hydrogen peroxide, Accelerated Hydrogen Peroixide	3 % or 6% Hydrogen peroxide, Peroxigard®	This product is a one-step disinfectant cleaner and deodorant designed for general cleaning, disinfecting, and deodorizing of hard, non-porous surfaces. Contact time 60 seconds****.
Blend of peracetic acid, hydrogen peroxide, and acetic acid	Spor Klenz	Respiratory irritant***. Contact time 10 minutes****. Division of Safety (DS) approved SOP is strongly recommended.
*The use of common brand names as examples does not indicate a product endorsement. ** Always follow manufacturer's instructions for dilution and expiration periods. All agents require personal protective equipment, and staff need to be aware of appropriate gloves and other items to ensure worker protection from chemical exposure. *** Please read the SDS for hazardous compounds and follow DS recommendations. **** This is the minimum time for infection control. For animals that are known to be infected, contact time must be determined based on the specific agent and aligned with the manufacturer's label and approved IBC registration document.		

Table 4. Recommended Sterilant for Surgical Instruments, Devices, and 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. We recommend the use of class V integrating indicators in surgical packs since these strips account for temperature, time, and pressure. These should be used in addition to autoclave tape which shows that peak temperature was reached at least momentarily.
Dry Heat	Hot Bead Sterilizer Dry Chamber	Fast Instruments must be cooled before contacting tissue. Only tips of instruments are sterilized with hot beads sterilizer.
Gas sterilization	Ethylene Oxide, Vaporized hydrogen peroxide (VHP) Chlorine dioxide	Gas/ Vapors are irritating to tissues, levels required for sterilization are dangerous to personnel life and health; all materials require safe airing time and monitoring. Appropriate sterilization indicators should be used to ensure sterility. DS clearance is required, approved SOP and trained staff when using gas sterilization***

AGENT	*EXAMPLES	COMMENTS
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. For the use of room sprayers or decon units, please refer to requirements for room sterilization due to hazards to personnel. General use for room cleaning requires standard lab PPE. DS clearance, approved SOP, and trained staff are required when using chlorine room sterilization***
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. DS clearance, approved SOP, and trained staff are required when using glutaraldehydes sterilization***
Blend of Peracetic acid, Hydrogen peroxide, and Acetic acid	Actril®	Respiratory irritant***. Several hours required for sterilization. Corrosive and irritating; only to be used in areas with strong ventilation. Instruments must be rinsed with sterile saline or sterile water before use. Use as room disinfectant or decontaminant requires DS review and approval, plus a risk assessment for PPE.
* 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. ** Alcohol is neither a sterilant nor high level disinfectant(25, 26) *** Please read the SDS for hazardous compounds and follow DS recommendations		

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Approved – 03/09/1994
Reapproved – 05/08/1996, 02/10/1999, 11/14/2001
Revised – 03/09/2005, 10/10/2007, 09/12/2012, 07/26/2017, 12/19/2019, 04/26/2023, 04/22/2026