



Animal Care and Use Program

Policy: Rodent Surgery

Objective:	To outline the proper procedures for the performance of rodent surgery
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Scope

1. In accordance with the *Guide for the Care and Use of Laboratory Animals* and 9 CFR, the Animal Welfare Act (AWA), all surgical procedures (survival and non-survival) on rodents must be performed using aseptic procedures. This includes the use of sterile instruments, and the aseptic preparation of the surgical site in order to prevent postoperative infections. A rodent surgical area can be a room or portion of a room that is easily sanitized, but must not be used for any other purpose during surgery.
2. Surgery can be classified as minor or major in nature.

Procedures

1. Overview

- a. Preoperative preparation includes the assessment of the animal's health, induction of anesthesia, preparation of the surgical site for aseptic surgery, the use of sterilized instruments and adherence to aseptic technique.
- b. Water should NOT be withheld unless required by the protocol.

2. Minor Surgery

- a. Minor surgery does not expose a body cavity and causes little or no physical impairment. Minor survival surgery includes wound suturing, peripheral-vessel cannulation, and subcutaneous implants. Minor procedures require aseptic technique and instruments, appropriate anesthesia and must be conducted on a work surface that can easily be decontaminated/sanitized using appropriate agents (see Table 1) or covered by impervious disposable drapes.
- b. Subcutaneous Implants
The number and size of subcutaneous implants should be the lowest number and smallest size possible. The subcutaneous implants must not impede normal mobility and physiologic function (i.e. eating, defecation, urination or respiration) in the animal.

3. Major Surgery

- a. Major surgery includes exposure of the cranial, abdominal or thoracic cavities. Procedures that cause physical or physiological handicap (i.e. stroke surgery, brain cannulas), or involves extensive tissue dissection or transection are considered major surgery. The use of aseptic technique to minimize the possibility of postsurgical infection is mandatory in such surgeries. Consultation with the Attending Veterinarian (AV) is recommended if there are questions regarding appropriate technique.
- b. The Surgery Room/Area
An area or portion of a room that is easily sanitized and not used for any other purpose during the time of surgery is acceptable. This must be kept free of clutter. Be aware that materials stored on open shelves above the surgery area are a likely source of contamination during surgery, i.e. dust dropping into the surgery area.
- c. Clean and disinfect the surface with a disinfectant before each surgery (See Table 1)
- d. Provide adequate lighting.

- e. Provide a heat source such as a covered heating pad, warm water bottle or heat lamp to prevent hypothermia of the animal.
4. Preparation of the animal should include clipping or shaving of the surgical site with enough border area to keep hair from contaminating the incision site. Hair removal should be performed in a location remote from the surgical area. The surgical site should be scrubbed at least twice with a germicidal scrub (see Table 4), being careful to scrub from in an enlarging circular pattern from the center of the shaved area to the outside. Finish with an alcohol wipe then apply chlorhexidine or povidone-iodine solution. A non-medicated ophthalmic ointment needs to be placed in the anesthetized animal's eyes to prevent drying if surgical procedures are expected to last more than 10 minutes. Animals do not close their eyes when anesthetized.
5. Depilatory cream may be used when indicated, however these products may complicate surgical recovery due to dermatitis caused by these products. To avoid contamination of the surgery area, hair removal should be done at another location and the loose hair should be vacuumed up or otherwise removed from the environment (tape is a practical and effective alternative for small amounts of hair.)
6. **Preparation of the Surgeon**
 - a. Wear a clean lab coat or scrub top and remove all jewelry (rings, bracelets, watches) from the hands and wrists.
 - b. A mask and hair bonnet or cap is required for all surgeries.
 - c. Use fresh sterile gloves at the initiation of surgery or when gloves become visibly damaged during surgery.
7. **Postoperative Care**
 - a. Check the animal regularly until it is fully ambulatory.
 - b. The animal must be monitored daily following surgery, assessing such parameters as appetite and wound healing. Any complications should be reported to the AV.
 - c. The three major threats to a rodent recovering from anesthesia are:
 - i. Hypothermia. This may be minimized by housing the animal in a warm room, use of a heating blanket, warm water bottle or other source of warmth.
 - ii. Dehydration. In prolonged or very invasive surgeries, 1-2 cc in mice and 10cc in rats of warm (not hot) sterile saline or LRS can be given subcutaneously after surgery to help prevent dehydration.
 - iii. Cagemates. House rodents singly after surgery. Rodents tend to cannibalize nonresponsive cagemates. Even if all of the rodents in a cage were anesthetized, some may be slower to recover than others, and may be injured by more alert animals.
 - d. Following the procedure, ensure that the animal is provided a source of warmth (i.e. warm gloves, heating pad, heating blanket, etc.) until fully recovered from anesthesia.
8. **Multiple, Major Survival Surgeries**

All efforts should be made to avoid multiple major survival surgery in animal studies. Two or more major survival surgeries are considered as "multiple." However, there are instances when multiple major survival surgical procedures are required. Such procedures must be described in the protocol, scientifically justified, and approved by the IACUC.
9. **Procedures for Surgeries on Multiple Rodents**

Sterile instruments and gloves must be used at beginning of surgery and handled in such a manner that they remain sterile. When using the "tips only" technique, the sterility of the instrument tips must be maintained throughout the procedure. (Generally, tips of instruments are exposed to heat in the bead sterilizer for ~15 seconds).

Instruments and gloves may be used for a series of similar surgeries provided they are maintained clean and disinfected with a high-level disinfectant between surgeries (see Table 2 or 3). Alternatively, fresh, sterile gloves and autoclaved or hot bead sterilized instruments should be used for each animal.

Table 1. Recommended Hard Surface Disinfectants (e.g. table tops, equipment) Always follow manufacturer's instructions.

NAME	EXAMPLES	COMMENTS
Alcohols	70% ethyl alcohol 70%-99% isopropyl alcohol	Contact time required is 15 minutes. Contaminated surfaces take longer to disinfect. Remove gross contamination before using. Inexpensive. Flammable.
Quarternary Ammonium	Roccal®, Cetylcode®	Rapidly inactivated by organic matter. Compounds may support growth of gram negative bacteria.
Chlorine	Sodium hypochlorite (Clorox® 10% solution) Chlorine dioxide (Clidox®, Alcide®)	<u>Corrosive</u> . Presence of organic matter reduces activity. Chlorine dioxide must be fresh (<14 Days old); kills vegetative organisms within 3 minutes of contact. A rinse with water or alcohol is required after solid surface disinfection.
Aldehydes	Glutaraldehyde (Cidex®, Cide Wipes®)	Rapidly disinfects surfaces. Toxic Exposure limits have been set by OSHA.
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.

Table 2. Recommended Instrument Sterilants

AGENTS	EXAMPLES	COMMENTS
Physical: 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).
Dry Heat ¹	Hot Bead Sterilizer Dry Chamber	Fast. Instruments must be cooled before contacting tissue.
Ionizing radiation	Gamma Radiation	Requires special equipment
Chemical: Gas Sterilization	Ethylene Oxide	Requires 30% or greater relative humidity for effectiveness against spores. Gas is irritating to tissues, so all materials require safe airing time. Carcinogenic. Use only for materials which cannot be sterilized with any other method.
Hydrogen Peroxide	(Sterad®)	Not useful for "delicate" items.
Chlorine ²	Chlorine Dioxide (Clidox®, Alcide®)	A minimum of 6 hours required for sterilization. Presence of organic matter reduces activity. must be freshly made (<14 days)
Aldehydes ²	Formaldehyde (2% sol.) Glutaraldehyde	For all aldehydes: many hours required for sterilization. Corrosive and irritating. Consult safety representative on proper use. Glutaraldehyde is less irritating and less corrosive than formaldehyde.

¹Instruments must be cleaned and rinsed before being placed in the bead sterilizer.²Instruments must be rinsed thoroughly with sterile water or saline to remove chemical sterilants before being used.

Table 3. Recommended Instrument Disinfectants

AGENT	EXAMPLES	COMMENTS
Alcohols Primary use is as a disinfectant- soak between animals when starting with sterilized instruments.	70% ethyl alcohol 70%-99% isopropyl alcohol	Contact time required is 15 minutes. Contaminated surfaces take longer to disinfect. Remove gross contamination before using. Inexpensive. Flammable. Low level disinfectant.
Chlorine ¹	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 minutes of contact.
Peracetic Acid/ Hydrogen Peroxide	Spor - Klenz®	Corrosive to instrument surfaces. Must be thoroughly rinsed from instruments before use.
Chlorhexidine	Nolvasan®, Hibiclens®	Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses.

¹Instruments must be rinsed thoroughly with sterile water or saline to remove chemical sterilants before being used.

Table 4. Skin Disinfectants Alternating disinfectants is more effective than using a single agent. For instance, an iodophore scrub can be alternated 3 times with an alcohol, followed by a final soaking with a disinfectant solution. Alcohol, by itself is not an adequate skin disinfectant. The evaporation of alcohol or alcohol based products can induce hyperthermia in small animals.

NAME	EXAMPLES	COMMENTS
Alcohols	70% ethyl alcohol 70-99% isopropyl alcohol	NOT ADEQUATE FOR SKIN PREPARATION! Contact time required is 15 minutes. Not a high level disinfectant. Not a sterilant. Flammable.
Iodophors	Betadine®, Prepodyne®, Wescodyne®	Reduced activity in presence of organic matter. Wide range of microbe killing action. Works best at 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 5. Suture Selection *

SUTURE	CHARACTERISTICS AND FREQUENT USES
Vicryl®, Dexon®	Absorbable; 60-90 days. Ligate or suture tissues where an absorbable suture is desirable.
PDS® or Maxon®	Absorbable; 6 months. Ligate or suture tissues especially where an absorbable suture and extended wound support is desirable
Prolene®	Nonabsorbable. Inert.
Nylon	Nonabsorbable. Inert. Recommended for skin.
Silk	Nonabsorbable. (Caution: Tissue reactive and may wick microorganisms into the wound). Silk is very easy to use and knot. Silk is not acceptable for suturing skin.

Chromic Gut	Absorbable. Versatile material. causes mild inflammation, but is absorbed more rapidly than synthetics. Chromic gut is not acceptable for suturing skin.
Stainless Steel: Wound Clips, Staples	Nonabsorbable. Requires instrument for removal from skin.

*Suture sizes for most general purposes for mice are 4-0 or 5-0 and for rats is 3-0 or 4-0. Sutures or staples must be removed from the skin after the incision is healed; this is generally 10-14 days.

References

1. Forman, L.A., 2000; Rodent Surgery guidelines, Northwestern University, Chicago, IL.
2. Slatter, D., 2002; *Textbook of Small Animal Surgery*, Philadelphia, W.B. Saunders and Co.
3. National Research Council. *Guide for the Care and Use of Laboratory Animals: Eighth Edition*. Washington, DC: The National Academies Press, 2011.
4. Animal Welfare Act (P.L. 89-544 -1966; amended 1970 [P.L. 91-579], 1976 [P.L. 94-279], 1985 [P.L. 99-198], 1990 [P.L. 101-624]).
5. *Guidelines for Rodent Survival Surgery*. Office of Animal Care and Use, National Institutes of Health: Animal Research Advisory Committee, September 26, 2017.
https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/b6-survival_rodent_surgery.pdf

Revision History

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