



THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

GUIDELINES:

Aseptic Technique for Rodent Survival Surgeries

The following Guidelines are provided to ensure correct aseptic technique for all rodent survival surgeries.

* **Surgical Area**

1. The surgical area should be isolated from active areas in the laboratory, doorways and ventilation supply ducts.
2. The area should be clean and uncluttered.
3. Surface areas should be easily sanitizable.
4. An animal preparation and recovery area, separate from the surgical area, should be provided. A heat source should be available for recovery if an extended wake-up time is anticipated. If a separate preparation area is not possible due to space constraints, cover the surgical area with a towel or drape and discard this after the animal has been prepared.
5. A heat source should be available during an extended surgery, since rodents rapidly lose body heat under anesthesia.
6. The surgery area should contain sufficient lighting.
7. Minimize the storage of cardboard and paper products directly above the surgery site. Sealable, plastic containers may be used for storage.
8. Chairs located in animal use areas should have an impervious, cleanable surface. No cloth chairs are allowed in areas animals will be used. Cloth chairs can be covered with disposable plastic bags when animals are present in the lab.

* **Animal Preparation**

1. Apply a bland ophthalmic lubricant to the eyes, since the blink reflex is lost during anesthesia. For extended procedures, reapplication of the ophthalmic lubricant should occur as needed in order to keep the eyes from drying out. Mineral oil may be used for lubrication, but must be applied every 20 minutes.
2. The area around the surgical site must be devoid of hair, since hair around the surgical site can act as a wick for bacterial infection. Hair removal may be achieved by either using a #40 clipper blade or a depilatory. In mice, plucking to remove hair is relatively easy once the mice are anesthetized.
3. Prepare the surgical site by using ethyl alcohol or isopropyl alcohol, followed by an Iodophor solution or Chlorhexidine scrub (Chlorhexidine is known to be irritating to skin).
4. Using cotton tipped applicators or gauze, start with the first alcohol application. Follow the alcohol application with the first Iodophor or Chlorhexidine application.
5. Starting in the center of the incision site, spiral outward in concentric circles toward the margins of the prepared area (never go back and forth over a cleansed area with the same gauze).

6. Repeat the alcohol and Iodophor or Chlorhexidine step for a total of three times each.
7. When using Chlorhexidine as the skin disinfectant, ensure complete removal with sterile saline or water from the site before incision or prior to skin closure.
8. Use a new gauze or cotton tipped applicator for each application.

* **Surgeon Preparation**

Surgical personnel should wear a clean lab coat, mask, bouffant cap, and sterile gloves. If performing multiple surgeries, new gloves should be donned between animals. Most aseptic surgery requires sterile gloves to be worn.

**Some (micro)surgery may not require the use of sterile gloves. (Some examples are blastocyst transfer, some stereotaxic procedures, and many mouse surgeries.)*

* **Placement of Drape Material**

The use of a drape is recommended to prevent contamination of the disinfected surgical site. This is especially true for procedures that require exteriorization of the viscera. Positioning of the drape over the surgical area should proceed with sterile gloves or instruments in order to maintain sterility.

**Some (micro)surgery may not require the use of drapes.*

**To determine if your (micro)surgery requires the use of sterile gloves and/or drapes, please contact The Office of Animal Care and Use at 966-5569 for further information.*

* **Instruments**

All instruments must be properly sterilized. Ensure the instruments are cleaned and free of all organic material before sterilizing. A sterility indicator must be placed inside or on the surgical pack to confirm proper sterilization. Acceptable methods include.

- 1) Autoclave
- 2) Gas sterilization with ethylene oxide
- 3) Cold sterilization (see the list below of FDA approved products)

Instruments should not be used on more than one rodent without resterilization. A new sterile pack should be prepared for each additional animal. Alternately, instruments may be resterilized by using a hot bead sterilizer or flash autoclaving between rodents.

* **Equipment Manipulation**

During some rodent surgeries there may be a need to manipulate certain types of equipment (microscopes, anesthetic machines, drills, etc.). Such equipment should be disinfected before surgery. If you are wearing sterile gloves to maintain asepsis and touch objects outside of the sterile field; your gloves are no longer sterile. Once surgery commences, adjustments and handling of equipment outside of the sterile field must be made using a piece of sterilized gauze, aluminum foil or commercially available sterile sleeve.

* Surgical Closure

The abdominal muscle/peritoneal layer and the skin must be closed separately. Appropriate suture material for each layer should be used. For closure of surgical incisions on the ventral surface (i.e. "underneath"), an interrupted suture pattern should be used in the muscle layer. When using sutures to close skin incisions, a monofilament material should be used (braided sutures used in skin tend to promote wound infection). An interrupted suture should also be applied when closing the skin. Wound clips or surgical staples may be used in the skin. However, clips or staples should not be used for closing skin on the ventral surface, since they may become contaminated with bedding. If clips, staples, or nonabsorbable sutures are used to close the skin, they should be removed seven to ten days after surgery. Commercially available tissue adhesive products for skin closure work well on small skin incisions which would normally require one or two clips or sutures.

If you need further information or if you need to review aseptic techniques, please visit <http://research.unc.edu/iacuc/> or call the Office of Animal Care and Use at 966-5569.

* Sterilants and Disinfectants

Hard Surface Disinfectants (the designated surgical area should be cleaned and disinfected prior to and after surgery; e.g., bench top, chairs, equipment): Always follow manufacturer's instructions.

A **disinfectant** is a germicidal chemical substance that kills microorganisms on inanimate objects, such as instruments and other equipment that cannot be exposed to heat.

Name	Examples *	Comments
Alcohols	70% ethyl alcohol, 70%-99% isopropyl alcohol	Contact time required is 15 minutes. Remove gross contamination before using. Flammable.
Quaternary Ammonium	Roccal(®), Cetylcide(®)	Rapidly inactivated by organic matter. Compounds may support growth of gram-negative bacteria.
Chlorine	Sodium hypochlorite (Clorox (®) 10% solution), Chlorine dioxide [Clidox(®), Alcide(®)]	Presence of organic matter reduces activity. Chlorine dioxide must be fresh (<14 Days old)
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(®)	Rapidly bactericidal and persistent. Effective against many viruses.

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

Skin Antiseptics

An **antiseptic** is a chemical agent that either kills pathogenic microorganisms or inhibits their growth. The term antiseptic is reserved for agents applied to the body.

Name	Examples *	Comments
Alcohols	70% ethyl alcohol, 70-99% isopropyl alcohol	Not adequate alone for surgical site preparation! Not a high level disinfectant.
Iodophors	Betadine(®), Prepodyne(®), Wescodyne(®)	Reduced activity in presence of organic matter. Wide range of microbe killing action.
Chlorhexidine	Nolvasan(®), Hibiclens(®)	Presence of blood does not interfere with activity. Rapidly bactericidal and persistent.

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

Instrument Sterilants (each surgery requires the use of sterilized instruments):

Always follow manufacturer's instructions.

Sterilization is the complete elimination of microbial viability, including both the vegetative and spore forms of bacteria.

Agents	Examples *	Comments
Steam sterilization (moist heat)	Autoclave-time and temperature dependant	e.g., 121°C for 15 min. vs. 131°C for 3 min (flash).
Dry Heat	Hot Bead Sterilizer, Dry Chamber	Instruments must be cooled before contacting tissue.
Chemical: Gas sterilization	Ethylene Oxide	All materials require safe airing time. Carcinogenic.
Hydrogen Peroxide	Sterad(®)	Not useful for "Delicate" items.
Chlorine ¹	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 ¹	Formaldehyde, Glutaraldehyde, Cidex®	For all aldehydes: many hours required for sterilization. Corrosive and irritating. Must be freshly made 14 or 28 day formulations.

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¹ **Instruments must be rinsed thoroughly with sterile water or saline to remove chemical sterilants before being used. These products can be caustic to both the animal tissues and instruments.**