

# UNC-Division of Comparative Medicine (DCM)

## Aseptic Technique Training Material

Please review before coming to Aseptic Technique class.

### Training Information

*Training and certification may be obtained in two ways:*

1. Each laboratory must designate a Laboratory Animal Coordinator (LAC) who may train research personnel in their laboratory in various animal-handling techniques. The LAC must be certified by the Division of Comparative Medicine (DCM) and demonstrate proficiency before training others within their lab. Please note, there are certain techniques that LACs are not allowed to train in.
2. For additional training please contact the DCM Training Team via the IACUC office at 966-5569 or [iacuc@med.unc.edu](mailto:iacuc@med.unc.edu). We offer training in both one on one and classroom settings.

For a look at IACUC Guidelines and training information, visit our websites:

<https://research.unc.edu/iacuc/training/> OR <https://research.unc.edu/comparative-medicine/training/>

To register for classes, click this link, and search under 'Office of Animal Care and Use':

<https://apps.research.unc.edu/events/index.cfm>

### Network of Laboratory Animal Coordinators (NLAC)

Also, consider joining the Network of Laboratory Animal Coordinators (NLAC) listserv. This listserv is an internal avenue for laboratory animal coordinators at UNC to make contacts, exchange ideas, ask questions, and make suggestions. The goal is to enhance communication between animal research laboratory personnel, the Institutional Animal Care and Use Committee, and the Division of Comparative Medicine. See the following link for more information and to subscribe:

<http://research.unc.edu/offices/nlac/>

### SURGERY COMMANDMENTS

1. Thou shalt carefully plan all aspects of surgical procedures in advance
2. Thou shalt ensure the animal is appropriately anesthetized
3. Thou shalt use aseptic techniques
4. Thou shalt minimize tissue trauma
5. Thou shalt minimize blood loss
6. Thou shalt keep the patient warm
7. Thou shalt ensure personnel have been sufficiently trained for all procedures
8. Thou shalt provide post-operative monitoring and supportive care
9. Thou shalt monitor and track outcomes, taking corrective action as necessary

### Objectives

1. General ideas to consider when planning survival surgery.
2. Teach how to assess an animal's surgical plan of anesthesia.
3. Teach methods for instrument, patient and surgeon preparation
4. Perform surgery using aseptic techniques (from incision to wound closure)
5. Teach proper post-surgical monitoring and recording.

## Instruments

*All instruments must be properly sterilized. Ensure the instruments are cleaned and free of all organic material before sterilizing. Utilize sterility indicators to confirm proper sterilization.*

Acceptable methods include:

1. Autoclave – ensure that autoclaves are monitored as required for proper functioning (bacterial indicator, test strips, etc.)! Details can be found here: [UNC Environment Health and Safety \(EHS\) Autoclave Decontamination](#).
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 re-sterilization. A new sterile pack should be prepared for each additional animal. Alternately, instruments may be re-sterilized by using a hot bead sterilizer or flash autoclaving between rodents.

## 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.



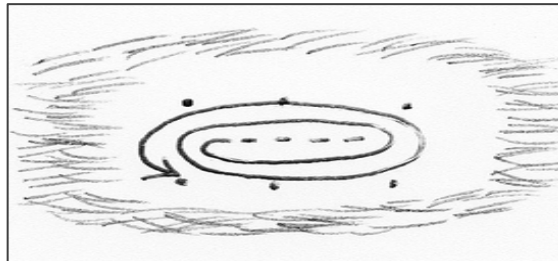
4. An animal preparation and recovery area, separate from the surgical area, should be provided. If a separate preparation area is not possible due to space constraints, cover the surgical area with a towel or drape and discard this after the animal has been prepped.



5. A heat source should be available anytime an animal is anesthetized (from induction to recovery) as rodents rapidly lose body heat under anesthesia.
6. Space near the surgery area should be available and contain sufficient lighting and room for hand scrubbing and donning of sterile gloves (where applicable).
7. Cardboard and paper products should not be stored directly above the surgery area. 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 should be covered with disposable plastic if they must be used when animals are present in the lab.)
9. The designated surgical area (e.g., bench top, chairs, equipment) should be cleaned and disinfected prior to and after surgery with a hard surface disinfectant. Always follow manufacturer's instructions.

### Animal Preparation

1. Apply a veterinary or pharmaceutical grade, 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.
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 anesthetized mice, plucking to remove hair is relatively easy.
3. Prepare the surgical site by using alternating applications of a 10% Iodophor (povidone-iodine) solution (Betadine®, Prepodyne®, Wescodyne®) or 2-4% Chlorhexidine scrub (Nolvasan®, Hibiclens®), followed by a skin antiseptic such as ethyl (70%) or isopropyl alcohol (70-99%). Please see Skin Antiseptic table at the end of this Standard for more details.
4. Using sterile cotton tipped applicators or gauze, start with the first Iodophor or Chlorhexidine application. 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 cotton tipped applicator or gauze).



5. Follow the Iodophor/Chlorhexidine application with the first alcohol application, repeating the concentric circle pattern.
6. Repeat the Iodophor/Chlorhexidine and alcohol steps for a total of **three times each**. Use a new sterile cotton tipped applicator or gauze for each application.
7. When using Chlorhexidine as the skin antiseptic, ensure complete removal with sterile saline or water from the site before incision or prior to skin closure. Chlorhexidine can be irritating to skin.
8. Be careful not to excessively wet the animal as this can exacerbate hypothermia.

## **Surgeon Preparation**

1. Surgical personnel should wear a clean lab coat, mask, bouffant cap, and sterile gloves.
2. If performing multiple surgeries, new sterile gloves should be donned between animals.
3. When wearing sterile gloves, hands should be scrubbed with an antimicrobial soap prior to donning gloves if able. This is an added precaution to reduce the risk of post-operative infection if the gloves tear during surgery.



## **'No Touch/Tips Only' Procedure**

Some (micro) surgery may not require the use of sterile gloves. (Some examples are blastocyst transfer, some stereotaxic procedures, and many mouse surgeries.) To determine if your (micro) surgery requires the use of sterile gloves and/or drapes, please contact the Office of Animal Care and Use (OACU) at 966-5569 for further information.

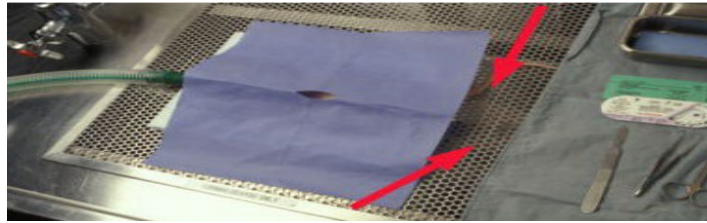
If sterile gloves are not used, a “No Touch/Tips Only” technique **must be used**. (This restricts the surgeon to using only the sterile working tips of the surgical instruments to manipulate the surgical field. The gloved hand must never touch the working end/tip of the instruments, the suture, suture needle, or any part of the surgical field. Sutures, catheters, and other sterile materials to be used in the surgery **must only** be handled with the instrument tips. Tissues must only be touched with instrument tips. Surgical personnel approved to utilize the ‘No Touch/Tips Only’ technique should wear a clean lab coat, mask, bouffant cap and gloves.

## **Placement of Drape Material**

1. 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.
2. Positioning of the drape over the surgical area should proceed with sterile gloves or instruments in order to maintain sterility.

## **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 sterile gloves touch objects outside of the sterile field, they 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.



## Be aware of space between sterile drape and sterile field

### 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 pattern should also be used to close the skin.

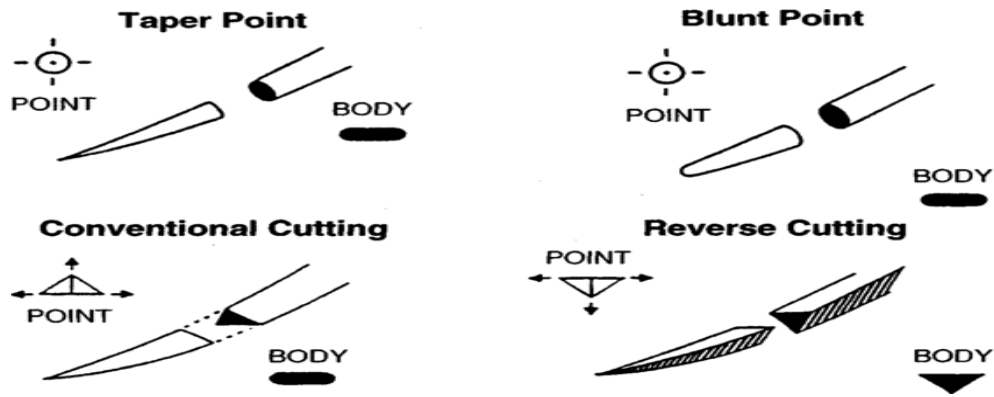
Wound clips or surgical staples may be used in the skin. However, clips or staples should not be used for closing skin on the ventral surface, since they may become contaminated with bedding. If clips, staples, or non-absorbable sutures are used to close the skin, they should be removed seven to fourteen 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.

### Suture Selection

SUTURE	CHARACTERISTICS
Vicryl, Dexon	Absorbable – 60-90 days. Suture tissues
PDS or Maxon	Absorbable – 6 months. Suture tissues
Prolene	Non-absorbable - Inert
Nylon	Non-absorbable – Inert. General Closure
Silk	Non-absorbable – Tissue reactive. May wick microorganisms into wound. Easy to use and knot. NOT ACCEPTABLE FOR SKIN SUTURE.
Chromic Gut	Absorbable – Versatile. Causes mild inflammation, yet rapidly absorbed. NOT ACCEPTABLE FOR SKIN SUTURE
Staples and wound clips	Non-absorbable – Requires special instrument for removal.

- **Suture Gauge Selection:** Use smallest gauge suture material that will perform adequately.
- **Cutting and Reverse-Cutting Needles:** Provides edge to cut through dense, difficult to penetrate tissue (e.g. skin)
- **Non-cutting, taper point or round needles:** No edges for cutting. Used for easily torn tissue (e.g. peritoneum, intestine).



### Wound Clips

Wound clips can be applied to an open incision site with a wound clip applicator, provided that the incision is not in a weight-bearing area. They **MUST** be sterile!



Wound Clips



Wound Clip Applicator



Wound Clip Remover

### Documentation

The USDA and PHS policies require proper documentation of animal care and use to assess compliance with research protocols and clinical care procedures. Dates of all observations, treatments and procedures must be recorded. Dates and times (including AM/PM) of all time-sensitive observations or treatments (post-operative evaluations, pain medication) must be recorded. Extent of records vary based on the nature of the procedure.

However, at a minimum, records of the procedure must consist of animal/cage/group ID, date of procedure, type of procedure, anesthetics/analgesics used (dose, route, and time), anesthesia chart (verification of toe pinch), drugs given (dose, time), general procedures (e.g., intubation, beginning and end of surgery, etc.). See <https://research.unc.edu/files/2017/02/UNC-IACUC-Rodent-Anesthesia-Analgesia-Procedure-Record.pdf>

Any deviations from the approved protocol due to emergency need must be documented, explained, and reported to the OACU. **All records must be available for review at any time by IACUC and external regulatory officials.**

## **Post-Operative Monitoring and Supportive Care**

1. Ensure that recovery occurs in a clean cage lined with a paper towel (rodents under the influence of anesthesia can aspirate corncob bedding).
2. Keep the patient warm until ambulatory.
3. Return to DCM facility when animal is in sternal recumbence and ambulatory in the cage.
4. Complete and place the pink-colored [Post-operative monitoring/analgesia card](#) (found in DCM animal housing rooms) on the cage. Cards can be removed once monitoring and/or analgesia administration as stated in approved application has been performed.
5. Provide all analgesics and fluid therapy as approved in your animal application.



Sternal Recumbence



Lateral Recumbence



Dorsal Recumbence    Ventral Recumbence

6. Monitor all animals for any visible signs of pain (e.g. hunched posture, ruffled fur, lethargy, resentment to being handled, and decreased appetite).
  7. Monitor animals for any signs of infection (e.g. swelling and redness around incision site, subtle change in behavior)
- NOTE: Some anesthetics (e.g. Xylazine, Medetomidine, and barbiturates) may be reversed by administration of an anesthetic/sedative antagonist (e.g. Yohimbine, Naloxone, or Atipamezole).

## **Factors influencing possibility of infection**

1. Foreign materials (e.g. non-absorbable suture, implants)
2. Increased surgery time
3. Use of incorrect suture (e.g. silk and cotton suture may act as a wick for bacteria into incision site)
4. Skin damage at surgical site (e.g. razor, dull clippers)
5. Not irrigating wound with sterile saline or antibiotic solution (this decreases the number of bacteria as well as removes blood clots and necrotic tissue)
6. Patient health (e.g. malnutrition, obesity, diabetes)
7. Tissue damage (e.g. improper instrument handling, heavy use of electro-cautery)

## **Relevant Links**

[Standard for Rodent Survival Surgery](#)

[DCM Veterinarian Recommended Formularies and Regimens](#)

[Standard for Humane Endpoints in Rodents](#)

[Standard on Assignment of Animals into Pain Categories](#)

[Standard on Pain Identification and Post-Operative Analgesia](#)

[Post-Procedure Monitoring and Analgesia Card](#)

[Isoflurane Vaporizer Monitoring Log Template](#)

[Isoflurane Drop Method Log Template](#)

[Anesthesia/Analgesia Drug Log Template](#)

## **Sterilants and Disinfectants**

[FDA-Cleared Sterilants and High Level Disinfectants](#)

## **Skin Antiseptics**

<b>Name</b>	<b>Examples*</b>	<b>Comments</b>
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. (i.e. 10% povidone-iodine)
Chlorhexidine	Nolvasan(®), Hibiclens(®)	This is an antiseptic/antimicrobial skin cleanser. Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Concentration of 2-4% is recommended.

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



## Disinfectants & Sterilants

Name	Examples*	Comments
Alcohols	70% ethyl alcohol, 70%-99% isopropyl alcohol	Remove gross contamination before using. Flammable. Read label for recommended contact time.
Quaternary Ammonium	Cetylcode(®)	Rapidly inactivated by organic matter. Compounds may support growth of gram-negative bacteria. Read label for recommended contact time.
Chlorine	Sodium hypochlorite (Clorox (®) 10% solution), Chlorine dioxide [Clidox(®), Alcide(®)]	Presence of organic matter reduces activity. Chlorine dioxide must be fresh (<14 days old). Read label for recommended contact time.
Aldehydes	Glutaraldehyde [Cidex(®), Cide Wipes(®)]	Toxic. OSHA has set exposure limits. Rapidly disinfects surfaces. Read label for recommended contact time.
Phenolics	Lysol(®), TBQ(®)	Less affected by organic material than other disinfectants. Read label for recommended contact time.
Chlorhexidine	Nolvasan(®), Hibiclens(®)	Rapidly bactericidal and persistent. Effective against many viruses. Read label for recommended contact time.

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

- Spor-Klenz is a hard surface antimicrobial product: <https://www.steris.com/about/hse/safety-data-sheets>
- Peroxigard is a hard surface disinfectant product: <https://www.peroxigard.com/sds/>

**For any further assistance or information, please contact the DCM Training team at [DCMTraining@unc.edu](mailto:DCMTraining@unc.edu).**

## GLOSSARY

- Antimicrobial: “destroy or inhibit the growth of microorganisms”
- Antiseptic: “preventing the growth of microorganisms”
- Asepsis: “the state of being free of pathogenic organisms”
- Bactericide: “destroys bacteria”
- Contamination: “process of infecting by contact or association”
- Disinfect: “to free from infection especially by destroying harmful microorganisms”
- Pathogen: “a specific agent (bacterium or virus) of disease.”
- Sanitize: “to make more acceptable by removing unpleasant or undesired features”
- Disinfectant: “a chemical that destroys vegetative forms of harmful microorganisms (as bacteria and fungi) especially on inanimate objects”
- Sterilant: “to free from living microorganisms”
- Sterile: “to free from living organisms and especially microorganisms”
- Sterilization: “to make sterile”

## **Supply and Vendor Information**

UNC-CH Hospital Pharmacy sells 100ml and 250ml bottles of isoflurane. All grant orders need to be submitted through online link below. Once ordered, it is reviewed by the pharmacy manager. If approved, orders are processed and shipped by the Shared Services Center in Durham.

<https://www.uncmedicalcenter.org/uncmc/patients-visitors/amenities/pharmacies/shared-services-center-pharmacy/>

### **Braintree Scientific**

Phone: 781-917-9526

Website: [www.braintreesci.com](http://www.braintreesci.com)

Description: Instruments, lab equipment, isothermal pads, tattoo paste

### **Fisher Scientific**

Phone: 800-766-7000

Website: [www.fishersci.com](http://www.fishersci.com)

Description: Lab equipment, chemicals, instruments, pharmaceuticals

### **Covetrus (formerly Henry Schein)**

Phone: 800-872-4346

Website: <https://northamerica.covetrus.com> (redirects from [www.henryscheinvet.com](http://www.henryscheinvet.com))

Description: Veterinary supplies, instruments, pharmaceuticals

\*Needs Vet License or Researcher DEA license\*

### **Kent Scientific**

Phone: 888-572-8887

Website: <http://www.kentscientific.com/>

Description: Surgical equipment, telemetry equipment

### **Med-Vet International**

Phone: 800-544-752

Website: <http://www.shopmedvet.com>

Description: Veterinary supplies and instruments (discounted)

\*Need Vet License\*

### **National Band and Tag**

Phone: 859-261-2035

Website: <https://www.nationalband.com/>

Description: ID tags, ear tags

### **Patterson Veterinary**

Phone: 800-225-7911

Website: [www.pattersonvet.com](http://www.pattersonvet.com)

Veterinary supplies, instruments, pharmaceuticals including Pentobarbital

\*Needs Vet License or Researcher DEA license\*

**Plas Labs**

Phone: 800-866-7527

Website: [www.plas-labs.com](http://www.plas-labs.com)

Description: Rodent restrainers, equipment

**Roadrunner Compounding Pharmacy**

Phone: 1-877-518-4589

**Roboz**

Phone: 800-424-2984

Website: [www.roboz.com](http://www.roboz.com)

Description: Specialize in instruments

**UNC-CH Materials Management and Distribution**

Phone: 966-5671

Description: Scientific Storeroom, General Storeroom, Chemical Storeroom

**Vetamac**

Veterinary anesthesia service and manufacturer

Phone: 800-334-1583

**Southern Anesthesia Surgical Inc.**

Phone: 800-624-5926

<https://www.sasrx.com/>

This is a human source company that has a Veterinary division, will set up an account without a vet license.

**DCM Vet Services**

Phone: 919-843-3407

Email: [hjoyce@email.unc.edu](mailto:hjoyce@email.unc.edu)

Description: Contact Heather Joyce (Vet Services Manager) if you need help finding and/or ordering drugs/veterinary supplies.

**UNC Physics Dept Instrument Sharpening**

\*Note: they do not sharpen to surgical grade requirements; will do guillotines Phone: 962-1183

Phillips Hall, Room 115, walk-ins are welcome <https://physics.unc.edu/instrument-shop/>