University Animal Care Committee Standard Operating Procedure		
Document No: 7.3	**Subject:** Aseptic Surgical Techniques (Mice)	
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Location: Queen's University

Responsibility: Principal Investigators, Research Staff, Veterinary Staff

Purpose: The purpose of this Standard Operating Procedure (SOP) is to describe the methods used to practice aseptic surgical techniques in mice

Introduction and Definitions: Asepsis refers to the state of being free from biological contaminants which could cause infection. To practice aseptic technique refers to the action of working within a sterile field to prevent infection.

To maintain good aseptic technique, the following (Halstead) principles should be adhered to:

- Strict asepsis during preparation and surgery
- Good haemostasis during the procedure to limit infection rate
- Minimize tissue trauma
- Good surgical judgment to ensure the elimination of dead space and adequate removal of material
- Minimize surgery time through knowledge of anatomy and technique
- Correct use of instruments and materials

Abbreviations: Animal Care Services **ACS**, Principal Investigator **PI,** subcutaneous **SC**, intravenous **IV**, intraperitoneal **IP,** intramuscular **IM**, per os **PO,** per rectum **PR**

1. **Materials:**
   All drugs, fluids, suture material and autoclaved items cannot be used if passed their expiry date*
   - Disinfectant surface spray (Accelerated Hydrogen Peroxide – Peroxigard)
   - PPE - hair cap, face mask, sterile/clean gown, surgical gloves, booties or dedicated footwear
   - Clippers and/or depilatory cream
   - Antimicrobial soap (4% chlorhexidine)
   - 2% chlorhexidine solution
   - 70% isopropyl alcohol
   - 10% Povidone-iodine solution
   - Iodine-based scrub

- Avagard 3M Surgical Hand Antiseptic
- Sterile towels
- Heat source (heat mat and/or surgical table)
- Sufficient sterile surgical packs (steam, gas, cold) for the number of procedures to be performed
- Surgical towels or peel packs for autoclaving instruments
- Sterile gauze
- Autoclave indicators
- Scalpel blade(s)
- Surgical drape(s)
- Sterile bowl with alcohol or cold sterilant
- Sterile bowl with sterile (distilled) water rinse
- Bead sterilizer (optimal)
- Lactated Ringers or 0.9% sodium chloride
- Sterile syringes
- Sterile needles
- Anesthetics (as per described in the Animal Use Protocol)
- Emergency drugs
- Oxygen source (if required)
- Extra pre-weighed scavenge canisters (if using passive scavenging system when using gas anesthetic)
- Suture material

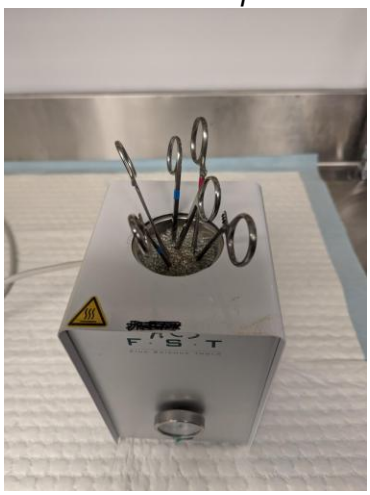
2. Procedures:

Surgical space and instrument care:

- Surgical area should be disinfected prior to starting surgery.
- Surgery should be conducted in a separate area from where the animal was shaved and prepped.
- Turn the bead sterilizer on for at least 30 minutes prior to use.
- All surgical instruments should be sterilized initially. This can be achieved by steam or chemical (vaporized hydrogen peroxide) sterilizing methods.
- Lay out instrument pack and remove first wrap so it is ready for the surgeon to access the inside sterile wrap or open the single use pack so surgeon can access instruments
- Have enough surgical packs for each surgical procedure. Packs can be used for up to 5 surgeries if cleaned between animals. Gold standard is a new pack per animal.
- If surgeries are conducted on multiple rodents and the lab does not have surgical

packs available for each animal, the instrument tips may be resterilized between surgeries using a bead sterilizer.

- With 70% isopropyl alcohol ensuring that all tissue, blood, and debris have been physically removed.
- Tool tips should be dry prior to placing them in the bead sterilizer.
- Allow 20-30 seconds of contact time with the hand/box open.
- Instruments are then placed on a sterile surface to cool prior to using on another animal.
- SEE PHOTO* Do not overcrowd the sterilizer and when tools are removed, ensure the handles are laid out together and the tips are laid out together. The tips are sterile and we do not want them contaminated by the handles. (See photos)
- **This procedure can be followed for a maximum of five surgeries before a new sterile pack is required.**



Preparing the animal for surgery:

- Shave enough hair from the patient to minimize the chance that it can contaminate the incision site. Shaved area should have large enough margins around where the incision will be but not too large to cause excessive heat loss.
- Remove any excess hair with a vacuum or tape.
- **First step of surgical scrub:** Prepare the area by scrubbing the area using either 4% chlorhexidine solution or iodine based scrub such as Betadine + detergent. This is intended to remove all oils and proteins. Manually scrub the surgical area and allow the solution to have a minimum of at least 25 minutes of contact time in total. Wipe

off with alcohol or distilled water on a gauze
- Administer lidocaine or bupivacaine if listed in AUP (refer to SOP 7.1 "Pain Management in Mice").

- **Second step of surgical scrub:** The intended incision site will be prepped with two consecutive applications alternating between both 2% chlorhexidine and 70% isopropyl alcohol **or** 10% povidone-iodine and 70% isopropyl alcohol. Allow the area to dry.
    - As an alternative, a commercially available chlorhexidine-alcohol solution (Baxidine or Avagard) can be applied in the same motion allowing for approximately 2-3 minutes of contact time. The solution may need to be applied more than once. Allow the solution to dry; do not wipe away.
- Total contact time of the antiseptic solutions should be no less than 5 minutes.
- Move animal to the surgical area placing the animal on a clean warm surface such as a sterile surgical drape. If necessary to stabilize the animal, secure to the surgical surface with tape or light weights.
- Administer analgesics as per described in the AUP.
- Conduct a final prep of the incision site, alternating between both 2% chlorhexidine and 70% isopropyl alcohol **or** 10% povidone-iodine and 70% isopropyl alcohol. Allow the area to dry.
- Open the surgical pack.

The Surgeon:
- Don a hair cap and face mask.
- Wash hands with an antibacterial soap and dry hands with sterile towel (if surgical sink access).
- Don clean lab coat/gown.
- Don surgical gloves using sterile technique.

    One pair of sterile gloves can be worn for a maximum of five surgical procedures before donning a new pair.
- Sterile gloves must be saturated with 70% alcohol following each surgical procedure.
- If there is a break in sterility a new pair of gloves must be worn.
- Place sterile drape across the incision site. Ensure that the animal can be monitored at all times throughout procedure.
- Place and maintain surgical instruments on sterile surfaces only.
- Skin incision is to be made using sterile scalpel blade/scissors. When making your

initial incision, attempt a smooth single cut. Multiple incision attempts with a scalpel blade or scissors can create a surface area for bacteria to grow.

- All suture material must be sterile and remain sterile throughout the procedure.
- Reuse of suture material for serial rodent surgeries (in one sitting) is permitted if the suture is soaked between animals in a 10% povidone iodine solution, then rinsed in sterile saline or water.
    - Sutures which can be re-sterilized include any monofilament (Prolene or Nylon/ Ethilon) or 'coated' sutures, e.g. some Vicryl or Ethibond. On the outside of the packets it states whether the suture is coated or not. Multi-filament or twisted fibres, such as Chromic, Silk, Mersilene and non-coated Ethibond must be discarded after use.

- Follow SOP 7.4 “Rodent Post-operative Care (Mice)” for recovery surgical procedures.

References:

Gibson Karen, Donald Alan W, Hariharan Harihar, McCarville Carole. *Comparison of two pre-surgical skim preparation techniques.* Can J vet Res 1997; 61: 154-156.

SOP Revision History:

Date	New Version
01/28/16	Triennial Review
02/28/19	Triennial Review
06/20/19	Update
05/01/22	Updated SOP format
10/22/25	Triennial Review