INTRODUCTION

The Animal Welfare Act and Guide for the Care and Use of Laboratory Animals require that all survival surgery be performed using aseptic procedures. This includes the use of surgical gloves, masks, sterile instruments, and aseptic technique.

Herein Principles of Aseptic Technique will be discussed with emphasis on the practical application of these principles in the research setting. It is the principal investigator's responsibility to ensure that appropriate aseptic conditions and practices are maintained. Any surgical procedures for research, teaching or testing purposes (other than veterinary care procedures performed by a qualified Vet) must be included in an Animal Subjects Approval Form and approved by the WSU IACUC prior to initiating the procedure. Requests for any variation from the details outlined in this policy should be described within the individual Animal Subjects Approval Form for review and approval by the WSU IACUC before the procedure can begin.

FACILITIES

Aseptic surgery should be conducted in dedicated facilities or spaces such that those areas are used only for aseptic surgeries and the storage of essential surgical equipment. The species, nature of the procedure (minor, major or emergency) and the potential for complications should be considered when determining the appropriate location. For many rodent, amphibian or fish surgeries, a clean and disinfected laboratory bench in a non-traffic area separate from other activities would be appropriate and field surgeries for minor agricultural animal or wildlife surgeries would be appropriate given the proper use of aseptic technique. For major surgery on most other species, a dedicated surgical suite would be appropriate.

A dedicated surgical facility or space should be located outside normal facility traffic patterns & personnel access should be restricted to essential surgical staff. There should be separate surgical preparation and recovery areas for the animals and scrub areas for the surgical personnel. The interior surfaces of the surgical space should be constructed of materials that are...
impervious to moisture and easily cleaned. Ideally, the ventilation system for the surgical area should provide a net positive pressure with respect to the surrounding facilities.

**EQUIPMENT**

The equipment in areas used for aseptic surgery should be easy to clean and portable to simplify sanitization of the area. The operating table or bench should be constructed with a durable surface material impervious to moisture which can be readily cleaned. Adequate lighting is essential for performing surgical procedures. Ancillary equipment such as respirators, electrosurgical units and ECG monitors should be included with the light fixtures in a routine equipment cleaning schedule.

**PERSONNEL**

Aseptic technique requires careful attention to a series of steps which begins with patient and instrument preparation and ends at final wound closure. Aseptic technique designs all actions and motions to protect the sterile field from contamination. The surgeon and surgical support staff must be adequately trained to perform each step correctly. Personnel should receive instruction on the indications for aseptic technique, the sources of potential contamination, patient, instrument and equipment preparation, sterilization systems, gowing and gloving techniques, and intraoperative aseptic management.

Assistance with employee training is available from the Office of the Campus Veterinarian, and/or a member of the faculty or research staff.

**STERILIZATION OF SURGICAL MATERIALS**

Specific sterilization methods should be selected on the basis of the physical characteristics of the materials to be sterilized. Sterilization indicators should be used to validate that materials have been properly sterilized. Please refer to [WSU IACUC policy #3- Sterilization of Instruments and Supplies for Aseptic Surgery](#) for appropriate methods of sterilization.

If sterile surgical supplies are stored, materials must be properly packaged, labeled with the date of sterilization and should be used within 6 months of the date. Contaminated or outdated
materials must be re-sterilized prior to surgical use. At the time of surgery, the surgical packs must be opened aseptically to maintain sterility.

**Part I – Rodent Survival Surgery**

**Purpose:**

Aseptic surgical procedures are designed to prevent post-surgical infection due to microbial contamination of the incision and exposed tissues. Prevention of infection improves the welfare of the animal and eliminates a source of uncontrolled variation in the experimental results.

The policy for aseptic surgery in rodents applies to mice of the genus Mus, rats of the genus Rattus as well as the USDA covered rodent species including but are not limited to the following: chinchillas, degus, gerbils, guinea pigs, deer mice, voles, hamsters and others. Please refer to Part II for the aseptic surgery policy for animals other than rodents.

**Procedures:**

**A. Surgical Area:**

- The surgical area should be separate from other laboratory activities and access restricted to essential surgical personnel. The area should be clean and uncluttered and work surfaces must be non-porous and sanitizable.
- Prepare the surgical area by removing all extraneous equipment or other materials.
- Clean the area with a disinfectant (10% bleach solution, Clidox) and place a clean towel or drape material to cover the work surface. There should be some means for provision of supplemental heat (heat pad, lamp, etc.) to prevent hypothermia in the anesthetized animal.
- Prepare your surgery area, arrange gas anesthesia mask, stereotaxic apparatus, etc. before unwrapping instruments and putting on gloves.

**B. Instruments, Suture Materials, Towels, Gauze Pads and Drapes:**

- All instruments that come in direct contact with the surgical site must be sterile. Refer to the [WSU IACUC policy #3-Sterilization of Instruments and supplies for Aseptic Surgery](#) for more information.
• Any instruments, sutures, etc. soaked in chemical sterilants must be rinsed off with sterile water or 0.9% NaCl before use.
• If performing surgery on more than one rodent, begin with at least 2 sets of sterile instruments. See section H for multiple animal surgeries.

C. Animal Preparation:

Rodents scheduled for survival surgery must have completed the required acclimatization period (refer to WSU IACUC Policy # 12 for Acclimation and Stabilization policy of animals used for research or teaching) or been released from quarantine by the Office of the Campus Veterinarian. Exceptions to this policy must be approved on an Animal Subjects Approval Form submitted to the IACUC.

• Evaluate prospective rodents to ensure that they are apparently healthy.
• Do not withhold food in rodents before surgery unless specifically mandated by the protocol or surgical procedure. Water must NOT be withheld unless required by the protocol. Withholding food for greater than six (6) hours in rats or mice must be discussed with a veterinarian and approved by the IACUC.
• Do the animal preparation in an area away from the surgical area (Note: animal preparation includes anesthetic induction, hair clipping and initial scrub).
• Induce anesthesia and check anesthesia level after required induction time using the toe pinch method.
• After the animal is anesthetized, apply a bland sterile ophthalmic ointment to the eyes to prevent drying, which could result in development of corneal ulcers. (Note: Animals do not close their eyes when anesthetized and they do not blink.)
• Remove hair from the surgical site. Electric clippers with #40 or #50 blade or depilatory cream may be used. The area to be shaved must be twice that expected for the surgical area in the event that a larger incision than planned may be required.
• Put on clean or sterile gloves and scrub the shaved skin with a chlorhexidine or povidone iodine soaked gauze/cotton. Start from the center of the shaved site (or start from where incision will be) and clean in concentric circles toward the edge of the shaved area. Discard the chlorhexidine or iodine soaked gauze and use an alcohol soaked gauze (70%
isopropyl alcohol) to remove excess chlorhexidine or iodine in a similar fashion as above (starting from the center working towards the edge).

D. Patient Surgical Scrub:

- Move the animal to the surgical area.
- Place animal on a clean absorbent pad, over a heating pad (if appropriate), or in appropriate stereotaxic apparatus.
- Position the animal. Do not overstretch the legs or bind them in such a way as to restrict circulation.
- Repeat chlorhexidine /sterile water or iodine/alcohol scrub at least two more times (as described above) for a minimum of 3 total scrubs.
- If possible, cover the animal with a sterile (recommended) drape with a fenestration (opening) over the proposed incision site. The drape minimizes contamination of the surgical area and surgical instruments. (To perform sterile draping, the surgeon must use sterile gloves).

E. Surgeon:

- Wear a clean lab coat and remove all jewelry (rings, bracelets, watches) on the hands and wrists.
- Don a face mask for all surgeries. A hair bonnet or cap is recommended to prevent the surgeon's hair from contaminating the surgical area.
- Wash and scrub hands with a disinfectant soap, or surgical scrub brush, and dry with clean towels.
- Wear sterile gloves.
- Change gloves if they become contaminated.
- Anything touching the surgeon's gloves, drape or the sterile field must be sterile. If forceps are used to check the toe pinch response, the tips are considered contaminated.
- Sterile gauze pads may be used to manipulate non-sterile objects.
F. Recommendations for Surgical Procedures:

1. Incision(s)
   - Check level of anesthesia again using toe pinch method.
   - Make the incision using a sharp scalpel or scissors.
   - Check level of anesthesia again using toe pinch method.
   - Control any hemorrhage through direct digital pressure, electrocautery, or with a hemostat and tying off vessels as appropriate.
   - Using a new scalpel or scissors, incise deeper layers of tissue, such as the abdominal wall. Take care to prevent damage to underlying structures.
   - Perform the intended surgical procedure. Work carefully. Avoid unnecessary crushing of tissues. If tissues are to be exposed for any length of time, they must be periodically lavaged with sterile saline, or covered with a saline-soaked gauze.

2. Closure of Incision(s):
   - Close the deeper tissue layers in one layer.
   - Depending on the procedure, a simple, continuous suture pattern with a 3-0 or 4-0 (for rats) or 4-0 to 5-0 (for mice) synthetic absorbable suture may be used or a simple interrupted pattern using natural absorbable (chromic gut) may be used.
   - Tighten all knots adequately. Only apply enough strength to the closure to appose tissue edges. Tissue should not be compressed.
   - Close the skin as a separate layer using simple interrupted suture pattern with monofilament non-absorbable suture such as nylon. Tissue adhesive or staples or wound clips may also be used. Uncoated silk is not appropriate because of its wick function, predisposing to postoperative infections.

H. For Multiple Animals Receiving Surgery:
   - After the first surgery, clean any blood or debris off of the instruments by rinsing or wiping with sterile saline or distilled water and insert each instrument into a hot bead sterilizer for the recommended time. Allow instruments to cool before using.
• Put on new sterile surgical gloves if they become contaminated by touching non-sterile surfaces or if they are soiled when starting surgery on a new animal. Because of the likelihood of contamination, gloves should be changed after every 3 animals.

• Follow all above procedures on the next animal. At any time in the surgery, if known or suspected contamination has taken place, the instrument should not be reused before re-sterilization.

I. Postsurgical Care:

• Recover each rodent in a separate cage with clean bedding or toweling. Conscious animals may injure an anesthetized animal.

• Recover the animal in a warm environment, for example in a clean bedded cage placed over a heating pad, a circulating warm water heater, or chemical hand warmers covered with a clean towel. A warm water bottle or warmed saline bag covered with towel or a heat lamp can also be used. Avoid direct contact of rodent with heat source. Use the lowest level of heat possible to prevent accidental burns.

• In prolonged or very invasive surgeries, administer warmed, balanced electrolyte solution (such as Lactated Ringers Solution = LRS) given intraperitoneally (IP) or subcutaneously (SC). Administer 0.5 -1.0 ml SC or IP to mice and 3- 5 ml SC or IP to rats. Larger rodent species may be have an indwelling IV catheter placed and receive fluids (LRS) via IV drip during the procedure. Alternatively, SC fluids may be administered at a rate of 4 ml/kg for every hour of surgery. More may be needed if there was much bleeding during surgery. Additional fluids should be given if the animal is dehydrated or not drinking.

• Monitor the color of pinnae (external ear) or footpad. If the color is too pink, this probably denotes overheating.

• Check respiration rate and depth every 10 to 15 minutes, until they have recovered their balance and can right themselves.

• Report any complications to the supervisor. The veterinarian must be consulted if recurring problems are not resolved.

• The animal must be monitored daily following surgery, assessing such parameters as appetite, and wound healing. Administer analgesics and other drugs as stipulated in the protocol or as recommended by the veterinarian.
Check on the animals the next morning. If they still appear lethargic, or do not appear to be eating or drinking, repeat IP fluid administration and analgesics.

Remove non-absorbable skin closure materials 10-14 days post surgery.

J. Records:

- Appropriate records of the surgical procedure, anesthesia and pre- and post-operative care should be maintained for all rodents and is mandated for USDA regulated rodents (i.e., gerbils, hamsters, guinea pigs, deer mice, voles, degus, chinchillas). All record notations must be signed/initialed and dated. A sample surgical record form is attached at the end of this document.
- The cage of the animal(s) should be marked by using the specific animal facility method indicating that the animal has undergone surgery.

REFERENCES:

- Forman, L.A., 2000; Rodent Surgery guidelines, Northwestern University, Chicago, IL.
- Ryden E. and Larsen D. 2004. Comparative Medicine Resources, New Jersey Medical School, UMDNJ Newark Campus
- IACUC Guidelines, University of California at San Francisco, 2005.

Part II –Survival Surgery for Non-Rodents

Purpose:

Aseptic surgical procedures are designed to prevent post-surgical infection due to microbial contamination of the incision and exposed tissues. Prevention of infection improves the welfare of the animal and eliminates a source of uncontrolled variation in the experimental results.

This portion of the policy applies to dogs, cats, non-human primates, swine, rabbits, birds, wildlife, agricultural animals and species other than those described in Part 1. Minor surgical procedures in agricultural animals or wildlife may be done under field or farm conditions as described within an IACUC approved protocol but still require appropriate aseptic technique.
Procedures:

A. Surgical Area:

- A dedicated surgical room should be located outside normal facility traffic patterns & personnel access should be restricted to essential surgical staff. A bench or counter-top within a laboratory is not sufficient.
- There should be separate surgical preparation and recovery areas for the animals and scrub areas for the surgical personnel.
- The interior surfaces of the surgical room should be constructed of materials that are impervious to moisture and easily cleaned. Ideally, the ventilation system for the surgical area should provide a net positive pressure with respect to the surrounding facilities.

B. Equipment

- All instruments that come in direct contact with the surgical site must be clean and sterile. Refer to the WSU IACUC policy #3-Sterilization of Instruments and supplies for Aseptic Surgery for more information.
- Any instruments, sutures, etc. soaked in chemical sterilants must be rinsed off with sterile water or 0.9% NaCl before use.
- It is highly recommended to use a new set of sterilized instruments and sterile gloves for each animal if performing serial surgeries. If known or suspected contamination has taken place, instruments should not be used before re-sterilization.

C. Animal Preparation:

Animal scheduled for survival surgery must have completed the required acclimatization period (refer to WSU IACUC Policy # 12 for Acclimation and Stabilization policy of animals used for research or teaching) unless indicated otherwise on the approved Animal Subjects Approval Form.

- Evaluate prospective animals to ensure that they are apparently healthy.
- Withhold food before surgery as appropriate for the species. Water should not be withheld unless required by the protocol.
- Do the animal preparation in an area away from the surgical area (Note: animal preparation includes anesthetic induction, hair clipping and initial scrub).
• After the animal is anesthetized, apply a bland sterile ophthalmic ointment to the eyes to prevent drying, which could result in development of corneal ulcers.

• Remove hair from the surgical site. Electric clippers with #40 blade may be used. The area to be shaved must be twice that expected for the surgical area in the event that a larger incision than planned may be required.

• Put on clean or sterile gloves and scrub the shaved skin with a chlorhexidine or povidone iodine soaked gauze/cotton. Start from the center of the shaved site (or start from where incision will be) and clean in concentric circles toward the edge of the shaved area. Discard the chlorhexidine or iodine soaked gauze and use an alcohol soaked gauze (70% isopropyl alcohol) to remove excess chlorhexidine or iodine in a similar fashion as above (starting from the center working towards the edge).

D. Patient Surgical Scrub:

• Move the animal to the surgical area.

• Place animal over a heating pad (if appropriate). The surgical approach will dictate actual animal position but some guidelines to consider are:
  1. The animal’s respiratory function should not be compromised
  2. Limbs should not be extended beyond their normal range of motion and restraint straps should be padded as needed to prevent impaired venous return in extremities.
  3. Ruminants are frequently positioned on a slight incline with the head dependent, to minimize the potential for aspiration of rumen fluids.

• Personnel who perform the presurgical skin preparation should wear a cap, mask and clean or sterile gloves when preparing the surgical scrub supplies and when opening pre-sterilized sponge and drape packs.

• Repeat chlorhexidine/sterile water or iodine/alcohol scrub at least two more times (as described above) for a minimum of 3 total scrubs (3 surgical scrubs alternating with 3 alcohol scrubs).

• Drapes serve to isolate the surgical site and minimize wound contamination. Cover the animal with a sterile drape with a fenestration (opening) over the proposed incision site. Drapes should be positioned without the fabric dragging across a non-sterile surface.
E. Surgeon:

- The surgeon should wear surgical scrubs and shoes or wear shoe covers. Head covers and face masks should cover all facial hair. Remove all rings, jewelry and wrist watches before scrubbing. Finger-nails should be trimmed short and cleaned with a disposable nail cleaner. Don a face mask for all surgeries.
- Scrub sinks equipped with leg or foot-operated faucets are ideal. Regular faucets must be turned on, adjusted and not touched again.
- The hands and forearms are washed for 30 to 60 seconds with a surgical scrub soap. Then a sterile brush is used to methodically scrub all surfaces of the hands, fingers and forearms down to the elbows. Both arms are rinsed and the process repeated starting with fingertips working down to the elbows. Contact times of 3 to 15 minutes and/or 5 to 20 strokes per surface are recommended. Dry the hands with a sterile towel.
- Put on sterile gown using appropriate gowning technique to maintain gown sterility.
- Put on sterile surgical gloves using appropriate gloving technique to maintain glove sterility.
- Arms and hands should be held above the waist at all times. Aseptic technique is maintained when the gowned and gloved surgical team only touches sterilized equipment within the sterile field.

F. Aseptic technique:

It is highly recommended that surgery be performed with a team of trained personnel so that the surgeon can focus of the surgery and other personnel can prepare and monitor the animal. The surgeon working alone faces logistical problems when attempting rigid aseptic protocol as defined above.

A proposed practical sequence of steps to minimize errors is presented as follows:

1. Assemble all sterilized supplies.
2. Change into scrubs.
3. Set up table, heat pads and gas machines, check equipment.
4. Weigh animal, induce anesthesia. Prepare animal by hair clip and shave, catheters placed as required.
5. Position and secure animal on the table.
6. Connect to gas machine, connect accessory monitors. Start I.V. lines as required.
7. Make certain that a stable anesthetic plane is attained.
9. Using one sterile glove, prepare surgical site with scrub solutions.
13. Put on gown and gloves.

G. Records:

- Appropriate records of the surgical procedure, anesthesia, anesthesia monitoring and pre- and post-operative care should be maintained for all animals and is mandated for USDA regulated species (i.e. dogs, cats, rabbits, primates, wildlife and agricultural animals used in biomedical research). All record notations must be signed/initialed and dated. A sample surgical record form is attached at the end of this document.
- The cage or pen of the animal(s) should be marked by using the specific animal facility method indicating that the animal has undergone surgery.

SUMMARY

The practice of aseptic technique, when performing survival surgical procedures, minimizes the chances that animal health or experimental data will be compromised by post-surgical infections. Aseptic techniques require that appropriate facilities and equipment be available and that the personnel involved be adequately trained. The key element in maintaining an aseptic environment is well-trained personnel who understand the principles of aseptic technique and utilize this knowledge on an ongoing basis.

This information was adapted, with permission, from: ESSENTIALS FOR ANIMAL RESEARCH: A PRIMER FOR RESEARCH PERSONNEL, Second Edition, B. Taylor Bennett, D.V.M., Ph.D.
REFERENCES


Contemporary Topics in Laboratory Animal Science Volume 45, Issue 6 November 2006; Hélène Héon, Nathalie Rousseau, Jane Montgomery, Gilles Beauregard, and Manon Choinière

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