1. PURPOSE

To ensure that rodent survival surgeries are completed using the basic rules of asepsis, gentle tissue handling, anesthetic maintenance, and proper post-operative care. To ensure that rodent survival surgeries are carried out in accordance with applicable regulations, university policies and best practices.

It is incumbent upon the investigator to care for all animals to minimize pain and distress and to optimize animal care. In addition, good animal care and use will optimize research results and minimize variability, thus making it possible to use fewer animals.

2. INTRODUCTION

2.1. Survival surgical procedures in all animals including rodents should adhere to Halsted’s Principles of Surgery. These include:

- Gentle tissue handling
- Accurate hemostasis
- Preservation of adequate blood supply
- Strict asepsis
- No tension on tissues
- Careful approximation of tissues
- Obliteration of dead space

2.2. Aseptic surgical procedures are designed to prevent post-surgical infection due to microbial contamination of the incision and exposed tissues.

2.3. Aseptic technique results in decreased inflammation and gentle tissue handling results in decreased catabolism enhancing recovery and reducing postoperative complications.

2.4. Infections in rodents can be sub-clinical, but still affect the behaviour and/or physiology of the animal.

2.5. Prevention of infection improves the welfare of the animal and eliminates a source of uncontrolled variation in the experimental results.

2.6. Research staff performing surgery must have completed the Carleton University Surgery Training Module and be competent in the surgical procedure to be performed.

2.7. Surgery should be scheduled early in the work-week to minimize the need for weekend monitoring.

3. PROCEDURE

3.1. Instruments, Suture Materials, Towels, Gauze Pads and Drapes:

3.1.1. All instruments that come in direct contact with the surgical site must be sterile.
3.1.2. Steam (autoclave), is required. Chemical sterilization is also an option. 
3.1.3. The instruments, sutures, etc. should be placed in a specially designed pack or wrapped in drapes or cloths, then steam autoclaved. Label packs with date of sterilization.

3.1.3.1. Any implants should be sterilized; fragile implants may be gas-sterilized or soaked in 2% glutaraldehyde (soak for 10 hours, or as otherwise directed) or other chemical sterilant (not disinfectant) – rinse off copiously with sterile water or 0.9% NaCl before implanting.

3.1.4. If performing surgery on more than one rodent, begin with at least 2 sets of sterile instruments.

3.1.5. Open the instrument pack.

3.1.6. Use sterile suture, drapes and gowns prepared by autoclaving (or they can be purchased sterile).

3.1.7. If the experimental design requires repetitive surgeries (i.e. performing the same surgical procedure on a number of rodents at the same time) proceed as follows between animals:

3.1.7.2. Clean all instruments thoroughly to remove all organic material rinse thoroughly and sterilize instruments. A bead sterilizer is recommended. (Note: alcohol is not a sterilant); ensure instruments are cool before using. It is recommended that a new set of sterile, autoclaved instruments be used on every five animals.

3.2. Animal Preparation:

3.2.1. Rodents scheduled for survival surgery must have completed the required acclimatization period, a minimum of 5 days is recommended for animals undergoing surgery, or been released from quarantine.

3.2.2. Evaluate prospective rodents, before surgery, to ensure that they are clinically healthy: good body condition and exhibiting normal behaviour.

3.2.3. 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 longer than six (6) hours in rats or mice must be discussed with a veterinarian and approved by the ACC.

3.2.4. A pre-surgical baseline weight is required – this information is also needed to determine drug dosages.

3.2.5. Animal should be prepared in an area away from the surgical area (Note: animal preparation includes anesthetic induction, hair clipping and initial scrub).

3.2.6. Induce anesthesia and check anesthetic depth after the required induction time by verifying lack of withdrawal upon firm toe pinch (toe pinch method).

3.2.7. After the animal is anesthetized, apply a 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.)

3.2.8. Remove fur from the surgical site using electric clippers. 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. Adhesive tape may be used to remove excess hair from the surgical site.

3.2.9. Aseptically prepare the surgery site:
3.2.9.3. Scrub the shaved skin with a chlorhexidine scrub 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.

3.2.9.4. Discard the chlorhexidine soaked gauze and use an alcohol soaked gauze (70% isopropyl alcohol) to remove excess chlorhexidine in a similar fashion as above (starting from the center working towards the edge).

3.2.9.5. Follow up with a chlorhexidine or iodine solution.

3.2.10. Repeat step 3.2.8, if performing stereotaxic surgery, after the animal has been placed in the ear bars.

3.3. Patient Surgical Scrub:

3.3.1. Move the animal to the surgical area.

3.3.2. Do not use the surgical area for any other purpose during the time of surgery.

3.3.3. Place animal on a clean absorbent pad, over a heating pad (if appropriate), or in appropriate stereotaxic apparatus.

3.3.4. Position with tape, if needed. Do not overstretch the legs or bind them in such a way as to restrict circulation.

3.3.5. Repeat chlorhexidine/alcohol/iodine skin preparation (as described above in 3.2.9).

3.3.6. Whenever 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 already be aseptically prepared including use of sterile gloves).

3.4. Rodent Anesthesia Monitoring

3.4.1. Refer to SOP: ANA-04 – Rat Anesthesia and ANA-03 – Mouse Anesthesia for details on anesthesia and anesthetic protocols.

3.4.2. Anesthetic monitoring of small rodents includes testing of rear foot reflexes before any incision is made, and continual observation of respiratory pattern, mucous membrane color and responsiveness to manipulations throughout the procedure. Monitor the rodent continually, every 10-15 minutes and note the following:

3.4.2.6. **Toe pinch method**: used to evaluate depth of anesthesia is useful but not enough in itself.

3.4.2.6.1. Use two fingers and give the toe/foot a good squeeze. If there is no withdrawal reaction, the animal is judged deep enough to commence surgery.

3.4.2.6.2. Remember that after this has been done the fingers are not sterile anymore.

3.4.2.6.3. A sterile gauze pad may be used to protect the sterile gloves. Alternatively, a hemostat may be used to squeeze toe/foot. In this case, one must be careful not to squeeze too hard.

3.4.2.6.4. After the hemostat/forcep has been used to squeeze toe, it is not sterile anymore and must not be used for surgery.
3.4.2.7. **Respiratory pattern:** Anesthesia will cause a distinct slowing of respiratory rate (RR). The surgeon must evaluate if RR becomes too slow and the anesthesia needs to be lightened and if the depth of respiration becomes too shallow. Increasing RR indicates the need for supplemental anesthesia.

3.4.2.8. **Mucous membranes (MM):** MM are evaluated by the color of the pinnae (ears) and toes.

3.4.2.8.1. Pale, bluish or cool this is an emergency, indicating that the animal does not have enough oxygen.

3.4.2.8.2. Pink is good

3.4.2.8.3. Red MM usually indicates that the animal is too warm. This is not likely to occur during surgery but may occur during recovery from anesthesia, especially if a heat lamp is used to keep the animal warm. In such a case, the animal recovering from anesthesia must be protected and the lamp moved.

3.4.2.9. **Reaction to surgical manipulation:** If the animal makes any kind of move in response to incision or manipulation of organs, surgery must be temporarily stopped and anesthesia supplemented.

---

**3.5. Surgeon:**

3.5.1. Wear a clean lab coat or scrub top and remove all jewellery (rings, bracelets, watches) on the hands and wrists.

3.5.2. Don a facemask and hair bonnet or cap for all surgeries.

3.5.3. Wash and scrub hands with a disinfectant soap, or surgical scrub brush, and dry with clean towels.

3.5.4. Done a sterile surgical gown

3.5.5. Wear sterile gloves (It is acceptable to use clean nitrile gloves disinfected with Accel and then rinsed thoroughly with sterile water or sterile saline.). Gloves should be donned after the animal has been fully prepped, immediately before commencing surgery.

3.5.6. Change gloves between animals or if they become contaminated.

3.5.7. Anything touching the drape or the sterile field must be sterile. If forceps are used to check the toe pinch response, the tips are considered contaminated.

3.5.8. Sterile gauze pads may be used to manipulate non-sterile objects.

---

**3.6. Surgical Procedure:** Please recall that only procedures approved in the ACC protocol can be performed

3.6.1. Check level of anesthesia again using toe pinch method.

3.6.2. Make the incision using a sharp scalpel or scissors.

3.6.3. Check level of anesthesia again using toe pinch method.

3.6.4. Control any hemorrhage through direct digital pressure.

3.6.5. Incise deeper layers of tissue, such as the abdominal wall. Take care to prevent damage to underlying structures.
3.6.6. 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.

3.7. **Closure of Incision(s):**

3.7.1. Close the deeper tissue layers in one layer.

3.7.2. 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. Note that silk is not appropriate because of its wick function, predisposing to postoperative infections.

3.7.3. Tighten all knots adequately. Only apply enough strength to the closure to appose tissue edges. Tissue should not be compressed.

3.7.4. Close the skin as a separate layer using simple interrupted suture pattern with monofilament non-absorbable suture such as nylon (silk is not appropriate due to wicking and poor tensile strength). Wound clips may also be used.

3.8. **For Multiple Surgeries:**

3.8.1. After the first surgery, clean the instruments by rinsing in saline or distilled water and insert each instrument into a hot bead sterilizer for the recommended time. Be sure to allow time for cooling after immersion in the hot bead sterilizer.

3.8.2. If gloves are soiled, change them; if not spray with disinfectant (Accel).

3.8.3. Follow all above procedures on the next animal. It is recommended that a new set of sterile, autoclaved instruments be used on every five animals.

3.8.4. If known contamination has taken place, the instrument should not be reused before re-sterilization.

3.9. **Postsurgical Care**

3.9.1. Recover each rodent in a separate cage with clean bedding placing the rodent on a clean paper towel inside the cage until it is sternal. This is to avoid aspiration of bedding and direct eye contact with bedding, while the animal is still anesthetized.

3.9.1.10. Additional eye gel can be applied at this time. Paper towel should be removed prior to placing the animal back in the holding room.

3.9.2. Recover the animal in a warm environment, for example in a clean bedded cage placed over a water circulating heat pad, covered with a clean towel. The cage should be placed half on the heat pad and half on a non-heated surface. Avoid direct contact of rodent with heat source.

3.9.3. In prolonged or very invasive surgeries, administer warmed balanced electrolyte solution (such as Lactated Ringers Solution = LRS, sterile saline may also be used) 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. More may be needed if there was much bleeding during surgery. Additional fluids should be given if the animal is dehydrated or not drinking.

3.9.4. Analgesia must be administered as described in the approved AUP, even if the animal doesn’t appear to be in pain.
3.9.5. Supportive care such as: food mash, hydrogel, kitten milk replacer, etc. may be provided to assist with the post-operative recovery period.

3.9.6. The Veterinarian needs to be consulted to discuss altering the dosage or type of drug to be used if there are concerns that an animal is in pain or if there are side effects of concern.

3.9.7. Check respiration rate and depth every 10 to 15 minutes, until they have recovered their balance and can right themselves. The animal must not be left unattended until it has recovered and is able to remain upright in a sternal position.

3.9.8. Report any complications to the Veterinarian or ACVS staff. The veterinarian must be consulted if recurring problems are not resolved.

3.9.9. The animal must be monitored daily for a minimum of 72 hours and up to one week (including weekends and holidays) following surgery (depending on progress during post-op recovery period), assessing parameters such as body weight, general condition, wound healing, etc.

3.9.9.11. Measure body weight and administer analgesics and other drugs as stipulated in the protocol or as recommended by the veterinarian using the Rodent Surgical and Post Operative Record (Appendix 1) monitoring sheet to document care during recovery.

3.9.10. If during the next few days after surgery the animal(s) appear lethargic, or do not appear to be eating or drinking, or seem painful, re-evaluate condition. If indicated repeat IP or SC fluid administration and analgesics and the veterinarian.

3.9.11. Remove skin closure materials 10-14 days post surgery.

3.9.12. During periods in which morbidity and mortality are expected to increase, animals must be evaluated a minimum of two times daily (every 8-12 hours). Those animals that are not expected to survive until the next scheduled evaluation should be humanely euthanized

3.10. Records:

3.10.1. Keep appropriate and complete records including: date and time, type of surgical procedure, body weight, anesthesia and pre- and post-operative care including dose, route and time of administration of analgesics and antibiotics administered (see Rodent Surgical and Post Operative record, Appendix 1).

3.10.2. All record notations must be signed/initialled and dated.

3.10.3. All records must provide the same level of detail included in the linked forms and must be kept in one easily accessed centralized location that is contiguous to the animal housing location.

3.10.4. The cage of the animal(s) should be marked with a red “surgery” cage card (Appendix 2); this card will be removed, by ACVS staff, when the animal is stable in the post-operative period.

4. REFERENCES

SOP: ANA-** - Mouse Anesthesia
SOP: ANA-04: Rat Anesthesia
Boston University: Rodent Surgery Guidelines, Sept 2011
Forman, L.A., 2000; Rodent Surgery guidelines, Northwestern University, Chicago, IL.
5. **APPENDIX 1 – Surgical Record**

### Rodent Surgical and Post-Operative Record

<table>
<thead>
<tr>
<th><strong>Principal Investigator:</strong></th>
<th><strong>Date:</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Surgeon:</strong></td>
<td><strong>AUP #:</strong></td>
</tr>
<tr>
<td><strong>Species:</strong></td>
<td><strong>Animal ID #:</strong></td>
</tr>
</tbody>
</table>

**Surgical Procedure:**

**Pre-Surgical Evaluation**

<table>
<thead>
<tr>
<th><strong>Injectable Anesthetic:</strong></th>
<th><strong>Amount (mg)</strong></th>
<th><strong>Volume (ml)</strong></th>
<th><strong>Route</strong></th>
<th><strong>Time</strong></th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th><strong>Inhalant Anesthetic:</strong></th>
<th><strong>Induction (%):</strong></th>
<th><strong>Maintenance (%):</strong></th>
<th><strong>Start time:</strong></th>
<th><strong>Finish time:</strong></th>
</tr>
</thead>
</table>

**Analgesics:**

- [ ] Buprenorphine
- [ ] Meloxicam (5mg/ml)
- [ ] Other: ____________________

<table>
<thead>
<tr>
<th><strong>Amount (mg):</strong></th>
<th><strong>Volume (ml):</strong></th>
<th><strong>Route:</strong></th>
<th><strong>Time of injection:</strong></th>
</tr>
</thead>
</table>

**Supplemental Fluids:**

- [ ] Yes
- [ ] No

<table>
<thead>
<tr>
<th><strong>Type:</strong></th>
<th><strong>Volume (ml):</strong></th>
<th><strong>Route:</strong></th>
<th><strong>Time of injection:</strong></th>
</tr>
</thead>
</table>

**Comments:**

Please ensure that all medications and procedures listed above are as stated in the approved animal use protocol.

Temperature, mucous membrane color, and depth of anesthesia should be monitored at least every 5-10 minutes during anesthesia.

---

### Rodent Postoperative Record

<table>
<thead>
<tr>
<th>DATE/TIME</th>
<th>BODY WEIGHT</th>
<th>ASSESSMENT/FINDINGS</th>
<th>COMMENTS/TREATMENT/OUTCOME</th>
<th>INITIALS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>