Johns Hopkins University
Animal Care and Use Committee

Guidelines on Survival Surgery of Mice and Rats[ab]

BACKGROUND: These guidelines apply to all survival surgical procedures performed on mice and rats at Johns Hopkins University[1]. Survival surgery on mice and rats must be performed using sterile techniques to minimize microbial contamination of exposed tissues. Minor surgical procedures such as wound suturing and peripheral vessel cannulation should be performed in accordance with standard veterinary practices[1].

Pre-Operative:

1. A dedicated surgical facility is not required for rodents. Rodent surgery spaces should have the following components: animal preparation area, surgical area, and holding and recovery area. If the animal preparation area is the same as the surgical area, practices such as the disinfection or changing of absorbent pads must be conducted to avoid contamination of the surgical site with hair clippings.
2. Surgery should be conducted in a clean and uncluttered area.
   The surfaces on which the surgery is going to take place must be non-porous/sealed, durable, and easily sanitized. The surface should be disinfected or covered with clean absorbent pads prior to surgery.
3. Access to the area by personnel not directly involved in the surgery should be limited when surgery is being performed.
4. Instruments and other items to be used for surgery must be sterilized using autoclaving or gas sterilization (ethylene oxide or vaporized hydrogen peroxide).
   a. If other methods of sterilization are required for research goals, they must be described and approved in the ACUC protocol.
   b. Standard surface disinfectant solutions, such as ethanol or Vimoba, are not sufficient to achieve sterilization of surgical instruments before survival surgery and should not be used for this purpose.
   c. "Cold sterilization" can be used for sensitive surgical equipment that cannot be autoclaved or gas-sterilized; this is achieved using immersion for several hours in glutaraldehyde (e.g. Cidex). After cold sterilization, instruments must be thoroughly rinsed with sterile saline or sterile water to remove toxic chemical residues before contact with animals.
5. Once animal is anesthetized, apply sterile, non-medicated ophthalmic ointment to eyes to prevent corneal drying.

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[a] For additional training call 410-955-3713 or 410-955-3273.
[b] Approved by the ACUC on: May 24, 2001, revised July 16, 2009, minor revisions September 25, 2012; reviewed 1/31/18; revised by GPS Sept 2025

6. An external heat source (preferably a feedback-controlled infrared, warm water circulating, or air-circulating heating device) should be utilized for thermal support throughout anesthesia and surgery to prevent hypothermia. Animals must never be in direct contact with the heat source. Electric coil heating pads and heat lamps are not recommended intraoperatively due to the risk of burns. Whenever possible, consider monitoring the animal's core body temperature via temperature probe.
7. Administer sterile isotonic fluids as indicated to replace possible fluid losses.
8. Provide preemptive analgesic/s as described in the protocol.
9. Remove hair from the surgical site(s), in a separate work surface, if possible, from where the surgery is conducted. If the same work surface is used for both animal preparation and surgery, once the animal is prepped, remove hair clippings and disinfect the surface and/or place a new, clean absorbent pad before surgery.
10. Prepare the surgical site(s) using at least 3 alternating cycles of dilute chlorhexidine or povidone iodine scrub and 70% isopropyl alcohol, 70% ethanol, or sterile saline. Avoid using chlorhexidine, alcohol products, or scrubs containing detergents near the eyes, which may lead to ocular damage. If the surgical site is near the eye, periocular areas should be aseptically prepared using 3 alternating cycles of sterile saline and povidone iodine solution.
11. All sterile instruments and other items must be placed on a sterile surface such as a drape or open autoclaved peel pouch to maintain sterility.
12. While not required, sterile drapes covering incision site/ surgical site are highly recommended in order to maintain a sterile surgical field. If drapes are not being used, extra precautions must be taken in order to maintain appropriate aseptic technique. Drapes can be cloth, paper, sterile stockinettes, 3M™ Steri-Drape™ Incise Drapes, or new and unused GLAD Press'n Seal® wrap.
    a. Sterilize drapes using an autoclave, vaporized hydrogen peroxide or ethylene oxide where appropriate (plastic products).
    b. Drapes can be used for wrapping instrument packs prior to sterilization as well as for creating a sterile field around the incision site.
13. Surgeons must wear a clean laboratory garment, a facemask, head cap/hat, and wash and dry their hands before donning sterile gloves.
    a. Assistants working in the immediate vicinity must wear a clean garment, head cap/hat, facemask and gloves.
    b. If working in a laminar flow hood, a facemask and head cap/hat are not necessary.
    c. Appendix 1 provides information on purchasing sterile gloves and how to sterilize regular exam gloves for surgery. Note that spraying laboratory gloves with disinfectants (e.g. ethanol, Vimoba) does not achieve sterilization and is not an effective method of sterilizing gloves before survival surgery.

Intra-Operative:

1. The animal(s) must be maintained in a surgical plane of anesthesia throughout the procedure. Check for proper anesthetic depth (e.g. lack of reaction to toe pinch, evaluation of respiratory rate) every 15-20 mins.
2. Begin surgery with sterile instruments and handle them aseptically throughout procedure.
3. Instruments may be used for a series of similar surgeries on the same day, provided they are maintained clean and disinfected using a hot bead sterilizer between animals. If other methods of disinfection are performed between animals, they must be approved in the

ACUC protocol. Note that if a hot bead sterilizer will be used to re-sterilize instruments, the instruments must still be autoclaved or gas-sterilized before the first surgery of the day.

4. Lay instruments on a sterile drape in between use.
5. Monitor and/or maintain the animal's vital signs.
6. Handle tissue gently and prevent tissue from drying.
7. Close surgical wounds using appropriate techniques and materials.

Post-Operative:

1. Move the animal(s) to a warm, dry area and monitor during anesthesia recovery.
2. Once animal(s) is fully recovered (ambulating, etc), return to home cage. If animal is to be singly housed, ensure this is stipulated in the protocol, and mark the cage card as required.
3. Provide analgesics as specified in the protocol.
4. Evaluate all post-surgical animals at least once a day for 7 – 10 days, or until the skin sutures or wound clips are removed, or the skin incision site is healed, whichever is latest.
5. Skin sutures and clips should be removed as soon as the wound is healed and no later than 14 days post-operatively.
6. Maintain a surgical and post-op record (see relevant section below).
7. Seek veterinary assistance in case of complications (infection, wound dehiscence, etc) not described in the protocol. Follow any treatment and/or monitoring plan prescribed by the veterinary personnel.
    a. Incision site dehiscence may be surgically repaired following veterinary personnel consultation or in accordance with the IACUC approved protocol. Daily post-operative monitoring for 7-10 days from date of incision repair is required.

Surgical and post-op record documentation

1. Appendix 2 provides an example of a surgery and post-op form for rodents. <u>Other forms may be used but the information in the sample form is minimally required.</u>
2. Surgical and post-operative records are required for each rodent cage that houses mice that have undergone surgery. The cage record can reflect all animals in the same cage. However, any individual animal with surgical complications and/or post-operative findings like pain/distress, dehiscence, infection, and hemorrhage should be identified on the record, with a notation of the finding and its remediation.
3. Each day's recording can only be recorded at the time that the post-operative monitoring is occurring.
4. Records must include anesthetics and analgesics administered for the surgical procedure and after for post-operative care. The records must also include the frequency of monitoring by any laboratory and veterinary personnel during the post-operative period. Information recorded for anesthetics and analgesics should include the dose, frequency of administration, and route of delivery.
5. If the animal is euthanized or dies within the post-operative period, this must be noted on the post-operative record.
6. Records must be on the cage until at least 7 days after the surgery or until sutures/wound clips are removed, whichever is latest. Records may also be kept close to where the animals are housed, but a system should be in-placed to cross-reference the records with the cages involved (i.e., the records clearly indicate the cages involved and the cages are labelled accordingly). After completion, keep records in the laboratory available for review for at least one-year post-surgery.
7. Records must be readably retrievable for review by investigative, IACUC, or veterinary personnel at all times.

References:

1. *Guide for the Care and Use of Laboratory Animals* (8th edition), 2011.
2. Animal Welfare Act Regulations.

Appendix 1: Sterile gloves for rodent surgeries

Please note that for rodent survival surgeries, sterile gloves specifically manufactured for surgery are not required, but are acceptable.

Commercially available, individually packaged pairs of exam gloves are available via:

- Amazon (www.amazon.com; search for "sterile exam glove")
- eSafetySupply (www.esafetysupply.com; follow menu Category -> Disposable Gloves -> Latex Gloves -> Medical/Exam Grade Latex Gloves -> Sterile Gloves

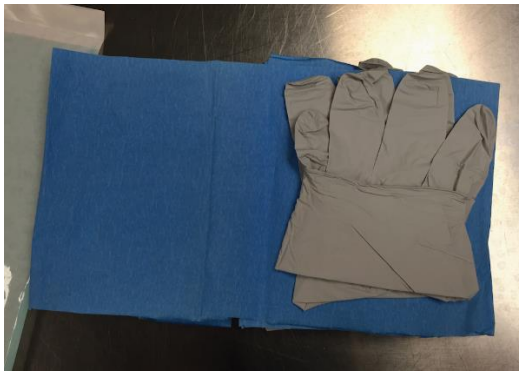
Regular exam gloves, packaged and autoclaved with appropriate quality assurance measures to ensure sterility (e.g., autoclave tape, etc.) are also acceptable. Below are instructions on how to do this.

Supplies Needed

- Clean latex or nitrile exam gloves
- Paper drape material
- Sterilization pouches
- Autoclave indicator strip
- Autoclave

Step-by-Step Instructions

1. Assure that autoclave is in good working order, with periodic testing, assessment, and appropriate record-keeping.
2. Fold the wrists of each glove up over the hand of the glove.
3. Cut drape material into rectangles to fold around each pair of gloves.
4. Wrap gloves, one pair per packet, with drape material to separate pairs of gloves or to separate gloves from instruments



5. Place an autoclave indicator within each glove.

6. Place up to 5 pairs of gloves in each sterilization pouch, or place one pair of gloves with a set of instruments to be used for the surgery (Note: contrary to the picture, place gloves ***ON TOP OF*** instruments to assist in maintaining sterility).

7. Autoclave gloves.
    a. Vacuum cycles
        i. Sterilize at 121°C for 20 minutes and dry 12 minutes
        ii. Sterilize at 121°C for 30 minutes and dry 12 minutes
        iii. Sterilize at 132°C for 6 minutes and dry 20 minutes
        iv. Sterilize at 132°C for 3 minutes and dry 1 minute (flash cycle used only for gloves in a peel pouch -- no instruments)
    b. Gravity cycle
        i. Sterilize 30 minutes and dry 10 minutes

Appendix 2: Sample Surgical Records

Instructions:

1. **Complete** the record **per cage** of animal/s that have undergone surgery.
2. For ***sections under italicized heading***, **indicate number of animals** that have abnormal findings and/or have received a treatment/intervention different than others.
3. **Perform post-op care** and **maintain the record on the cage** for at least 7 days post-surgery or until "End" (see Post-op care table), whichever is the latest.
4. **Consult** the rodent veterinarian and veterinary technicians for adverse events (e.g., unexpected or increased mortality) and complications/treatments/interventions outside of what are described in your ACUC-approved protocol.
5. **Fill out a new form** should a surgical repair/revision be performed. Both initial surgery and repair surgery records must be present in the cage while the cage is still under post-op monitoring. Surgical revision must be specifically approved in the protocol or by a veterinarian.
6. After completion, **keep records** available for review for at least **one-year post-surgery**.

Rodent Surgical and Post-op Record

PI: _____ Protocol number: _____ Surgeon: _____
Procedure: _____ # of animals in cage: _____ Date of procedure: _____
For each of the following, provide the dose (mg/kg or %) administered per animal:
Anesthetics: _____ Pre-emptive analgesia[1]: _____
Anesthetic and Surgical notes:[2]

_____
_____

Post-op care:

Date/Time	*Observations[3] and Treatments[4]/Interventions[5]*	Initials

[1]If no pre-emptive analgesia is given, write "**NA**". Note that this must be justified in the ACUC-approved protocol.
[2]Include 1) analgesics and other treatments; and 2) complications like redosing of injectable anesthetics because of poor anesthesia, severe blood loss during the surgery, and prolonged anesthetic recovery. Otherwise, write "**None**" to mean that the procedures were uneventful.
[3]Assess the overall condition of the animal and the incision site – normal vs. abnormal. If abnormal, provide info.
[4]Include analgesics (provide dose) and wound treatment.
[5]Include euthanasia. At end of post-op care, write "**End**". This means that the incision is healed and sutures/clips have been removed.