

University of Michigan
Unit for Laboratory Animal Medicine

Guidelines for the Performance of Survival Surgery on Rodents

I. Introduction

This document is intended to provide guidelines for investigators who perform survival surgical procedures utilizing rodents. The importance of utilizing appropriate surgical techniques should not be underestimated. They are designed to reduce post-surgical complications (e.g., infections and wound dehiscence), improve animal survival rates, and hasten return to the basal physiological functions that were present in the animal prior to surgery. Therefore, by observing appropriate procedures for aseptic surgery, the investigator will help ensure that the post-surgical rodent will be a suitable research subject.

The following guidelines are based on the recommendations and regulations outlined in the Guide for the Care and Use of Laboratory Animals (The Guide), the Animal Welfare Act, the PHS Policy on Humane Care and Use of Laboratory Animals, and NIH Guidelines for Survival Rodent Surgery. In most cases, the best results will be obtained by following the recommended guidelines. However with prior approval from the University Committee on Animal Care and Use (UCUCA) or the Unit for Laboratory Animal Medicine (ULAM), it may be acceptable to deviate from these guidelines. Investigators are encouraged to discuss alternatives with a ULAM veterinarian (764-0277) or the UCUCA Coordinator of Research Animal Standards and Staff Development (763-8028). The Coordinator can also assist individuals seeking additional training in any of the techniques or procedures discussed.

Additional information related to rodent surgeries can be found in other UCUCA documents addressing issues including, but not limited to, anesthesia guidelines for multiple species, a policy on endstage illness and humane endpoints, and post-surgical monitoring of rodents. These and other useful documents can be accessed at <http://www.ucuca.umich.edu/UCUCADoc.html>.

Surgical Procedures

Surgical procedures are categorized by The Guide as either major or minor procedures based on the anatomical site and the degree of physiological impairment. A major surgery penetrates and exposes a body cavity or produces substantial impairment of physical or physiologic functions. Examples of a major surgery include laparotomy, thoracotomy, craniotomy, joint replacement, and limb amputation. A minor surgery does not expose a body cavity and causes little or no physical impairment. Examples of a minor surgery include wound-suturing, and peripheral-vessel cannulation.

Surgical procedures can also be divided into survival and non-survival procedures. In a survival surgery, the animals are allowed to recover from anesthesia while in a non-survival surgery, the animal is euthanized prior to recovery.

Both major and minor survival procedures must be performed using aseptic technique and instruments as well as appropriate anesthesia. However, aseptic technique may not need to be as strictly followed for non-survival surgeries.

Aseptic Technique

Asepsis implies the freedom of living tissue from pathogenic organisms. Aseptic surgery may be defined as surgery performed in an environment sufficiently free of microorganisms such that significant infection with pathogenic organisms does not occur. In order to conduct an aseptic surgery, the environment, the equipment and instruments, the animal subject, and the surgeon must be adequately prepared. In addition, the surgeon and equipment must remain free of contamination throughout the length of the surgery.

The following guidelines are designed to assist investigators both with the preparation for and the performance of aseptic surgery in rodents. Although some deviations from strict aseptic procedures are considered acceptable for rodent surgery, the basics of aseptic technique should be observed when performing survival surgical procedures. Any questions about allowable deviations from aseptic technique can be directed to UCUCA (ucuca.office@umich.edu).

II. Preparation of the Environment

The Surgery Room/Area

Use of a dedicated operating room is not required when performing rodent surgeries. However, it is required that the room, or portion of the room, that is used is easily sanitized and is not used for any other purpose during the time of surgery. Although this area may be used for other purposes when surgery is not being conducted, preparation for surgery will be facilitated if the area is kept reasonably free of clutter. It is highly advisable that shelves, equipment, and air vents not be located directly over the surgical area as dust, debris and storage items may fall from these areas into the surgical field. However, if due to space constraints, this configuration is unavoidable, a covering (i.e., poster-board or heavy plastic draping material) can be applied to the shelves to prevent contamination of the surgical area.

Preparation of the Surgery Area

The surface upon which surgery will be performed should be cleaned and disinfected on a regular basis, or at least shortly before each surgery day. For maximum efficacy, the disinfectant should be liberally applied. The surface should not be wiped dry, but instead should be allowed to air-dry. The following disinfectants can be used for this purpose. Please note that use of some of the disinfectants listed (ex. alcohol) are not appropriate for destroying or inactivating all viral agents.

Disinfectants Recommended for Preparation of the Surgical Area.

Disinfectant	Examples	Comments
2% glutaraldehyde plus 7.05% phenol	Sporocidin [®]	toxic
2% aqueous glutaraldehyde	Cidex [®] , Cide-Swipes [®]	toxic
chlorine (sodium hypochlorite, chlorine dioxide)	Clorox [®] , Exspor [®] , Clidox [®]	corrosive; presence of organic matter reduces activity; chlorine dioxide must be freshly made
70% ethyl alcohol or 85% isopropyl alcohol		contact time required is 15 minutes. contaminated surfaces take longer to disinfect; remove gross contamination before using.
Quarternary Ammonium	Roccal-D [®] or Virex II [®]	rapidly inactivated by organic matter; compounds may support growth of gram negative bacteria

Preparation of Surgical Instruments

Surgical instruments must be sterilized before use in a rodent survival surgery. Sterilization differs from disinfection. Sterilization implies the destruction of all microorganisms and their pathogenic products, whereas disinfection implies destruction of vegetative forms of pathogenic bacteria, but not necessarily viruses, fungi, or spore-forming bacteria. It is important to note that many agents that are excellent disinfectants are not effective sterilants for surgical instruments. The following techniques and agents can be used to sterilize instruments and other materials that will come into direct contact with the animal's internal tissues. A “recommended” method should be utilized in place of an “acceptable” method when possible.

RECOMMENDED Means of Sterilizing Surgical Instruments

- Single-use autoclaved surgical packs. Saturated steam under pressure (autoclaving) requires 30-40 min at 250° F and 15 psi.
- Hot bead sterilizer (sterilization in 10 seconds) (www.stoeltingco.com/physio or www.braintreesci.com). Instruments should be cleaned prior to their placement in a hot bead sterilizer. Only the portion of the instrument inserted into the sterilizer will be sterilized. Any portions of the instruments that do not contact the beads (such as the instrument handles) will not be sterilized by this process. The beads should be replaced approximately every six months. To extend the life of the unit, it is not recommended that the unit be left on for long periods of time (i.e. overnight).

ACCEPTABLE Methods for Sterilization of Surgical Instruments

- Cold liquid sterilization – requires submersion of the instruments in the following solutions for the designated periods of time:

Solution	Required Contact Time	Comments
10% povidone iodine solution	24 hours	
2% aqueous glutaraldehyde (Cidex [®])	10 hours	toxic
2% glutaraldehyde plus 7.05% phenol (Sporocidin [®])	10 hours	<ul style="list-style-type: none"> • requires immersion for 10 hr in a 1:8 aqueous solution • rinse in <u>sterile</u> water or <u>sterile</u> saline before use • corrosive for metal instruments.
Chlorine dioxide (Clidox-S)	6 hours	<ul style="list-style-type: none"> • requires immersion for 6 hr in a 1:5:1 solution (1 part Clidox activator : 5 parts water : 1 part Clidox base) • the newly mixed solution must be allowed to sit for 15 minutes prior to use • once mixed, the solution is effective for 14 days after which time a new solution must be made • highly corrosive for metal instruments. • rinse in <u>sterile</u> water or <u>sterile</u> saline before use
Chlorhexidine solution (Nolvasan [®] , Hibiclens [®])	24 hours	<ul style="list-style-type: none"> • rinse in <u>sterile</u> water or <u>sterile</u> saline before use

Following cold sterilization, the instruments must be thoroughly rinsed in sterile saline or sterile water prior to use in order to remove all solution that can be caustic to tissue. It should be noted that most liquid sterilants are corrosive and, with repeated use, will damage surgical instruments.

- Ionizing radiation (gamma-rays)
- 10% ethylene oxide in CO₂ - requires 8-10 hours at 131-156°F
 - articles must be placed in a well-ventilated area for at least 48 hr following sterilization so that any residual ethylene oxide gas can dissipate
 - the Central Sterile office of the University of Michigan Hospitals (936-6162) can perform this service. All items submitted to Central Sterile that are to be used in animal research must be marked with a sticker (preferably bright or fluorescent orange) that says, “FOR ANIMAL USE ONLY.”

Alcohol should not be used for the sterilization or disinfection of surgical instruments as it is inactive against bacterial spores and many viruses, does not penetrate surfaces well, and requires a prolonged contact time.

Any devices that will be implanted (intraventricular catheters, mini pumps, electrodes, etc) must be disinfected or sterilized prior to implantation.

Devices or equipment (i.e., animal restraining devices, monitoring equipment, stereotaxic devices, etc) that will be required in the surgical field should also be disinfected prior to each use. This includes disinfection between use with each animal when used in consecutive surgeries.

Procedure for Multiple Surgeries

Although the use of sterile instruments is highly desirable for all survival surgeries, it may not be feasible under circumstances where many surgeries will be performed in a single session. Under these circumstances, investigators should begin with at least 2 sets of sterile instruments. Starting the surgical session with multiple sets of sterile instruments will enable the surgeon to continue to work while the used instruments are being disinfected. After the sterile instruments have been used, they should be thoroughly cleaned to remove all organic material and then dried if possible. The instruments should then be treated with a high-level disinfectant at an appropriate concentration and for an appropriate length of time before being used with the next animal. The following disinfectants are recommended for this purpose:

Disinfection/Sterilization of Surgical Instruments Used for Multiple Surgeries

- Hot bead sterilizer (sterilization in 10 seconds) (www.stoeltingco.com/physio or www.braintreesci.com). Instruments should be cleaned prior to their placement in a hot bead sterilizer. The beads should be replaced approximately every six months. To extend the life of the unit, it is not recommended that the unit be left on for long periods of time (i.e. overnight).
- 2% glutaraldehyde plus 7.05% phenol (Sporocidin[®]) - requires immersion for 10 min in a 1:18 aqueous solution (1 part Sporocidin[®] : 18 parts water); rinse in sterile water or sterile saline before use.
- Chlorine dioxide (Clidox-S) - requires immersion for 5 minutes in a 1:5:1 solution (1 part Clidox activator : 5 parts water : 1 part Clidox base). The newly mixed solution must be allowed to sit for 15 minutes prior to use. Once mixed, the solution is effective for 14 days after which time a new solution must be made. Chlorine dioxide is highly corrosive for metal instruments.
- 2% aqueous glutaraldehyde (e.g., Cidex[®]) - requires immersion for 10-30 minutes, depending on the manufacturer's recommendations; rinse in sterile water or sterile saline before use.

Alcohol should not be used for the sterilization or disinfection of surgical instruments as it is inactive against bacterial spores and many viruses, does not penetrate surfaces well, and requires a prolonged contact time.

III. Preparation of the Animal

Preparation of a rodent for surgery requires attention to both the specific surgical site and the physiological status of the animal as a whole. It is vital that the animal's body temperature be maintained within acceptable limits as hypothermia is a significant cause of perisurgical mortality in rodents. The normal body temperature of a mouse is approximately 96.4° – 99.7°F (35.8° – 37.6°C). The normal body temperature of a rat is approximately 96.6° - 99.5°F (35.9° - 37.5°C). The normal body temperature of a guinea pig is approximately 98.6° – 103.1°F (37.0° - 39.5°C).

An ophthalmic ointment (e.g., Puralube[®] or Lacrilube[®]) must be applied to the eyes of any animals receiving injectable anesthetics or to those animals anesthetized with gas anesthetics for greater than 5 minutes.

Hair must be removed from the area surrounding the incision site if the incision will extend more than one centimeter in length. This may be accomplished with animal clippers, a razor, or a depilatory agent such as Nair[®]. If a depilatory agent will be used, all of the substance

must be thoroughly removed from the skin after use to prevent skin irritation. In order to avoid contaminating the area of the lab in which surgery will be performed, hair removal should be performed at a separate location such as a nonadjacent portion of the lab bench. Loose hair must then be removed from both the animal and the environment. This can be done through use of a vacuum or sticky/Scotch tape (which is a practical and effective alternative for removing small quantities of hair).

After hair removal, the animal should be placed on a clean, absorbent surface, such as an absorbent pad, to minimize heat loss during surgery.

A heating source must be provided when the duration of anesthesia will be greater than 20 minutes or for any procedure in which a body cavity will be exposed (ie. thoracotomy, laporatomy). Use of a recirculating warm water blanket (ThermoCare Inc., Gaymar) or a self-regulating heating pad (Fine Scientific Tools) specifically designed for use with rodents is highly recommended. Electric heating pads (i.e., drug store heating pads) not specifically designed for medical use are only acceptable for use when placed under the caging unit. Use of warm water bottles, microwaveable heating packs (eg. Snuggle Safe – R.C. Steele Inc), and slide warmers is acceptable. To decrease the risk of burns, the heat source should never be in direct contact with the animal. Instead, a pad must be placed between the animal and heat source. Alternate heat sources may be used only with prior approval by UCUCA (ucuca.office@umich.edu) or the ULAM veterinary staff. Heat sources should not be so hot as to elevate the animal's body temperature beyond its normal physiologic range.

If it is not possible for the animal to be placed in close proximity of a heating source, bubble wrap can be placed under the animal to prevent dissipation of body heat through the resting surface.

Once the animal has been stabilized in the area in which surgery will be performed, the skin must be cleaned and/or disinfected prior to any surgical manipulations. This may be achieved by cleaning the area with three alternating scrubs of an appropriate disinfectant and rinse solution (see below). Materials (such a gauze pads) should not be used for more than one scrub. The scrub should be performed so that the proposed incision site is scrubbed first and the scrub then continued in a spiral pattern which radiates out from the proposed incision line. If dirt or debris is still visible after three alternating scrubs, additional scrubs must be performed. However, it should be noted that each additional scrub may contribute to the animal becoming hypothermic.

Disinfectants Recommended for Use for Skin Preparation

- iodophors (e.g., Betadine[®] scrub or Prepodyne[®] scrub)· Prolonged contact with these substances may irritate the skin. Therefore, these substances should be rinsed from the skin with sterile saline or sterile water prior to surgery.
- chlorhexidine scrub (e.g., Nolvasan[®], Hibiclens[®])
- phenolics (e.g. PhisoHex) must be mixed each time prior to use
- 0.25% triclosan (e.g. Septisol NPD)
- 70% ethyl alcohol is acceptable for use only when the animal's hair will not be removed and the surgical incision will be less than 1 cm in length

Alternate skin disinfectants may be used only with prior approval from UCUCA (ucuca.office@umich.edu) or the ULAM veterinary staff.

Appropriate Rinse Solutions

- warm, sterile saline or sterile water. Since rodent can quickly and easily become hypothermic, the use of sterile saline or sterile water is preferable over the use of alcohols that, upon evaporation, can quickly induce hypothermia.
- 70% ethyl alcohol
- 85% isopropyl alcohol

After disinfection of the skin, larger rodents (i.e. rats and guinea pigs) should be covered with a sterile drape containing a hole over the proposed incision site. Due to their small size, “draping” very small rodents (i.e. mice, hamsters, gerbils) may consist solely of placing a sterile drape under the animal. When used in either fashion, the drape helps minimize contamination of the surgical site and also serves as a clean surface upon which instruments, sponges, etc. can be placed during surgery.

If desired, clear adhesive drapes (3M[™] Steri-Drape[™] Incise Drape) can be used which completely cover the animal without impeding visual assessment of the animal. However, care must be taken to ensure that the drape does not obstruct the animal's breathing.

IV. Preparation of the Surgeon

Ideally, surgeons should wear shoe covers, a surgical cap, a mask, sterile gloves and a sterile gown. Immediately prior to surgery, the surgeon should scrub his/her hands and arms for 10 minutes with a disinfectant soap. However, this degree of sterility is neither required nor necessarily practical for rodent surgery. To perform rodent surgeries, the surgeon must, at a minimum, perform the procedures listed below.

Minimally Required Preparations for the Surgeon

- wash hands prior to surgery
- wear a mask
- wear either sterile surgical gloves or clean exam gloves. Exam gloves must be from either a previously unopened box or an opened box kept covered and used only for this purpose. In order to decrease the level of contamination, a new pair of gloves should be worn for handling each animal, even if multiple surgeries will be performed in a single session
- wear a clean scrub shirt, lab coat, or isolation gown

V. Suture Materials and Wound Closure

Selection and use of appropriate suture materials is imperative for successful wound closure and healing. Sutures are either absorbable or nonabsorbable dependant upon the materials from which they are manufactured.

For routine surgical procedures in rodents, commercial suture materials with swaged (attached) needles in sterile packets are ideal but not required. Materials should be selected that are the correct size and have appropriate absorption (if absorbable) and handling characteristics for the intended procedure and animal species. Silk sutures should be avoided when closing skin incisions. Silk is braided and bacteria (skin microflora) penetrating the interstices of the braid may lead to infections. Suture material should be sterile as they are a foreign material and provide a substrate where bacteria may proliferate. Wound clips or skin sutures must be removed 7-14 days after placement unless previously approved through UCUCA.

Proper wound closure is essential to avoid wound dehiscence. Surgery in which a body cavity is opened requires a two-layer closure in which the body wall is closed separately from the skin.

VI. Required Documentation

Intra- and post-operative documentation is required from laboratories that perform recovery surgical procedures on select species of rodents. A ULAM Surgical Record Form is attached to this document. Records for rodents, other than laboratory rats and mice, should include the following information: animal identification, pre-surgical weight, date of surgery, anesthetic/analgesic dosage and route, type of surgical procedure performed, description of any complications, length of surgery, and documentation of 5-7 days of post-operative monitoring.

It is strongly encouraged that intra- and post-operative surgical records be created for rats and mice undergoing major recovery surgical procedures (i.e., penetration of a major body cavity such as laparotomy or craniotomy). Some basic information should include, date of surgery, pre-surgical weight, anesthetic/analgesic administration, type of surgery, and documentation of 5-7 day post-operative monitoring.

VII. Postoperative Care

It is the investigator's responsibility to oversee the animal's anesthetic recovery. This involves regularly checking the animal until it is fully ambulatory. Animals recovering from anesthesia should be rotated from side to side every 15-20 minutes until they are able to remain upright. After this, the animal should be examined by the laboratory staff for postoperative complications at least once a day for 7-10 days or until skin sutures or wound clips are removed. A veterinarian (764-0277) or veterinary technician (936-1037) should be contacted if the animal or surgical site appears abnormal. This would include, but is not limited to, excessive swelling, redness, dehiscence (opening) or drainage of the surgical site.

Analgesics (pain-relieving medication) should be provided as approved in the animal use protocol (UCUCA Form 8225).

Aside from bleeding or another direct complication of surgery, the common complications affecting rodents recovering from anesthesia are hypothermia, dehydration, and injury from cagemates.

Hypothermia: The risk of hypothermia can be minimized by keeping the animal in a warm environment and on a bedded or padded surface. An external heat source such as a recirculating warm water blanket (Baxter k-mod by ThermoCare Inc. or Gaymar) or a self-regulating heating pad (Fine Scientific Tools) specifically designed for use with rodents is highly recommended. Heat lamps can be used only if the animal is very carefully observed to ensure that the animal does not become overheated. For additional information regarding heating sources, please refer above to the section "Preparation of the Animal."

Dehydration: To help prevent dehydration, 1-2 cc of warm (not hot) sterile 0.9% NaCl (or equivalent) per 100 gm of body weight can be administered subcutaneously or intraperitoneally before, during, or after surgery. Additional fluids may be required under certain circumstances such as if 1) the animal suffers significant blood loss, 2) the animal will be anesthetized for prolonged periods (such as greater than 2 hours), 3) the animal is prone to dehydration due to any factors not related to surgery (such as if the animal is diabetic), or 4) the animal has not recovered from anesthesia within several hours.

Injury from other animals: In general, a rodent that has been anesthetized (especially with injectable anesthetics) should not be put back in a cage with other rodents until it is fully ambulatory. This is because rodents tend to cannibalize nonresponsive cagemates. Even if all of the rodents in a cage were anesthetized, some will be slower to recover than others. These animals may be injured by more alert animals. Animals may be housed together following anesthesia but prior to recovery only if they are continually observed (at least once every 2-3 minutes) by a member of the research lab.

Rodents that are housed individually may be returned to the animal room before they have recovered from anesthesia if they are checked regularly until they are fully ambulatory. A sign indicating that the animal was recently anesthetized should be posted on the cage so that other

people observing the animal will not mistakenly think that it should be reported to the ULAM veterinary staff.

During the anesthetic recovery, it is important that the animals be separated from bedding within the cage as animals have previously choked on bedding at this time. This may be accomplished by placing a pad or a towel between the animal and bedding. Alternatively, the bedding can be removed, and an absorbant pad placed in the cage. Once the animal has recovered, the pad can be replaced with fresh bedding.

Skin sutures or wound clips should be removed 7-14 days after surgery unless otherwise approved by UCUCA.

Attached to this document is a summary of the recommended guidelines for rodent surgery. This summary can be posted as a quick reference in the rodent surgery area of the laboratory.

Summary - Guidelines for the Performance of Survival Surgery on Rodents

PREPARATION OF THE SURGERY AREA

1. Area is a room or portion of a room that is easily sanitized and not used for any other purpose during the time of surgery - keep it free of clutter.
2. Clean or disinfect the surface upon which surgery will be performed with one of the environmental disinfectants listed in the Requirements for the Performance of Survival Surgery on Rodents.

PREPARATION OF SURGICAL INSTRUMENTS

1. Sterilize surgical instruments with one of the sterilizing agents listed in the Requirements for the Performance of Survival Surgery on Rodents.

For multiple surgeries performed during a single session:

- a. Start with at least 2 sets of sterile instruments.
- b. Between animals, disinfect instruments with one of the high level instrument disinfectants listed in the Requirements for the Performance of Survival Surgery on Rodents.

PREPARATION OF THE ANIMAL

1. Prior to taking the animal to the surgery area, remove hair (unless incision is less than 1 cm in length) with animal clippers, razor, or depilatory cream; vacuum or otherwise remove the loose hair.
2. To keep the animal warm, place it on a clean absorbent surface; placement of a heating blanket, warm water bottle, or equivalent under the pad is strongly recommended.
3. Clean and disinfect the animal's skin by scrubbing with one of the skin disinfectants listed in the Requirements for the Performance of Survival Surgery on Rodents.
4. Put an ophthalmic ointment or sterile eye drops in the anesthetized animal's eyes to prevent drying.
5. Cover the animal with a surgical drape.

PREPARATION OF THE SURGEON

1. Wear a mask, sterile gloves or clean, unused standard latex lab gloves, clean scrub shirt or lab coat; a new pair of gloves should be worn for each animal.

2. Wash hands before surgery.

POSTOPERATIVE CARE

Immediately after surgery :

Check the animal regularly until it is fully ambulatory.

To minimize hypothermia, place the animal in a warm room on a bedded or padded surface; provide additional warmth with something such as a warm water blanket or warm water bottle.

To help prevent dehydration, give 1-2cc of warm 0.9% NaCl (or equivalent) per 100gm of body wt. subcutaneously; give more if there was much bleeding during surgery or if the animal has not recovered from anesthesia within several hours.

To avoid cannibalism by cage mates, house rodents individually until they are fully ambulatory.

After the immediate postoperative period, check the animal at least once a day to assure freedom from postoperative complications.

Contact a veterinarian (4-0277) or a veterinary technician (6-1037) if the animal or surgical wound appears abnormal.

University of Michigan Surgical Record Form

Animal Id _____ spp. _____ Protocol # _____

Surgeon _____ Pre-surgical Weight of animal _____

Date of Surgery _____ Pre-operative Agent _____ (time _____)

Induction Agent _____ (time _____) Anesthetic (type/dose/route) _____

Anesthesia Start Time _____ Surgery Start Time _____

Description of Surgery (Procedures and Complications):

Surgery Completion Time: _____ Extubation time: _____

Immediate Post-operative monitoring (minimum 15 minutes until recovery): _____ (initials/time)

Recovery Time: _____ Comments:

Post-operative Records

Date/Time	Surgical Site OK?	Eating?	Drinking?	Post-op Analgesia	Normal Feces?	Problems?	Initials
	Y/N	Y/N	Y/N		Y/N		
	Y/N	Y/N	Y/N		Y/N		
	Y/N	Y/N	Y/N		Y/N		
	Y/N	Y/N	Y/N		Y/N		
	Y/N	Y/N	Y/N		Y/N		
	Y/N	Y/N	Y/N		Y/N		
	Y/N	Y/N	Y/N		Y/N		