

IACUC Policy on Survival Rodent Surgery

9/2010MW

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General: The following principles apply to survival rodent surgery and are described in the following:

ILAR Guide for the Care and Use of Laboratory Animals

<https://grants.nih.gov/grants/olaw/Guide-for-the-Care-and-Use-of-Laboratory-Animals.pdf>

NIH OACU ARAC Guidelines

http://oacu.od.nih.gov/ARAC/documents/Rodent_Surgery.pdf

CDC Healthcare Infection Control Practices Advisory Committee (HICPAC)

http://www.cdc.gov/hicpac/Disinfection_Sterilization/table_4.html

- There are two methods of surgery described in this Policy. Both are acceptable methods of surgery for rodents. Please write your protocol accordingly, when describing which method of surgery you will perform.
 - Aseptic Survival Surgery Using Surgical Gloves
 - Aseptic Survival Surgery Using the Tips-only Technique
- Appropriate attention to pre-surgical planning, personnel training, aseptic and surgical technique, animal well-being, and animal's physiologic status during all phases of a protocol will enhance the outcome of surgery.
- A dedicated surgical facility is not required.
- All survival surgery will be performed by using aseptic procedures, including masks, sterile gloves (unless using tips-only technique), sterile instruments, and aseptic techniques.
- It is important for research personnel to be appropriately qualified and trained in all procedures to ensure that good surgical technique is practiced. Prior to working independently on survival surgeries, the individual must complete didactic training modules through CITI and JOVE. The individual must also demonstrate proficiency during Post-Approval Monitoring to assure that the individual is appropriately qualified and trained in the procedure. Contact Michael Wong at wongmich@hawaii.edu to request access to JOVE.
 - Good technique includes:
 - Asepsis
 - Gentle tissue handling
 - Minimal dissection of tissue
 - Appropriate use of instruments
 - Effective hemostasis
 - Correct use of suture materials and patterns or other wound closure techniques
- Investigators should work closely with their veterinarian to assure that the challenges of surgery are adequately addressed. Protocol specific situations

may require variances to the policy. These situations should be discussed with the veterinary staff under IACUC policy 15 and should be reflected within the protocol under survival surgery asepsis.

Aseptic Surgery Using Sterile Gloves

A. Personal Protective Equipment

- 1) Clean lab coat or scrub top
- 2) Mask
- 3) Hair Bonnet
- 4) Shoe Covers
- 5) Gloves for preparing animal for surgery and disinfecting space where surgery is done.
- 6) Sterile surgical gloves during surgery Using sterile surgical gloves allows you to touch all areas of the sterile surgical field and surgical instruments with your gloved hand.

B. Pre-Operative

- 1) Prepare instruments by sterilizing prior to use. Multiple methods are available for sterilizing instruments prior to contact with animal tissues. These include autoclaving, dry heat sterilizing, ethylene oxide, or chemical agents.
 - a. Autoclaving or dry heat sterilizing are preferred methods.
 - b. Alcohol is not a recognized method for sterilizing instruments.
 - c. When chemical sterilization is used, adhere to the following:
 - i. The use of chemicals classified as sterilants. Chemicals classified as disinfectants are not adequate.
 - ii. The items being sterilized must allow for all surfaces to be exposed to sterilant (inside and out), impervious to moisture, and relatively smooth.
 - iii. The items must be exposed to the prescribed amount of time required by the manufacturing company or the CDC.
 - iv. The sterilant must be clean, mixed properly, and used within its activated shelf life.
 - v. Instruments must be rinsed with sterile water or sterile saline prior to use on animal tissue.
- 2) Surgery should be conducted in a disinfected, uncluttered area that promotes asepsis during surgery. A separate prep area and surgery area should be present.
 - a. Disinfect the **space where surgery is done** by cleaning with a disinfectant. Appropriate contact time must be used.
 - b. Commonly used disinfectants are quaternary ammonium compounds, chlorine dioxide-based sterilant (Clidox), chlorhexidine (Nolvasan), 70% alcohol, or other antimicrobial agent. Disinfectants must be prepared and used according to the manufacturer's recommendations.
- 3) Anesthetize the animal according to approved IACUC protocol methods.

- 4) Apply a sterile ophthalmic lubricant to protect the corneas of the research animals.
- 5) Prepare the animal by removing hair from the surgical site. Perform this procedure in an area separate from where the surgery is to be conducted (prep area).
- 6) Prepare the surgical site(s) with an appropriate skin disinfectant.
 - a. Alternating a surgical scrub (iodophor or chlorhexidine) with 70% alcohol (sterile water can substitute for alcohol) will allow for good surgical site skin disinfection.
 - b. Starting at the incision site, using gauze or cotton tip applicators apply the surgical scrub in a circular motion moving outwards away from the incision site. Repeat with alcohol in a circular motion from the incision site. Repeat this procedure 3 times.
- 7) Heating pads or heat lamps should be utilized to prevent hypothermia during surgery. Care should be used to prevent overheating or burning of animals.
- 8) Transfer the animal to the heated surgical area, being careful not to touch the prepped surgical site.
- 9) Surgeons should wash and dry their hands before aseptically donning sterile surgical gloves.

C. Operative:

- 1) The animal must be maintained in a surgical plane of anesthesia throughout the procedure.
 - a. Anesthesia is considered adequate when the animal stays still quietly, is unresponsive to external stimuli, and has constant heart and respiratory rates. In rodents the absence of the reflexes suggests a fair anesthetic depth.
- 2) Drape the surgical area with a sterile drape: precut drapes, sterile gauze, or clear drapes are all acceptable.
- 3) Begin surgery with sterile instruments and sterile surgical gloves. A sterile field must also be maintained for the sterile instruments, this can include the inside of the autoclaved instrument holder, a sterile drape, or the inside of sterile gloves packaging. Sterile instruments and gloves must not touch anything outside the sterile field, otherwise they will be considered non-sterile. Sterile gloves will allow the surgeon to manipulate tissues with sterile gloved hands or instruments.
- 4) Instruments and gloves may be used for up to 5 similar surgeries provided they are maintained clean and sterilized between animals. Instrument tips can be sterilized within a bead sterilizer. This requires placing instrument tips for 10-15 sec within heated beads. Instrument must be allowed to cool for approximately 30-60 sec prior to reuse. Sterile surgical gloves can be disinfected with alcohol as long as they have not touched anything non-sterile. Note, that the surgeon cannot touch the alcohol container unless it is also sterile. Please use a second person to help with spraying alcohol on

surgical gloves or pick up the alcohol container with sterile gauze, discarding the gauze once used, or change sterile gloves between animals.

- 5) Monitor and maintain the animal's vital signs, i.e. respiratory rate and pattern, heart rate, blood pressure, temperature, and color of ears, nose, mucous membranes, feet.
- 6) Close surgical wounds using appropriate techniques and materials. Note needles and suture material must also remain sterile.

a. **WOUND CLOSURE SELECTION**

- i. **Suture gauge selection:** Use the smallest gauge suture material that will perform adequately.
- ii. **Cutting and reverse cutting needles:** Provide edges that will cut through dense, difficult to penetrate tissue, such as skin.
- iii. **Non-cutting, taper point or round needles:** Have no edges to cut through tissue; used primarily for suturing easily torn tissues such as peritoneum or intestine.
- iv. The Guide recommends using sterile suture to reduce the likelihood of infection. These may be purchased as individually wrapped packages such as those shown here:
- v. Sutures from these packs can be used for more than one surgery in a given day if the suture is kept on a sterile field. Sutures from a sterile pack must not be saved once surgeries are done for the day. If using suture material that is not from a sterile pack, it must be sterilized prior to use. A sterilant that does not breakdown the tensile strength of the suture must be used. (Ethanol is not adequate to sterilize sutures and needles, and should not be used for this purpose. See definition of sterilant below and also the Pre-Operative section above regarding sterilizing instruments) If a cold sterilant is used, it must be thoroughly rinsed with sterile water prior to use. Alternatively sterile wound clips may be used in lieu of suture, when appropriate.

D. Post-Operative:

- 1) Move the animal to a warm, dry area and monitor it during recovery. If placed on a heating pad, be sure the animal has the option to move to a non-heated portion of the cage. Monitor the animal and return it to its resident housing cage only after it has recovered from anesthesia (e.g., the animal can maintain itself in sternal recumbency).
- 2) Place a postoperative tag behind the cage card, noting the date and contact information for the surgeon.
- 3) Generally, remove skin closures 10 to 14 days post-operatively.
- 4) Surgical records must be maintained, even if the surgery went without problems. Examples of surgical records are found at the end of the policy.
- 5) Maintain post-operative observations at least daily for 3 days after the surgery. Records should show that the animal was observed post-operatively

and the state of the animal, including if there are no issues noted during the observation.

E. Analgesia

- 1) Analgesia is required for all surgeries. It is recommended to be given pre-operatively or intra-operatively to maximize the analgesic affects. Follow-up post-operative analgesia (analgesia that is given once the initial surgical analgesia has worn off) is required when an animal shows further post-operative pain. For examples of drugs and doses see Policy on Rodent and Rabbit Anesthesia and Analgesia.
- 2) Withholding analgesia is allowed only when scientifically proven justified and approved by the IACUC. Under this situation, these rodents would fall under Pain Category E.

F. IACUC Approved Protocols

- 1) Surgery techniques performed within the vivarium including methods of aseptic technique must match what is written within the IACUC protocol. Deviations must be amended within the IACUC protocol and reviewed by the IACUC.
- 2) The following may be copied and pasted for maintaining a **full sterile aseptic surgery**. Please amend to meet your protocol requirements:

The surgeon will wear a clean lab coat or scrub top, mask, hair bonnet, shoe covers, non-sterile gloves when preparing an animal for surgery and disinfecting surgical area, and sterile surgical gloves during surgery. The space where the surgery will be done will be disinfected with Clidox (list a different disinfectant if one will be used) The rodent will be anesthetized, eyes lubricated with sterile ophthalmic ointment, then shaved or have hair removed chemically, separate from the surgery area. The skin will be aseptically prepared by scrubbing with iodine or chlorhexidine scrub working in a circular motion from the middle out, followed by alcohol in the same pattern. This will be repeated three times. The packaging for the sterile instruments will be opened. The animal will be draped using sterile drapes. The corners on the drapes will be handled to maintain the drapes sterility. The surgeon will don sterile gloves and only then will the surgeon handle the sterile instruments. If the surgeon touches anything non-sterile with the sterile gloves, they will be changed. The surgery will proceed as listed in the protocol. The animal's vital signs will be monitored while under anesthesia. The animal will be given analgesics either pre-, intra-, or immediately post-operatively. The animal will be observed until it is able to gain sternal recumbency (normal posture) prior to returning it to the housing rack. Surgical and post-operative records will be kept and the animal observed for at least 3 days post-operatively with observations noted, even if the animal appears normal. Follow up analgesia will be given as needed.

Aseptic Surgery Using the Tips Only Technique

Using clean exam gloves and a “tips only” technique restricts the surgeon to using only the sterile working ends of the surgical instruments within the surgical field. The gloved hands must never touch the sterile ends of the instruments, the suture, suture needle, or any part of the sterile surgical field. This technique is useful when working alone or manipulating non-sterile objects. All techniques described above are applicable except for the following:

A. Personal Protective Equipment

- 1) All PPE is the same as above with the exception of use of sterile gloves. Clean exam gloves can be used in its place.

Pre-Operative

- 1) Instrument tips can be sterilized within a bead sterilizer, requires 10-15 sec within heated beads. Instrument must be allowed to cool for approximately 30-60 sec prior to reuse. Once sterilized, remove instruments from bead sterilizer and place sterile tips on sterile gauze or other sterile field. Do not place the instrument tips on the surgery table or other non-sterile field. Place all sterile equipment such as scalpels and suture material on to the sterile field as well, opening them in a manner that prevents contamination.
- 2) Changing to new clean exam gloves is necessary after animal has been prepped for surgery.

Operative

- 1) Sterile drapes must be handled by the edges to prevent contamination.
- 2) Remember that only the sterile tips of the instruments can come into contact with the sterile field.

All further steps are the same as above, including post-operative procedures and analgesia.

The following may be copied and pasted for maintaining aseptic **tips only technique surgery**. Please amend to meet your protocol requirements

The surgeon will wear a clean lab coat or scrub top, mask, hair bonnet, shoe covers, non-sterile gloves when preparing an animal for surgery and disinfecting surgical area, and new gloves during surgery. The space where the surgery will be done will be disinfected utilizing a product such as Clidox (list a different disinfectant if one will be used) The rodent will be anesthetized, eyes lubricated with sterile ophthalmic ointment, then shaved or have hair removed chemically, separate from the surgery area. The skin will be aseptically prepared by scrubbing with iodine or chlorhexidine scrub working in a circular motion from the middle out, followed by alcohol in the same pattern. This will be repeated three times. The instrument tips will be sterilized in a bead sterilizer. Once sterilized the instrument tips will be kept on a sterile field. The animal will be draped using sterile drapes. The corners on the drapes will be handled to maintain the drapes sterility. The surgeon will don new gloves. The surgery will proceed as listed in

the protocol. The animal's vital signs will be monitored while under anesthesia. The animal will be given analgesics either pre-, intra-, or immediately post-operatively. The animal will be observed until it is able to gain sternal recumbency (normal posture) prior to returning it to the housing rack. Surgical and post-operative records will be kept and the animal observed for at least 3 days post-operatively with observations noted, even if the animal appears normal. Follow up analgesia will be given as needed.

DEFINITIONS:

- Surgical procedures are categorized as major and minor.
 - MAJOR SURGERY: Any surgical intervention that penetrates and exposes a body cavity or produces substantial physical or physiological impairment (such as laparotomy, thoracotomy, craniotomy, joint replacement, or limb amputation)
 - MINOR SURGERY: Any surgical intervention that does not expose a body cavity or causes little or no physical impairment (such as wound suturing, peripheral-vessel cannulation, or routine farm animal procedures such as castration, dehorning, repair of prolapses, or outpatient procedures done in a veterinary clinical practice). Minor procedures still require aseptic technique and instruments, as well as anesthesia.
- ASEPTIC SURGICAL PROCEDURES: Surgery performed using procedures that limit microbial contamination so that significant infection or suppuration does not occur.
- STERILIZATION/STERILANT: The process whereby all viable microorganisms are eliminated or destroyed. The criterion of sterilization is the failure of organisms to grow if a growth supporting medium is supplied.
- DISINFECTION: The chemical or physical process that involves the destruction of pathogenic organisms. All disinfectants are effective against vegetative forms of organisms, but not necessarily spores.

NON-Survival Rodent surgeries

An animal is euthanized before recovery from anesthesia. At minimum the surgical site must be clipped, surgeon should wear gloves, and the instruments and surrounding area should be clean.

Surgical Records

For the convenience of the surgeon, examples of surgery forms can be found below. There is a short and a long form. Either version is acceptable. Surgeons are also allowed to use their own records to record their surgeries, which may be different from the forms below. However, these records should include the date of the surgery, anesthetics and analgesics given including dose or amount, and health monitoring records/observations both during and post operatively, including the 3 days after surgery. Records must be available for review during Post Approval Monitoring or upon request of the IACUC or veterinarians.

Surgical Forms
(short form)

Surgery Form

Date:_____ Procedure:_____ Survival Surgery: Yes No

Surgery Start Time:_____ Surgery End Time:_____

Drugs/Medications (please list dosage)_____

Post Surgery (Recovery)- IF SURVIVAL

Animal Health Description:_____

(long form)

Surgery Form

Date: _____ Cage Card ID: _____ PI Name: _____ Protocol: _____
Survival Surgery: Yes No Sex: Male Female
Species: Mouse Rat Other: _____ Strain: _____
Surgical Procedure(s): _____

Pre Surgical Evaluation

Overall Health of Animal Good Average Poor Comments: _____

Pre-Anesthetics/Analgesics/Other Drugs-Time administered and dosage: _____

Surgical Evaluation

PROCEDURE DETAILS PLEASE SEE PROTOCOL

Start Time: _____ am/pm End Time: _____ am/pm Surgeon Name: _____
Heat Source Present: Yes No Fluids Given: _____
Sutures Used: _____ Absorbable NonAbsorbable
Anesthesia during Surgery: Isoflurane TBE Ketamine/Xylazine
 Other: _____ Dosage: _____
NOTES (complications/other): _____

Post Surgical Evaluation for Survival Surgeries

Overall Health of Animal Good Average Poor Comments: _____

+/- Medications/Dosage: _____

Additional Health Monitoring

Date/Time/Health of Animal: _____