Policy 303 Aseptic Survival Surgery in Rodents, Birds, and Amphibians

303.1 Purpose: The following guidelines represent the baseline minimum requirements for aseptic survival surgery and post-procedural monitoring in rodents, birds, and amphibians. Please contact the SOM or Yerkes DAR veterinarian for questions related to aseptic technique and surgery as needed. This policy does not apply to fish and the requirements are listed in the approved IACUC protocol.

303.2 Facilities: Surgical procedures should be performed in the animal procedure rooms within the animal facility, unless otherwise approved by the IACUC (refer to the IACUC Animal Housing and Study Areas policy for details). Prior to initiation of surgical procedures, the work surface should be sanitized in compliance with the IACUC Sanitation of Research Equipment Used with Animals policy. Work areas should be clean and uncluttered during surgery, and unnecessary foot traffic should be minimized. Surgical areas should be free of hair and other debris. Separate, designated spaces are recommended for animal preparation and surgery, so that the surgical area is kept free of hair and debris.

303.3 Surgeon training: Training requirements for surgeons are detailed in the IACUC Education and Training policy.

303.4 Instrument and materials:

a. All instruments should be cleaned and then sterilized prior to use in surgery.
   i. Any method of sterilization (steam, gas, cold chemical, hot bead) is acceptable if done properly. Steam or gas sterilized instruments are recommended for the first surgery of the day because these processes are less prone to errors and incomplete sterilization than other sterilization methods.
   ii. Supplies that are autoclaved or gas sterilized should have a sterilization indicator on the outside of the package (e.g., temperature-sensitive autoclave tape or indicator integrated into the bag exterior).
   iii. Autoclave validation programs are managed by EHSO and the individual animal resources programs.
   iv. Sterilized instruments that are properly wrapped in sterile packaging and protected from dust accumulation (e.g., in a closed cabinet) may be stored for later use if the packaging remains intact.
   v. Cold sterilants should be labeled as a sterilizing agent and used in accordance with the manufacturer label regarding contact times. Alcohol is not a sterilant and therefore is not acceptable as a sole method for sterilizing instruments.

b. All other materials introduced into the animal during surgery should be sterile (e.g., implants, gauze, cotton swabs, etc.)
   i. Dental cement is an exception, as it is not often labeled as sterile. It should be formulated for animal or human use and mixed in a sterile container.
c. See Appendix 3 regarding acceptable methods of skin closure. Plain or chromic gut suture should not be used in surgery due to its inflammatory properties.

303.5 **Surgeon preparation:**

a. Hands should be washed and free of gross contamination.

b. Gloves should be worn when performing surgical procedures.
   i. Clean gloves are acceptable instead of sterile gloves if using the sterile-tip technique. With the sterile-tip technique, only the sterile instrument tips/supplies touch the surgical field; the surgeon’s hands do not enter the surgical field.

c. Surgeon should wear a surgical mask and gown or lab coat during all surgeries.
   i. The surgeon should also wear a surgeon cap if the procedure is considered “major” as defined by *The Guide for The Care and Use of Laboratory Animals* or if a cap requirement is denoted in the approved IACUC protocol.

303.6 **Animal Preparation:** Animals should be anesthetized using agents and doses listed in the corresponding IACUC protocol.

a. Birds and Mammals:
   i. Hair and feathers should be removed from the surgical site following the induction of anesthesia. Hair should be removed using clippers or a depilatory agent.
   
   ii. After hair/feather removal, gross contamination and excess loose hair/feathers should be removed with a combination of two antiseptics.
      • Alcohol is recommended as one of the antiseptics, with betadine or chlorhexidine as the other antiseptic. Skin disinfection should be accomplished with three applications of a combination product (e.g., chlorhexidine and alcohol) or three applications each of the two antiseptics, alternating between the antiseptics and ending with the antiseptic that is not alcohol.
      • Deviations from this standard antiseptic protocol must be specifically outlined and approved in the IACUC protocol.
   
   iii. Ocular ointment should be placed on the eyes to prevent exposure keratitis.
   
   iv. Prone subjects should be restrained appropriately using tape or fine rope used in a non-occluding manner as a means of affixing limbs to the surgical table or platform.
   
   v. A sterile drape should be used unless exempted by the IACUC.
      • For those procedures that require suturing, the drape should be an appropriate size to maintain sterility of the suture.
   
   vi. The animal should be placed on an appropriate heating surface for procedures lasting longer than 15 minutes.

b. Amphibians:
   i. Gross debris should be rinsed off the skin at the surgical site as the first step of surgical preparation.
   
   ii. The skin should be disinfected with 0.5% povidone-iodine rinse followed by rinse with 0.9% sterile saline.
   
   iii. A sterile drape with a fenestration should be used for amphibians so that suture material can remain sterile. The drape should be water resistant and adhesive-free to avoid skin and mucus layer irritation (thus products such as Ioban drapes or Press and Seal Drape should not be used).
   
   iv. *Xenopus laevis* do not require heat support during surgery or recovery.
303.7 Surgery on multiple animals:
   a. If the same set of instruments will be used for multiple animals, instruments should be cleaned and re-
      sterilized prior to starting on each subsequent animal.
      i. The initial cleaning should remove all gross debris and is best accomplished by using
         clean gauze soaked with sterile saline.
      ii. Instruments should then be sterilized using methods as described above in “Instrument and
          materials preparation”. Exceptions are allowed for Hamilton needles and suture material as
          described below.
      iii. Hamilton needles should be sterile for the first animal of the day and IACUC recommends
           that researchers use a sterile needle for each animal. Alternatively, the needle can be flushed
           and wiped with a sterile solution (e.g., sterile saline or water) between animals.
      iv. Suture material should also be sterile for the first animal of the day and IACUC recommends
          that researchers use sterile suture material for each animal. Alternatively, one suture pack can
          be used for a maximum of three consecutive animals by wiping the suture with ethanol in
          between animals.
   b. A single drape can be used for up to two animals if it remains clean. However, the drape should be
      discarded after the first animal if it is damaged or significantly contaminated with blood, hair, or other
      debris.
   c. Gloves should be changed before starting surgery on an animal if they were used for animal preparation, or at
      any point if they are damaged.

303.8 Post-operative recovery:
   a. Animals should be placed in a clean recovery cage (or tank for amphibians).
      i. Recovering animals should be separated from ambulating animals to prevent injury during
         recovery.
   b. Heat should be provided using an approved heating source so that half of the cage is heated and half is at
      ambient room temperature to create a gradient, except for *Xenopus laevis*.
      i. A temperature-regulated heating source should be used unless justification is provided for a
         heat lamp.
   c. Animals should be monitored at least every 15 minutes until fully recovered from anesthesia, at which
      time the animals can be moved into their housing room. Subsequently, the animals should be provided
      care and observed at the frequency required in the approved protocol. These immediate and
      subsequent observations should be documented as detailed in this policy (303.9). Any exceptions must
      be approved by IACUC.
   d. Post-operative care such as analgesia or fluids should be administered as described in the approved
      IACUC protocol. The postoperative management of the incision sites, including suture or staple
      removal (generally performed 7-14 days post-surgery), should be consistent with the approved
      protocol.

303.9 Surgical and post-operative monitoring and record keeping:
   a. A cage card record (Appendix 1) and lab log record (Appendix 2) are required for surgical and post-
      operative monitoring.
      i. One cage card record (Appendix 1) is required per cage of animals for both non-ACT and ACT
         rodents.
      ii. For non-ACT species such as mice, rats, birds, and amphibians, only one lab log record
         (Appendix 2) is required per surgery day for each surgeon.
      iii. For USDA (ACT) covered species such as voles, spiny mice, gerbils and guinea pigs, one lab
log (Appendix 2) is required per cage of animals.

iv. Researchers can develop an alternative to the examples in the appendices provided the forms include a minimum of the information in the templates provided.

b. The animals must be monitored for the time period described in the IACUC protocol. This time will be a minimum of 3 (three) days after the day of the surgical procedure which counts as day zero, or until time of death if this occurs prior. Longer monitoring periods might be necessary for more invasive surgery. These protocol-required observations must be documented on the cage card record.

c. The cage card record must be maintained on the cage for as long as post-operative observations are required per the IACUC protocol.

i. Afterwards, or when the animals/cage is euthanized, the card must be removed from the cage and added to the surgery lab log as part of the permanent record.

d. Inclusion of the surgery type and date on the permanent cage card greatly facilitates communication between the animal resources program and the researchers if complications arise after the temporary surgery cage card record has been removed.

e. Cage card and lab log records must be maintained in a central location and readily available upon request.

* NOTE: Major survival surgery penetrates and exposes a body cavity, produces substantial impairment of physical or physiological functions, or involves extensive tissue dissection or transection.

303.10 Appendices
Appendix 1: Survival Surgery Cage Record Card Template
Appendix 2: Survival Surgery Lab Log Record
Appendix 3: Surgical Incision Closure Options

303.11 Document Properties
Authored by: IACUC
Administering Division/Department: IACUC Office
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Review/Revised: 10/06/2021
Version: v.20211006
Effective Date: Upon approval of any new protocols or 3-yr renewals
APPENDIX 1: Survival Surgery Cage Card Record Template

a) Front Side of Card

<table>
<thead>
<tr>
<th>Date:</th>
<th>Cage Card Number:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgeon &amp; phone number:</td>
<td></td>
</tr>
<tr>
<td>Procedure:</td>
<td></td>
</tr>
<tr>
<td>Time at which all animals have recovered:</td>
<td></td>
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</tbody>
</table>

**ANALGESICS: check or write-in drug name**

<table>
<thead>
<tr>
<th>Sustained release buprenorphine</th>
<th>Standard buprenorphine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sustained release meloxicam</td>
<td>Standard meloxicam</td>
</tr>
<tr>
<td>Other:</td>
<td></td>
</tr>
</tbody>
</table>

**ANESTHETICS: circle or write-in drug name**

<table>
<thead>
<tr>
<th>Isoflurane</th>
<th>Ketamine/Xylazine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other:</td>
<td></td>
</tr>
</tbody>
</table>

Other Medications or Notes

b) (Back side of card)

<table>
<thead>
<tr>
<th>Date/Time</th>
<th>Animal Condition**</th>
<th>Analgesia drug</th>
<th>Dose (mg/kg)</th>
<th>Initials</th>
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</thead>
<tbody>
<tr>
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Note: This record should be maintained on the cage for as long as post-operative observations are required per the IACUC protocol. Observations are required for a minimum of 3 days after the day of surgery or longer if required per the approved IACUC protocol. After that, the card should then be transferred to the lab surgery notebook as an official monitoring record.

**1= Active   2= Inactive   3= Moribund 4= Found Dead**
Appendix 2: Survival Surgery Lab Log Record
Note: Affix the cage card log to this document after removing the cage card log from the cage

<table>
<thead>
<tr>
<th>Principal Investigator:</th>
<th>Date:</th>
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<tbody>
<tr>
<td>Surgeon</td>
<td>IACUC Protocol #:</td>
</tr>
<tr>
<td>Species/Strain</td>
<td></td>
</tr>
<tr>
<td>Animal ID Number(s)</td>
<td></td>
</tr>
<tr>
<td>Body weight(s):</td>
<td></td>
</tr>
<tr>
<td>Surgical Procedure Description:</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ANESTHETIC DRUG(S) ADMINISTERED</th>
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<tbody>
<tr>
<td>Drug/Dose(mg/kg)</td>
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<table>
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<tr>
<th>ANALGESICS DRUG(S) ADMINISTERED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug/Dose(mg/kg)</td>
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<table>
<thead>
<tr>
<th>SURGERY NOTES AND OBSERVATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time Surgery Started:</td>
</tr>
<tr>
<td>Intra-operative monitoring Performed (Y/N):</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>SURGICAL NOTES: (add surgical notes here or indicate Not Applicable)</td>
</tr>
</tbody>
</table>

Surgical Recovery Monitoring
(To be done at least every 15 minutes until the animal is awake and ambulatory):

<table>
<thead>
<tr>
<th>Time</th>
<th>15'</th>
<th>30'</th>
<th>45'</th>
<th>60'</th>
<th>75'</th>
<th>90'</th>
<th>105'</th>
<th>120'</th>
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</thead>
<tbody>
<tr>
<td>Initials</td>
<td>notes</td>
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Notes on recovery
Appendix 3: Surgical Incision Closure Options

1. Tissue adhesive: Tissue adhesive meant for use in human or veterinary medicine is appropriate for incisions that are small (< approximately 1 cm) and in areas of low tension (e.g., the abdomen or back). It can be used for head incisions in the head and neck area provided the surgeon is careful to protect the animal’s eyes.

2. Wound clips: Stainless steel wound clips are appropriate for use in rodents and larger species to close incisions in body areas with sufficient excess skin to allow for a closure without tension on the incision. Wound clips are not appropriate for closure of incisions the head/neck area of mice.

3. Suture:
   a. Suture type: Monofilament suture is recommended because it wicks less bacteria into the incision than multifilament sutures. If a multifilament suture is indicated, silk should not be used in the incision because of its inflammatory properties in skin closures. Plain or chromic gut should not be used in surgery due to its inflammatory properties.
   b. Suture size: 4-0 or 5-0 is typically used for mice and 3-0 to 4-0 for rats.