UNIVERSITY STANDARD

Title

UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL
STANDARD ON ASEPTIC TECHNIQUE FOR RODENT SURVIVAL SURGERY

Introduction

PURPOSE
The standards and procedures described below provide guidance to all researchers and animal handlers for ensuring adequate aseptic technique for all non-USDA regulated rodent (i.e., mice and rats) survival surgeries.

SCOPE OF APPLICABILITY
All personnel engaged in the performance of survival surgery in rodents. Individuals must obtain training in specific surgical techniques prior to conducting surgery in live animals.

The UNC-CH IACUC expects that anyone involved in animal work at the University will comply with this Standard. Requests for exceptions to this Standard must be reviewed and approved by the IACUC. Unless recommended by the Division of Comparative Medicine (DCM) Veterinary Staff, for acute or emergency situations, ANY deviation from this Standard must be included in the approved IACUC protocol prior to implementation in live animals.

Standard

Preoperative Practices

A. Prepare surgical space:

1. The area in which surgery is conducted should be isolated from active areas in the laboratory, doorways and ventilation supply ducts.
2. The area should be clean and uncluttered.
3. Surface areas should be easily sanitizable.
4. An animal preparation and recovery area, separate from the surgical area, should be provided. If a separate preparation area is not possible due to space
constraints, cover the surgical area with a towel or drape and discard this after
the animal has been prepared.
5. A heat source should be available anytime an animal is anesthetized (from
induction to recovery) as rodents rapidly lose body heat under anesthesia.
6. Space near the surgery area should be available and contain sufficient lighting
and room for hand scrubbing and donning of sterile gloves (where applicable).
7. Cardboard and paper products should not be stored directly above the surgery
area. Sealable, plastic containers may be used for storage.
8. Chairs located in animal use areas should have an impervious, cleanable surface.
   No cloth chairs are allowed in areas animals will be used. (Cloth chairs should be
   covered with disposable plastic if they must be used when animals are present in
   the lab.)
9. The designated surgical area (e.g., bench top, chairs, equipment) should be
   cleaned and disinfected prior to and after surgery with a hard surface disinfectant.
   Always follow manufacturer's instructions. See Disinfectant & Sterilants tables at
   the end of this Standard.

B. Prepare the Animal:

1. Apply a bland ophthalmic lubricant to the eyes, since the blink reflex is lost during
   anesthesia. For extended procedures, reapply the ophthalmic lubricant as needed
   in order to keep the eyes from drying out.
2. The skin around the surgical site must be devoid of hair, since hair can act as a
   wick for bacterial infection. Hair may be removed using either a #40 clipper blade
   or a depilatory. In anesthetized mice, plucking to remove hair is relatively easy.
3. Prepare the surgical site by using alternating applications of skin antiseptics such
   as ethyl or isopropyl alcohol, followed by an Iodophor solution or Chlorhexidine
   scrub (Solution = 10%, scrub = 7.5%). Please see Skin Antiseptic table at the end
   of this Standard for more details.
4. Using sterile cotton tipped applicators or gauze, start with the first alcohol
   application. Starting in the center of the incision site, spiral outward in concentric
   circles toward the margins of the prepared area (never go back and forth over a
   cleansed area with the same gauze).
5. Follow the alcohol application with the first Iodophor or Chlorhexadine application
   repeating the concentric circle pattern.
6. Repeat the alcohol and Iodophor or Chlorhexidine step for a total of three times
   each. Use a new gauze or cotton tipped applicator for each application.
7. When using Chlorhexidine as the skin antiseptic, ensure complete removal with sterile saline or water from the site before incision or prior to skin closure. Chlorhexidine can be irritating to skin.
8. Be careful not to excessively wet the animal as this can exacerbate hypothermia.

C. **Place Drape Material**

1. The use of a sterile drape is recommended to prevent contamination of the disinfected surgical site. This is especially true for procedures that require exteriorization of the viscera.
2. Positioning of the drape over the surgical area should proceed with sterile gloves or instruments in order to maintain sterility.

D. **Surgeon Preparation***

1. Surgical personnel should wear a clean lab coat, mask, bouffant cap, and sterile gloves.
2. If performing multiple surgeries, new sterile gloves should be donned between animals.
3. When wearing sterile gloves, hands should be scrubbed with an antimicrobial soap prior to donning gloves if able. This is an added precaution to reduce the risk of post-operative infection, if the gloves tear during surgery.

E. **‘No Touch/Tips only’ Procedure:**

Some (micro)surgery may not require the use of sterile gloves. (Some examples are blastocyst transfer, some stereotaxic procedures, and many mouse surgeries.) To determine if your microsurgery requires the use of sterile gloves and/or drapes, please contact The Office of Animal Care and Use (OACU) at 966-5569 for further information.

If sterile gloves are not used, a “No Touch/Tips only” technique must be used. (This restricts the surgeon to using only the sterile working tips of the surgical instruments to manipulate the surgical field. The gloved hand must never touch the working end/tip of the instruments, the suture, suture needle, or any part of the surgical field. Sutures, catheters, and other sterile materials to be used in the surgery must only be handled with the instrument tips. Tissues must only be touched with instrument tips.)
Surgical personnel approved to utilize the ‘No Touch/Tips Only’ technique should wear a clean lab coat, mask, bouffant cap and gloves.

**F. Instrument Preparation:**

All instruments must be properly sterilized. Ensure the instruments are cleaned and free of all organic material before sterilizing. A sterility indicator must be placed inside or on the surgical pack to confirm proper sterilization. Acceptable methods include:

1) Autoclave – Autoclaves should be validated for efficacy on a routine basis
2) Gas sterilization with ethylene oxide
3) Cold sterilization (See instrument sterilizing method table at the end of this Standard)

Ideally, a new sterile pack should be prepared for each additional animal.

Instruments should not be used on more than one rodent without resterilization. If instruments are to be used in subsequent surgeries (only allowable on surgeries conducted on the same day), instrument tips must be re-sterilized between surgeries, so that the tips (the part touching the animal) are always sterile. Remove any gross debris prior to placement of instruments in the sterilizer. (If using a bead sterilizer, remember to let the instruments cool before touching tissue.)

Do not allow the tips of instruments to touch non-sterile surfaces.

**Intraoperative Practices**

**G. Equipment Manipulation**

During some rodent surgeries there may be a need to manipulate certain types of equipment (microscopes, anesthetic machines, drills, etc.). Such equipment should be disinfected before surgery. If sterile gloves touch objects outside of the sterile field, they are no longer sterile. Once surgery commences, adjustments and handling of equipment outside of the sterile field must be made using a piece of sterilized gauze, aluminum foil or commercially available sterile sleeve.
H. Surgical Closure

The abdominal muscle/peritoneal layer and the skin must be closed separately. Appropriate suture material for each layer should be used. For closure of surgical incisions on the ventral surface (i.e., "underneath"), an interrupted suture pattern should be used in the muscle layer. When using sutures to close skin incisions, a monofilament material should be used (braided sutures used in skin tend to promote wound infection). An interrupted suture pattern should also be used to close the skin.

Wound clips or surgical staples may be used in the skin. However, clips or staples should not be used for closing skin on the ventral surface, since they may become contaminated with bedding. If clips, staples, or non-absorbable sutures are used to close the skin, they should be removed seven to fourteen days after surgery. Commercially available tissue adhesive products for skin closure work well on small skin incisions which would normally require one or two clips or sutures.

I. Documentation

The USDA and PHS policies require proper documentation of animal care and use to assess compliance with research protocols and clinical care procedures. Dates of all observations, treatments and procedures must be recorded. Dates and times (including AM/PM) of all time-sensitive observations or treatments (post-operative evaluations, pain medication) must be recorded. Extent of records vary based on the nature of the procedure. However, at a minimum, records of the procedure must consist of: Animal /cage/group ID, date of procedure, type of procedure, anesthetics/analgesics used (dose, route, and time), anesthesia chart (verification of toe pinch), drugs given (dose, time), general procedures (e.g., intubation, beginning and end of surgery, etc.). See https://research.unc.edu/files/2017/02/UNC-IACUC-Rodent-Anesthesia-Analgnesia-Procedure-Record.pdf

Any deviations from the approved protocol due to emergency need must be documented, explained, and reported to the OACU. All records must be available for review at any time by IACUC and external regulatory officials.
EXCEPTIONS
Requests for exceptions to this Standard must be reviewed and approved by the IACUC.

Definitions and Tables

IACUC: Institutional Animal Care and Use Committee
DCM: Division of Comparative Medicine
University Standard: The minimum acceptable limits or rules used to achieve Policy implementation, enforceable by the IACUC.
Antiseptic: A chemical agent that either kills pathogenic microorganisms or inhibits their growth. Antiseptics are antimicrobial substances that are applied to living tissue/skin to reduce the possibility of infection or sepsis.
Disinfectant: A germicidal chemical substance that kills microorganisms on inanimate objects, such as instruments and other equipment that cannot be exposed to heat.
Sterilization: The complete elimination of microbial viability, including both the vegetative and spore forms of bacteria.

Skin Antiseptics Table:

<table>
<thead>
<tr>
<th>Name</th>
<th>Examples</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohols</td>
<td>70% ethyl alcohol, 70-99% isopropyl alcohol</td>
<td>Not adequate alone for surgical site preparation! Not a high-level disinfectant. Contact time required 15-20 minutes</td>
</tr>
<tr>
<td>Iodophors</td>
<td>Betadine(®), Prepodyne(®), Wescodyne(®)</td>
<td>Reduced activity in presence of organic matter. Wide range of microbe killing action. Contact time required 10 minutes or read the label.</td>
</tr>
<tr>
<td>Chlorhexidine</td>
<td>Nolvasan(®), Hibiclens(®)</td>
<td>Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Contact time required 15 minutes or read the label.</td>
</tr>
</tbody>
</table>

*The use of common brand names as examples does not indicate a product endorsement.
<table>
<thead>
<tr>
<th>Name</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Alcohols</td>
<td>70% ethyl alcohol, 70%-99% isopropyl alcohol</td>
<td>Remove gross contamination before using. Flammable. Contact time required 15-20 minutes.</td>
</tr>
<tr>
<td>Quaternary Ammonium</td>
<td>Cetylcid(®)</td>
<td>Rapidly inactivated by organic matter. Compounds may support growth of gram-negative bacteria. Contact time required 5-10 minutes or read the label.</td>
</tr>
<tr>
<td>Chlorine</td>
<td>Sodium hypochlorite (Clorox (®) 10% solution), Chlorine dioxide [Clidox®, Alcide®]</td>
<td>Presence of organic matter reduces activity. Chlorine dioxide must be fresh (&lt;14 Days old). Contact time required 10-15 minutes or read the label.</td>
</tr>
<tr>
<td>Aldehydes</td>
<td>Glutaraldehyde [Cidex®, Cide Wipes®]</td>
<td>Toxic. OSHA has set exposure limits. Rapidly disinfects surfaces. Contact time required 5-10 minutes or read the label.</td>
</tr>
<tr>
<td>Phenolics</td>
<td>Lysol®, TBQ®</td>
<td>Less affected by organic material than other disinfectants. Contact time required 10 minutes or read the label.</td>
</tr>
<tr>
<td>Chlorhexidine</td>
<td>Nolvasan®, Hibiclens®</td>
<td>Rapidly bactericidal and persistent. Effective against many viruses. Contact time required 10-15 minutes or read the label.</td>
</tr>
</tbody>
</table>

*The use of common brand names as examples does not indicate a product endorsement.*
## Instrument Sterilant Methods and Agents Table:

<table>
<thead>
<tr>
<th>Method</th>
<th>Common use</th>
<th>Exposure Time/Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Steam Sterilization</strong></td>
<td>Steam autoclave is the most common use. Used to sterilize surgical instruments and equipment that is tolerant to moisture and heat</td>
<td>30-40 minute (high-pressure) cycle at 121°C for 15 min. vs. 131°C for 3 min (flash). Contact of materials with the steam is essential</td>
</tr>
<tr>
<td><strong>Dry Heat- Oven</strong></td>
<td>Used to sterilize items sensitive to moisture</td>
<td>2 hours; Allow appropriate cooling time. Not suitable for plastic materials</td>
</tr>
<tr>
<td><strong>Dry Heat- Glass Bead</strong></td>
<td>Used to re-sterilize tips of clean instruments</td>
<td>10-15 seconds; Requires glass beads to be heated to 200-240 °C. Allow appropriate cooling time before use. Must be used in conjunction with another sterilization method. The instruments must be clean and dry before using the sterilizer. <strong>Only instrument tips in contact with the hot beads for the specified time can be considered sterilized. All other portions must be considered ‘dirty’ and are not to be placed within the sterile field. Please follow the manufacturer’s recommendation for the sterilization time and temperature combination.</strong></td>
</tr>
<tr>
<td><strong>Gas- Ethylene Oxide (EtO)</strong></td>
<td>Used on medical and pharmaceutical products that cannot support conventional high temperature steam sterilization such as devices that incorporate electronic components, plastic packaging or plastic containers</td>
<td>May need up to 15 hours; Carcinogenic. Very limited availability</td>
</tr>
<tr>
<td><strong>Liquid Chemical- Peracetic Acid</strong></td>
<td>Spor-Klenz® is an example. Useful for heat-sensitive, nonporous materials when access to sterilization equipment is limited</td>
<td>5 ½ hours; Corrosive to metal instruments. Irritation to tissues. <strong>Rinse</strong> with sterile water or saline prior to use on animal tissues</td>
</tr>
<tr>
<td><strong>Liquid Chemical- Chlorine</strong></td>
<td>Cldox-S® and Alcide® are examples. Used on nonporous materials, heat-sensitive materials when access to sterilization equipment is limited</td>
<td>6 hours; Remove gross debris. Corrosive to metal instruments. <strong>Rinse</strong> with sterile water or saline prior to use on animal tissues</td>
</tr>
</tbody>
</table>
Liquid Chemical-Glutaraldehyde
Cetylce-G® is an example. Used on nonporous, heat-sensitive materials when access to sterilization equipment is limited
10 hours; **Rinse thoroughly** with sterile water or saline prior to use on animal tissues
*Some agents (i.e. Cidex Plus), have shorter contact times.

Liquid Chemical-Chlorhexadine
Nolvasan® is an example. Used on nonporous, heat-sensitive materials when access to sterilization equipment is limited.
24 hours; **Rinse** with sterile water or saline prior to use on animal tissues

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### Related Requirements

**EXTERNAL REGULATIONS AND CONSEQUENCES**

**UNIVERSITY POLICIES, STANDARDS, AND PROCEDURES**

For more detailed guidance, please refer to the University Policy on the Care and Use of Vertebrate Animals for Research, Training and Teaching Purposes.

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### Contact Information

<table>
<thead>
<tr>
<th>Subject</th>
<th>Contact</th>
<th>Telephone</th>
<th>Email</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protocol or Standard Questions</td>
<td>Office of Animal Care and Use</td>
<td>919-966-5569</td>
<td><a href="mailto:iacuc@med.unc.edu">iacuc@med.unc.edu</a></td>
</tr>
<tr>
<td>Surgery or Animal Related Questions</td>
<td>Department of Comparative Medicine</td>
<td>919-962-5335</td>
<td></td>
</tr>
</tbody>
</table>
Important Dates

- Effective Date and title of Approver: 10/9/2009; UNC IACUC
- Revision and Review Dates, Change notes, title of Reviewer or Approver: Revised: 04/16/2010; Revised: 09/28/2012; Updated: 04/2014; 04/2018; UNC IACUC

Approved by: UNC IACUC

Dr. Roland Tisch
UNC IACUC Chair

4/2018