SURGERY & MEDICAL RECORD GUIDELINES FOR NON-RODENT ANIMAL SPECIES USED IN BIOMEDICAL RESEARCH

This document is designed to provide guidance to research personnel when planning for surgical procedures on non-rodent species (e.g., dogs, cats, rabbits, ferrets, pigs, sheep) used in biomedical research at Michigan State University and is based upon the Guide for the Care and Use of Laboratory Animals (Guide). All personnel performing surgical procedures must be appropriately trained and listed on an IACUC-approved form. Modifications to or departures from these guidelines must be included and scientifically justified in the Animal Use Form (AUF).

The following topics are covered in this guideline:

- Surgical Facilities
- Surgeon Preparation and Aseptic Technique
- Instruments / Recommended Instrument Sterilants
- Preparation of Surgical Site / Recommended Skin Disinfectants
- Antibiotic Use
- Analgesia
- Maintenance of Anesthesia
- Monitoring Vital Signs
- Heat Loss
- Fluid Loss
- Incision Closure / Recommended Suture Selection
- Post-Operative Care
- Medical Records

Surgical Facilities

Aseptic surgery should be conducted in either a dedicated operating room/suite or an area that provides separation from other activities and that is clean, uncluttered, and easily sanitized. The basic concept of aseptic surgery is to ensure that anything that comes in contact with the surgical site is sterile. The procedures outlined here are to ensure that sterility is achieved when beginning the procedure and maintained throughout the procedure.

The functional components of the surgery facility should include:

- Surgical support area with a surgeon’s scrub location.
- Animal preparation area (for hair removal and initial skin preparation).
- Surgical room
- Post-operative recovery area (in a quiet location) where the animals can be continuously observed until fully recovered from anesthesia.

Areas that support these functions should have minimal traffic flow and separate related non-surgical activities from the surgical procedures in the operating room, either by placement of physical barriers (walls) or by distance between areas. In addition, surgery areas are to be limited to access only for people involved in performing the procedure.

Operative procedures conducted at field sites need not be performed in dedicated facilities, but must be performed using aseptic procedures.

Per the USDA Animal Care Policy #3, nonsurvival surgery does not require aseptic procedures or dedicated facilities; however, there may be situations where aseptic technique may be warranted. Factors such as the degree of tissue trauma, surgical site (e.g., gastrointestinal surgery), harvesting of tissues or extended duration may influence the decision to use aseptic procedures.
Surgeon Preparation and Aseptic Technique

Personnel must don the appropriate personal protective equipment prior to the start of surgery; this includes a surgical face mask, hair bonnet, sterile surgical gown and sterile surgical gloves. The purpose of the surgeon’s scrub is to remove dirt and, as much as is possible, decrease the bacterial flora on the surgeon’s hands and arms. Some of the agents used during a surgeon’s scrub have residual activity which helps decrease bacterial levels under surgical gloves. To effectively perform a surgical scrub, it is recommended that a sink with a foot/knee pedal be available. Sterile towels should be used to completely dry hands and arms.

- The surgeon should not touch anything that is not sterile once gloves are put on.
- If sterile gloves come in contact with a non-sterile surface, they must be changed before touching the animal again.
- ALWAYS put sterile equipment on a sterile surface (sterile surgical tray, sterile towel or drape, or sterile gauze) when not in use.
- Do not let equipment (instruments, catheters, implants, etc.) become contaminated.
- The surgeon should restrict his/her contact with the surgical site, using only sterilized equipment until the incision is closed.
- The tips of sterilized instruments should be used to manipulate and handle tissues. Minimize exteriorizing organs, but if necessary, place on sterile gauze and keep moist with sterile saline.
- If instruments are being used on multiple animals, a hot-bead sterilizer may be considered for use to sterilize instruments between animals.

Instruments

- Instruments, supplies and devices must be sterilized prior to use (see Table 1). Instruments used should be wiped clean of blood and tissues with sterile gauze and then sterilized after each surgery.
- Surgery packs should be labelled with a sterilization indicator and date of sterilization.
- Certain surgical procedures may be permitted to use cold-sterilants (such as Alcide, Cidex) for their instrument treatments if approved by the IACUC and manufacturer guidelines are followed.

Table 1

<table>
<thead>
<tr>
<th>RECOMMENDED INSTRUMENT STERILANTS</th>
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<tbody>
<tr>
<td><strong>AGENT</strong></td>
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<tr>
<td>Steam Sterilization</td>
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<tr>
<td>Ionizing Radiation</td>
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<tr>
<td>Chemical (Gas Sterilization)</td>
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Preparation of Surgical Site

- Prepare animal in a location separate from the surgical area.
- Remove hair, preferably using electric clippers made for this purpose. Avoid skin abrasions and thermal injuries.
- Dispose of removed hair to avoid hair from becoming airborne and potentially contaminating the surgical site.
- Sterile gauze soaked with skin disinfectant can be used for scrubbing (see Table 2).
- Begin aseptic skin preparation for surgery along the planned incision line with disinfectant soaked gauze and extend outward in a circular pattern to maintain clean (shaved area) to dirty (haired area) approach; do not go over the incision site twice with the same gauze.
Table 2

<table>
<thead>
<tr>
<th>RECOMMENDED SKIN DISINFECTANTS</th>
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<tr>
<td>An iodophor scrub can be alternated up to 3 times with room temperature sterile saline/alcohol, followed by a final prep with an iodophor solution. Chlorhexidine scrub can be used without an alternate scrub. Final application should be left on skin for at least 2 minutes. Remove excess liquid with dry sterile gauze.</td>
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<tr>
<th>NAME</th>
<th>EXAMPLES</th>
<th>COMMENTS</th>
</tr>
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<tbody>
<tr>
<td>Iodophors</td>
<td>Betadine®</td>
<td>Inactivates a wide range of microbes but their activity is reduced in the presence of organic matter.</td>
</tr>
<tr>
<td>Prepodyne®</td>
<td></td>
<td></td>
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<tr>
<td>Wescodyne®</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorhexidine</td>
<td>Hibiclens®</td>
<td>Rapidly bactericidal and persistent. Effective against many viruses. Excellent for use on skin.</td>
</tr>
<tr>
<td>Nolvasan®</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol</td>
<td>70% ethyl alcohol; 70-99% isopropyl alcohol</td>
<td>Not adequate for use alone. Not a disinfectant/sterilant. For use to remove excess scrub.</td>
</tr>
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</table>

**Antibiotic Use**

Prophylactic antibiotics (given immediately prior to surgery in order to maintain a therapeutic blood level during surgery) decrease wound infection and may be considered for use. Consult with veterinary staff (carvets@msu.edu) to determine appropriate therapeutic options, intervals and relevant dosages to deliver.

**Analgesia**

Appropriate attention to animal welfare necessitates the use of drugs to alleviate pain and distress associated with research procedures. The basic assumption of the US Government Principles (1985) is that if a procedure would be likely to cause pain or distress in a human, then it is likely to also do so in animals. The MSU IACUC has determined that analgesia must be provided in all such cases unless non-administration has been scientifically justified in IACUC Animal Use Form (AUF) and approved by the MSU IACUC.

Analgesia is most effective if it is administered prior to commencement of the surgery (pre-emptive), followed by additional analgesics given post-operatively. Multi-modal therapy (e.g., use of a local skin anesthetic and a NSAID or opioid) provides broader coverage of pain receptors and is recommended. The level, type and duration of analgesia will depend upon the procedure performed. For post-operative analgesia, minimally invasive procedures need less potent or shorter-acting analgesics and may respond to a single dose of analgesic. More invasive procedures (e.g. laparotomy, orthopedic surgery) may require multiple doses of analgesic for up to 72 hours post-operatively. Specific criteria for evaluating pain and provision of analgesia must be developed through veterinary consult and described in the AUF. It is the responsibility of the investigator and staff to evaluate the animal on a routine basis and determine whether additional treatment is needed based upon the criteria described in the AUF.

**Maintenance of Anesthesia**

The animal must be maintained at an appropriate depth of anesthesia beginning immediately before the surgical procedure is initiated, through the conclusion of the procedure, and until the post-operative analgesics have taken effect. For most species, the following techniques can be used to ascertain that the animal is appropriately anesthetized.

- **Jaw tone**: gentle attempts to open the mouth should be met with little to no resistance.
- **Toe or ear pinch**: brief clamping of the web of skin between toes or claws, or outer ear pinna, with a hemostat or fingers. Firmly pinching should not elicit a withdrawal response from an animal at a surgical depth of anesthesia.
- **Palpebral reflex**: gently tapping the medial canthus (inner corner of the eyelids, near the nose) of the animal’s eye should not elicit a blink or eye flutter. This technique is not always reliable in all animals. Movement of the eyelids is an indication that the depth of anesthesia is not sufficient to do surgery.
Each animal responds differently when under anesthesia, therefore it may be necessary to modify your use of anesthetics during the procedure. All routinely used anesthesia options must be described in the Animal Use Form. Anesthetists must be trained in not only delivering the anesthetic to the patient, but also in identifying anesthetic related problems.

- Increases and decreases in vital signs may require modifications in anesthetic dosing.
- If, at any time, an animal begins to respond to pain or attain an anesthetic depth that is too light, stop the procedure and adjust the inhalant anesthetic level or give a supplemental dose of injectable anesthetics. Reassess the animal before resuming work.
- Animals must be continually monitored by the anesthetist providing appropriate anesthesia and life support for the duration of the procedure.
  
  Anesthetized animals must NEVER be left alone during the procedure.
- In order to maintain sterility during complex surgical procedures and to properly monitor the animals, it may be necessary to include a second person in the procedure, e.g. a surgical assistant or anesthetist.

**Monitoring Vital Signs**

CAR veterinary staff are always available to assist in choosing an appropriate method of monitoring for your species.

The anesthetist should continuously monitor (e.g. record parameters every 5 min) the animal patient’s basic physiological function for the duration of the procedure—from induction through recovery. The following vital signs should be monitored during all procedures:

- Respiratory rate can be assessed by watching the rising and falling of the chest. Subjective changes should be addressed by altering the depth of anesthesia.
- Heart rate.
- Body temperature (see **Heat Loss** section below).

**Heat Loss**

- Maintenance of normothermia (a clinically appropriate body temperature) is critical for welfare and recovery of animals from anesthesia. Hypothermia often occurs due to anesthesia-induced vasodilation and from surgery via opened body cavities.
- Warm water recirculating blankets, warmed (body temperature) fluid bags, warming blankets, and warming discs must be used or the animal will become hypothermic.
- When using warming devices, they should be covered such that no animal is placed directly on a warmed surface.
- Electric heating pads should never be used due to inconsistency of heating and history of thermal burns in small animals. Microwaveable discs must also be monitored to ensure they are not TOO HOT before exposing an animal to them.
- Heat lamps must be used with caution to avoid overheating.
- No matter the type of heat source provided, ensure that the animals have a non-heated part of the recovery area which they can access to avoid hyperthermia.

**Fluid Loss**

- Fluid loss occurs as a result of evaporation from body cavities and blood loss.
- All animals lose fluid during anesthesia.
- Reduce fluid loss by:
  - Irrigating the operative field with warm sterile saline (be careful not to wet drapes).
  - Controlling blood loss.
  - Administering warm sterile fluids post procedurally, as needed.
Incision Closure

When closing skin and tissue layers after surgery, appropriate suture material and suture pattern for each layer should be used. For strength of closure and to avoid dehiscence, the fascial layer and the skin must be closed separately. See Table 3 for suggested skin closure materials.

Wound clips or surgical staples may be used in the skin. If clips, staples, or nonabsorbable sutures are used to close the skin, they should typically be removed 10-14 days after surgery (unless an exception is approved in the AUF). Commercially available tissue adhesive products for skin closure work well on small skin incisions which would normally require one or two clips or sutures.

Table 3

<table>
<thead>
<tr>
<th>SUTURE</th>
<th>PROPERTIES</th>
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<tbody>
<tr>
<td>Vicryl ®(polyglactin 910)</td>
<td>Braided material; absorbable within 60-90 days. Use to ligate or suture tissues where absorbable suture is needed.</td>
</tr>
<tr>
<td>Dexon™ (polyglycolic acid)</td>
<td>braided material; absorbable within 60-90 days. Use to ligate or suture tissues where absorbable suture is needed.</td>
</tr>
<tr>
<td>PDS® (polydioxanone), Maxon™ (polyglyconate)</td>
<td>Monofilament material; absorbable within 6 months. For use in ligation or suturing where an extended wound support is needed.</td>
</tr>
<tr>
<td>Prolene® (polypropylene)</td>
<td>Monofilament material; nonabsorbable. Inert. Used in soft tissue procedures.</td>
</tr>
<tr>
<td>Ethilon® (nylon)</td>
<td>Monofilament material; nonabsorbable. Inert. Good tensile strength and minimal tissue reactivity.</td>
</tr>
<tr>
<td>Silk</td>
<td>Braided material; nonabsorbable. (Caution: can cause tissue reactions and may wick microorganisms into the wound). Used in soft tissue procedures, not for skin closure.</td>
</tr>
<tr>
<td>Chromic Gut</td>
<td>Monofilament material; absorbable. Soft tissue and ligation procedures; may cause moderate tissue reaction.</td>
</tr>
<tr>
<td>Stainless Steel Wound Clips, Staples</td>
<td>Nonabsorbable. Requires special instrument for steel/staple insertion and removal.</td>
</tr>
<tr>
<td>Surgical Adhesive, Vet Bond</td>
<td>Skin adhesive. Area needs to be dry and free of blood; can cause an exothermic reaction at time of placement on skin so limiting the volume applied is essential.</td>
</tr>
</tbody>
</table>

Post-Operative Care

Intubated animals must be monitored continuously until 5 minutes after endotracheal tube extubation (respiratory complications are most likely to occur during the first 5 minutes after extubation). Endotracheal tubes should not be removed until the animal exhibits a gag reflex or is observed to swallow.

- Check heart and respiration rates, color of mucus membranes and response to toe pinch.
- Check condition of surgical site, if possible.
- Isolate and monitor the animal continuously until the animal is conscious and then every 10-15 minutes until fully ambulatory. Body temperature must be maintained. Place animal in a clean, dry, warm area on a padded
surface and provide warmth with a circulating water blanket, warm water bottle, heat discs, or other method (see Heat Loss section).

- Post-surgical animals should be seen at least daily by a member of the investigator’s staff or other identified trained individual to ensure that there are no complications.
- If the principal investigator or staff finds that an animal appears ill, is not eating or drinking, or the surgical wound appears abnormal, contact the CAR veterinary staff (carvets@msu.edu) immediately.
- Animals should be monitored for signs of pain and distress (see below). Administration of analgesics must be consistent with the description provided in the IACUC approved AUF.
- Once animal is normal and skin sutures/stapes are removed, specific post-surgery care and its documentation are no longer required.

Post-operative clinical signs that may be associated with pain and distress can include the following, which should be recorded in the medical records for the specific animal:
- Decreased food and water consumption, weight loss.
- Self-imposed isolation/hiding.
- Rapid, open mouth breathing.
- Biting, aggression.
- Unkempt appearance (rough, dull hair coat).
- Abnormal posture/positioning (hunched back, head-pressing), movements, twitching, trembling.
- Dehydration, skin tenting, sunken eyes.

Medical Records

Medical records are to be maintained on all vertebrate animals undergoing survival surgery.

At a minimum, medical/anesthetic records are to include the following information:

- Animal identification, AUF number, and species.
- Surgical procedure and date/time procedure was performed.
- Name of the persons doing the procedure.
- Time, route, dose and name of all substances administered.
- List of physiological parameters monitored along with the frequency of monitoring:
  - Note the initial values of parameters once a surgical plane of anesthesia has been reached.
  - Documentation of any significant changes in those parameters during the course of surgery/anesthesia.
  - Documentation of recovery from surgery [NOTE: not necessary for nonsurvival procedures]
- Date sutures removed.
- Method of euthanasia.
- Emergency contact information.

Post-operative observations and treatments must be recorded and initialed on each date performed. At a minimum, there must be daily entries as to the animal’s condition for 3 days following the procedure, including information describing analgesic drugs as they are administered, body weights, and food/water consumption, as outlined in the appropriate AUF. Please ensure that all daily entries are legible.

Each page of surgical records should be initialed. Surgical / anesthesia records or copies must be accessible in the investigator’s lab or surgical area at all times. If recorded in laboratory notebooks, those notebooks must remain in the laboratory.

Record retention: Retention of all surgical records is the responsibility of the Investigator. In general, regulatory agencies require medical records and record of disposition be kept for 1 year. However, some funding agencies and publishers may require a longer period of record retention. Check with your agencies and editors for their requirements.
References