Guidelines for Rodents and Bird Survival Surgery

BASIS:

Note: For the purpose of these guidelines, the term "rodent" includes rats, mice, guinea pigs; please note that rabbits and tree shrews are not rodents. For information on survival surgery in nonrodent species, please refer to the Guidelines on Survival Surgery in Nonrodent Species.

DEFINITIONS:

Aseptic Technique
Aseptic technique is used to reduce microbial contamination to the lowest possible practical level and includes preparation of the animal, such as hair removal and disinfection of the operative site; preparation of the surgeon, such as the provision of decontaminated surgical attire, surgical scrub, and sterile surgical gloves; sterilization of instruments, supplies, and implanted materials; and the use of operative techniques to reduce the likelihood of infection.

Survival Surgery
Any surgical procedure from which an animal regains consciousness for any period of time.

Major Survival Surgery
Any survival surgical procedure that penetrates and exposes a body cavity, requires the use of more than a single application of a short-term anesthetic, or produces substantial impairment of physical or physiologic functions. Examples of major surgery include laparotomy, castration, ovariectomy, thoracotomy, craniotomy, joint replacement, spinal transection, and limb amputation.

Multiple Survival Surgeries
Multiple survival surgical procedures are not permitted on animals unless scientifically justified. For additional information, please refer to the Guidelines on Multiple Survival Surgeries.

GENERAL GUIDELINES:
Location of Surgery
A surgical area for rodents and birds can be a room or portion of a room that is easily sanitized and not used for any other purpose during the time of surgery. However, because dedicated surgical facilities provide advantages with regard to veterinary
oversight, the IACUC requires the use of a dedicated surgical facility, such as a procedure room within the vivaria, whenever possible. **An investigator’s laboratory may be used as a survival surgery area provided such use is scientifically justified by the investigator and the location is inspected and approved by the IACUC.** In selecting a surgical location, the investigator should bear in mind that “...the number of personnel and their level of activity have been shown to be directly related to the level of bacterial contamination and the incidence of post-operative wound infection.” (the Guide, p. 78-79). Thus, every attempt should be made to sufficiently separate the surgical area from other areas in the room to minimize unnecessary traffic and decrease the potential for contamination of the wound. Further, the location should be designed to include the following three areas.

1. An area should be designated for preparation of the animal, including weighing, hair or feather removal, and initial skin disinfection. The prep area should be sufficiently separate from the surgery table to minimize the potential for contamination of the surgery area by aerosols generated during animal preparation.

2. A separate area should be set aside for the conduct of the surgical procedures (i.e., from skin incision to wound closure). The surgical table and immediate surrounding areas must be constructed of a material that can be washed with soap and water and then disinfected using appropriate agents (see Table 1 below). The immediate surgical area should be disinfected prior to and between surgeries to decrease dust-borne contamination and may not be used for other purposes during the time of surgery.

3. Finally, a separate recovery area should be established. This should be a quiet, undisturbed location where the animals can be observed.

**Table 1. Recommended Hard Surface Disinfectants**
(e.g., table tops, equipment) Always follow manufacturers’ instructions.

<table>
<thead>
<tr>
<th>Agents</th>
<th>Examples</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohols</td>
<td>70% alcohol</td>
<td>Minimum contact time required is 15 minutes. Contaminated surfaces take longer to disinfect. Remove gross contamination before using. Inexpensive. Flammable.</td>
</tr>
<tr>
<td>Agents</td>
<td>Examples</td>
<td>Comments</td>
</tr>
<tr>
<td>------------</td>
<td>-------------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Physical:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Steam sterilization (moist heat)</td>
<td>Autoclave</td>
<td>Effectiveness is dependent upon the temperature, pressure and time (e.g., 121.6 °C for 15 min. vs. 131 °C for 3 min.).</td>
</tr>
<tr>
<td>Chemical:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gas sterilization</td>
<td>Ethylene Oxide</td>
<td>Requires 30% or greater relative humidity for effectiveness against spores. Gas is irritating to tissue; all materials require safe airing time. Carcinogenic. Suitable for catheters and implants.</td>
</tr>
</tbody>
</table>

Surgical Instruments
Surgical instruments must be sterile. A list of acceptable methods for instrument sterilization is included in Table 2 below.

Table 2. Recommended Methods of Instrument Sterilization
Always follow manufacturers’ instructions.
Chlorine Dioxide  Clidox®, Alcide®  A minimum of 6 hours required for sterilization. Corrosive. Presence of organic matter reduces activity. Must be freshly prepared (<14 days). Must be thoroughly rinsed from instruments using sterile distilled water before use.

Aldehydes  Gluteraldehyde  Many hours required for sterilization. Corrosive and irritating. Consult Biosafety Officer on proper use. Must be thoroughly rinsed from instruments using sterile distilled water before use.

Surgical instruments may be used on more than one animal; however, **any item used on multiple animals must be carefully cleaned and disinfected between animals** (see Table 3 below). Hot bead sterilizers are preferred for this purpose, although soaking in disinfectant is also acceptable. Because the effectiveness of disinfection is directly dependent upon the contact time with the disinfectant, the surgeon should anticipate the number of surgical instruments required to guarantee uninterrupted conduct of the procedures while affording ample contact time. Disinfectants should be replaced when contaminated with body fluids or tissues.

**Table 3. Recommended Instrument Disinfectants**

Always follow manufacturers’ instructions.

<table>
<thead>
<tr>
<th>Agents</th>
<th>Examples</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry heat</td>
<td>Hot bead sterilizer</td>
<td>Fast. Instruments must be cooled before contacting tissue.</td>
</tr>
<tr>
<td>Alcohols</td>
<td>70% alcohol</td>
<td>Minimum contact time required is 15 minutes. Contaminated surfaces take longer to disinfect. Remove gross contamination before using. Inexpensive. Flammable.</td>
</tr>
<tr>
<td>Chemicals</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>---------------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Chlorine</td>
<td>Sodium hypochlorite (Clorox® 10% solution) Corrosive. Presence of organic matter reduces activity. Chlorine dioxide must be fresh (&lt;14 days old). Kills vegetative organisms within 3 min. Must be thoroughly rinsed from instruments using sterile distilled water before use.</td>
<td></td>
</tr>
<tr>
<td>Chlorine dioxide (Clidox®, Alcide®)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peracetic Acid/ Hydrogen Peroxide</td>
<td>Spor-Klenz® Corrosive to instrument surfaces. Must be thoroughly rinsed from instruments using sterile distilled water before use.</td>
<td></td>
</tr>
<tr>
<td>Aldehydes</td>
<td>Gluteraldehyde Minimum contact time required is 15 min. Corrosive and irritating. Consult Biosafety Officer on proper use. Must be thoroughly rinsed from instruments using sterile distilled water before use.</td>
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</tbody>
</table>

**Pre-Surgical Evaluation & Treatment**

Pre-existing health conditions may negatively affect the immediate or long-term success of the surgical procedures. Performing pre-surgical evaluations will help ensure that the animals are not overtly ill. This should include visual inspection of the animal and assessment of the behavioral status of the animal. The animal should be alert and behaving normally, and should have a smooth coat and clear eyes. Physical or behavioral abnormalities should be brought to the attention of the Comparative Medicine veterinary staff.

Withholding food or water is generally not necessary in rodents or birds unless specifically mandated by the protocol or surgical procedure (e.g., gastrointestinal surgery). Discuss withholding food or water for more than six hours with a Comparative Medicine veterinarian.

In some cases, it may be preferable to initiate antibiotic or analgesic treatment prior to surgery. All antibiotic or analgesic treatment regimens should be discussed with a Comparative Medicine veterinarian.

**Surgical Preparation**

Preparation of the animal should include removal of hair/feather from the surgical site with a generous border (at least 1 cm) to avoid contaminating the incision site. Hair or feather removal should be performed in a location separate from the surgical area. The surgical site should be scrubbed with a germicidal scrub (e.g., Hibiscrub® or Betadine®), being
careful to scrub from the center of the site toward the periphery. The site should then be rinsed with 70% alcohol. At least three alternating preparations of germicidal scrub and rinse are considered adequate. Finally, the area should be draped with sterile drapes, which will not only help to prevent contaminants from entering the surgical field, but will also provide a sterile area on which to lay sterile instruments during surgery.

The surgeon must thoroughly wash his or her hands with a bactericidal scrub. The use of sterile surgical gloves is required. Gloves dipped in bleach are not acceptable for this purpose. A surgical mask must be worn. Wearing a clean lab coat is mandatory; however, a sterile gown is preferable, especially for major surgeries or surgeries where materials are implanted.

**Anesthesia**

The anesthetic regimen for any surgical procedure should be determined in consultation with a Comparative Medicine veterinarian and must be described in the approved research protocol. Generally, gas anesthesia (e.g., isoflurane or halothane) is recommended for longer procedures that would require multiple injections of anesthesia. In any case, it must be determined that the animal is fully anesthetized prior to initiating the procedure and that a consistent plane of anesthesia is maintained throughout the duration of surgery. Anesthetic depth may be monitored in a number of ways (e.g., respiration rate, corneal reflex, positive toe pinch) and may vary depending upon the species and the anesthetic agent used. For rodents and birds, it is generally not necessary or feasible to monitor the heart rate.

For guidance in selection and use of anesthetics, please contact a Comparative Medicine veterinarian.

**Surgical Procedures**

All surgical procedures must be conducted as described in the IACUC approved protocol. Evaluation of the animals during surgery is critical. In addition to monitoring anesthetic depth as described above, maintaining normal body temperature is of particular importance, as anesthetic agents can directly or indirectly induce hypothermia. Water-circulating heat pads are recommended for this purpose. Using electric heat pads may overheat or burn the animal; if these are used, the pad should be set on low, a light cloth covering or bubble wrap should be placed between the animal and the pad, and the animal must be observed frequently for signs of hyperthermia. Even the use of rectal thermostat heating blankets can and do result in burned and overheated animals. Because heat lamps may cause severe hyperthermia or other thermal injury, their use is prohibited. Remember, the animals have been given anesthetics and/or analgesics to prevent them from feeling pain; this will interfere with their ability to perceive and react to pain, including burns.
To prevent corneal desiccation, bland ophthalmic ointment must be placed in the eyes following the onset of anesthesia. If animals will undergo survival stereotaxic surgery, blunt ear bars must be used to prevent damage to the tympanic membrane.

Paralytic agents may not be used without anesthesia. If a neuromuscular blocking agent is required for the surgical procedures, please refer to the *Guidelines on Neuromuscular Blocking Agents*.

**Suture Selection**
Use an absorbable suture material for body wall closure or other internal wound closures, and a nonabsorbable monofilament suture material for skin closure. Subcuticular suture placement, although more technically challenging, is acceptable for skin closure and may be performed using absorbable materials. The smallest gauge suture material should be used as practicable; typically 3-0 or 4-0 material is acceptable. A list of acceptable suture materials is included in Table 4 below.

Table 4. Acceptable Suture Materials

<table>
<thead>
<tr>
<th>Suture</th>
<th>Characteristics and Frequent Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vicryl®, Dexon®</td>
<td>Absorbable; 60-90 days. Suitable for internal wound closure.</td>
</tr>
<tr>
<td>PDS®, Maxon®</td>
<td>Absorbable; 6 months. Suitable for internal wound closure where extended wound support is desirable.</td>
</tr>
<tr>
<td>Prolene®</td>
<td>Nonabsorbable. Suitable for skin closure.</td>
</tr>
<tr>
<td>Nylon</td>
<td>Nonabsorbable. Suitable for skin closure.</td>
</tr>
<tr>
<td>Stainless Steel</td>
<td>Nonabsorbable. Suitable for skin closure. Requires instrument for removal from skin.</td>
</tr>
<tr>
<td>Wound Clips, Staples</td>
<td></td>
</tr>
</tbody>
</table>

Because silk and chromic gut cause significant tissue inflammation, these materials are not acceptable for wound closure.

Sutures, staples, or wound clips must be removed 7-14 days following surgery. Suture removal prior to euthanasia is not necessary for those animals euthanized within 14 days of surgery. Foreign substances (e.g., suture material) remaining in the incision for an extended period of time serves as a nidus of irritation and infection. Please contact a Comparative Medicine veterinarian to examine incisions that do not appear to be healing.
For guidance in suture selection and use, please contact a Comparative Medicine veterinarian.

**Post-Operative Recovery**
Observation during post-surgical recovery is imperative. The animal, whether it will recover in or out of its ‘home’ cage, must be kept warm. Water-circulating heat pads are recommended for this purpose. Using electric heating pads may overheat or burn the animal; if these are used, the pad should be set on low, and a light cloth covering or bubble wrap placed between the pad and the animals/cages, and the animal must be observed frequently for signs of hyperthermia. Turn somnolent animals periodically to prevent burns or other thermal injury. Provisions must also be made for a conscious animal to escape the heat source when it becomes too warm. Because heat lamps may cause severe hyperthermia or other thermal injury, their use is prohibited.

A recovering animal must be watched continuously until in sternal recumbency and showing a degree of purposeful movement. **Unconscious animals must never be unattended.** To prevent undue risk, rodents should be housed individually following surgery until they are ambulatory.

**Post-Operative Analgesic and Antibiotic Treatment**
As described in the *Guidelines on Post-Operative Care and Analgesia*, analgesia must be provided to all animals following survival surgery, unless scientific justification for withholding such agents is approved by the IACUC as part of the investigator’s research protocol, or if a veterinarian examines the animal and determines that analgesic administration is no longer necessary.

A list of commonly used analgesics is included in Table 5 below. Comparative Medicine has assembled a more exhaustive list; please contact a Comparative Medicine veterinarian for more details.

**Table 5. Frequently Used Analgesics**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Species</th>
<th>Dose</th>
<th>Route</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opiate drugs:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buprenorphine</td>
<td>Mice</td>
<td>.05-.1 mg/kg</td>
<td>s.c.</td>
<td>Every 12 hours</td>
</tr>
<tr>
<td></td>
<td>Rats</td>
<td>.01-.05 mg/kg</td>
<td>s.c.</td>
<td>Every 12 hours</td>
</tr>
<tr>
<td></td>
<td>Birds</td>
<td>.01-.05 mg/kg</td>
<td>i.m.</td>
<td></td>
</tr>
</tbody>
</table>
Non-steroidal drugs (NSAIDs):

<table>
<thead>
<tr>
<th>Drug</th>
<th>Species</th>
<th>Dose</th>
<th>Route</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carprofen</td>
<td>Mice &amp; Rats</td>
<td>5 mg/kg</td>
<td>s.c.</td>
<td>Once daily</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>Mice &amp; Rats</td>
<td>5 mg/kg</td>
<td>s.c.</td>
<td>Once daily</td>
</tr>
<tr>
<td>Flunixin meglumine</td>
<td>Mice &amp; Rats</td>
<td>1.1-2.5 mg/kg</td>
<td>s.c.</td>
<td>Every 12 hours</td>
</tr>
</tbody>
</table>

The use of local pain-relieving drugs such as Xylocaine® (Lignocaine) or Marcaine® (Bupivacaine), in addition to systemic analgesia, may be indicated for some procedures resulting in significant disruption of the skin (e.g., Alzet pump placement, catheter exteriorization), as these drugs help to block the onset of the pain cascade due to disruption of the dermal nerve cells. Local analgesics are not intended for use in lieu of systemic analgesics, unless the withholding of systemic analgesia is scientifically justified.

Post-operative antibiotic treatment should be discussed with a Comparative Medicine veterinarian to determine whether routine administration of antibiotics is necessary. In general, post-operative antibiotics should be provided if the animal will survive long enough to develop severe infection, but may also depend upon other factors such as the invasiveness of the procedure and the immune status of the animals. The administration of antibiotics prior to commencing a procedure can further minimize post-operative infections. There is no substitute for using aseptic technique.

**Long-Term Recovery and Monitoring**

Post-surgical observations include a minimum daily observation, including weekends and holidays, of the condition of the animal and the surgical site. Animals should be observed for continued recovery, which may include state of arousal; indices of pain or discomfort; condition of the surgical wound; appetite; hydration status; capillary refill time; mucous membrane color; or fecal and urine production.

Some surgical manipulations may require an extended period of post-operative monitoring. The Comparative Medicine veterinary staff, in consultation with the investigator, can determine the appropriate duration and extent of monitoring. Situations necessitating prolonged monitoring periods include animals with chronic debilitating disease states (e.g., diabetes mellitus), animals undergoing organ transplantation or...
immunosuppressive therapy, and animals with chronically implanted instruments or catheters.

The on-call veterinarian pager number (pager #96545) should be kept readily available in the event that post-surgical complications are observed.

**Record-keeping Requirements**
In accord with recommendations of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International, surgical records are required for rodents and birds. These records should include the administration of anesthetics, fluids, and any drugs; details of the procedure, including intraoperative monitoring; daily post-operative recovery observations and treatment, including administration of analgesics and antibiotics; monitoring of incision healing, including suture/staple removal if applicable; and the initials of the individual performing these tasks. All medications, including the name, dose, route, and time of administration should be recorded. Additionally, any adverse outcomes should be noted. A sample *Surgical and Post-Operative Evaluation Record for Mice, Rats, and Birds* is included with these guidelines. Additionally, any adverse outcomes should be noted.

To facilitate the veterinary staff's evaluation of post-operative healing and to ensure sutures are appropriately removed, **mark each cage card with the date of surgery.** If animals will be provided antibiotics in the drinking water, please communicate your needs to the Comparative Medicine staff at the respective animal facility and special treatment tags (orange *Animal under veterinary treatment/observation*) will be placed on the cages of animals requiring treatment.

All records relating to surgical procedures and post-operative care are subject to review during IACUC inspection or audit.

**Bibliography**
Abdi, S; Lee, DH; Park, SK; Chung, JM. Lack of Preemptive Analgesic Effects of Local Anaesthetics on Neuropathic Pain. British J of Anaesthesia 85(4):620-623


Varma, S; Lumb, WV; Johnson, LW; Ferguson, HL. 1981. Further Studies with Polyglycolic Acid (Dexon) and Other Sutures in Infected Experimental Wounds. American Journal of Veterinary Research 42(4):571-574