

## Guidelines for Survival Surgical Procedures in Rodents, Birds, and Cold-Blooded Vertebrates

The University of Texas at Austin  
Institutional Animal Care and Use Committee (IACUC)

*These guidelines have been written to assist faculty, staff, and students in performing vertebrate animal procedures in a humane manner and complying with pertinent regulatory requirements. Under some circumstances deviations from these procedures may be indicated but such variances must be approved in advance by the IACUC.*

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This document provides information to be used when planning and performing survival surgical procedures in rodents, birds, and cold-blooded vertebrates used for research, teaching, or other purposes at The University of Texas at Austin. It is organized into five sections:

Section A – Definitions

Section B – Requirements

Section C – Specific Considerations

Section D – Additional Sources of Information on Disinfectant, Sterilants, and Wound Closure Materials

Section E – Acknowledgements

### Section A – Definitions

**Survival surgery** is defined as a surgical procedure after which the animal will be allowed to recover consciousness, even for a short time.

**Minor surgery** is a procedure that involves surgical manipulation but does not meet the definition of major surgery. Typical examples include operative procedures in which only skin or mucous membranes are incised, e.g. vascular cutdown for catheter placement or implantation of minipumps in subcutaneous tissue. Also included are minimally invasive means of accessing a body cavity, such as needle biopsy or the introduction of instruments using a trocar.

**Major survival surgery** is defined as a survival surgical procedure that involves penetration and exposure of a body cavity (abdomen, thorax or cranium) or will produce a substantial physical or physiologic impairment.

**Multiple major survival surgery** occurs when two or more separate major survival surgical procedures are performed on a single animal. It is allowable only under certain circumstances, the most common being a situation in which each surgical manipulation is an essential and related component of a single study. Cost alone is not an adequate justification for performing multiple survival surgeries on the same animal. Multiple major survival surgeries must be specifically justified by the PI and approved by the IACUC as part of an approved protocol.

**Aseptic (sterile) surgical techniques** are well-established methods used to avoid the introduction of microbial contamination into tissues exposed and/or manipulated during surgery. More details are provided below.

### Section B – Requirements

1. **Non-survival surgery** does not require aseptic (sterile) techniques if the subjects are not anesthetized long enough to show evidence of infection. However, personnel present in the area must observe reasonable

cleanliness practices for both themselves and the animals. Eating, drinking or smoking is not acceptable in surgery areas.

2. **Survival surgical procedures** may be performed in either a dedicated animal procedural room or in a suitably located and equipped laboratory area, subject to approval by the IACUC. These procedures generally cannot be done appropriately within an animal holding room. The bench or tabletop areas used for animal prep, surgery, and recovery should contain only the ancillary laboratory, diagnostic, or clinical equipment and supplies required to support the procedure being performed. Equipment used on an infrequent basis and bulk supplies should be stored elsewhere. The area should be free of physical or chemical hazards potentially arising from splashes, spills, etc. A low-traffic area of the laboratory away from doorways, hoods, etc. is optimal. The National Research Council's (NRC) *Guide for the Care and Use of Laboratory Animals* states "For most rodent surgery, a facility may be small and simple, such as a dedicated space in a laboratory appropriately managed to minimize contamination from other activities in the room during surgery."
3. **Aseptic (sterile) techniques for survival surgery** are required for ALL species. However, some modifications are allowed when rodents, birds and cold-blooded vertebrates are involved. For example, the use of surgical drapes in small species may not be necessary because a) the incisions are very small and organs are rarely exteriorized during the procedures and b) drapes may actually impair the ability to assess breathing patterns or other monitoring criteria used to assure proper anesthetic depth. Another difference is that chemical disinfection of the surgical site may not be appropriate for the slime-coated and absorptive skin of amphibians and fish. The need for gowning of the surgeon is also considered optional for surgery involving rodents, birds, and cold-blooded vertebrates. However, the surgeon should wear a surgical mask. Unless a strict "Tips Only" method is being used (as described below), sterile surgical gloves ARE required.

## **Section C – Specific Considerations**

### **1. Preparation of Surgical Facilities and Instruments**

#### **a. Surgery Facilities**

Prior to surgery:

- Clean and disinfect the surface upon which surgery will be performed. Use soap and water to clean the area if visibly soiled, followed by the use of a hard surface disinfectant that is appropriately-labeled for hospital, laboratory or veterinary use. Use of absorbent "chucks" or bench lining paper can contain liquids and facilitate cleanliness in surgical areas, but these disposable items should be changed and discarded at the end of the surgical procedure.

#### **b. Surgical Instruments**

Surgical instruments must be sterilized prior to use for survival surgery. Organic material must be removed by cleaning prior to sterilization. Methods used for sterilization may vary, but all must conform to established medical standards for complete sterilization. Options include:

- Steam sterilization at proper pressures and exposure times
- Ethylene oxide gas (ETO) used in a specialty chamber
- Dry heat sterilization at proper temperature and exposure time
- Prolonged immersion in a hospital-grade formaldehyde- or glutaraldehyde-based cold sterilant following label directions (alcohol immersion is NOT acceptable)
- Hot bead sterilizer (acceptable for use in procedures where a "tips-only" method of aseptic

technique is appropriate)

When performing surgeries on multiple animals and re-using instruments during a single session, repeated full sterilization is NOT needed, but instruments must be disinfected between animals. This will require thoroughly removing all organic material (e.g., blood) from instruments and then immersing the instruments in an appropriate disinfectant (e.g., 60-90% ethyl or isopropyl alcohol or a product labeled as an instrument disinfectant) between animals. NOTE: Disinfectant residue must be removed prior to use by allowing adequate drying time (for alcohols) or including a sterile water rinse (for disinfectants).

## 2. Preparation of the Animal

Prior to taking the animal to the surgery area:

- a. Remove hair from the surgical site, e.g., with clippers, a razor, or very careful use of depilatory cream (which can quickly injure the thin skin of small animal). Carefully vacuum or otherwise remove loose hair and debris. Do not use scissors only to remove hair.
- b. Clean and disinfect the surgical site using an appropriate surgical prep technique (e.g., scrubbing in a gradually enlarging circular pattern from the interior of the shaved area towards the exterior) and an effective antiseptic. Although chlorhexidine or povidone iodine-based antiseptic solutions are considered the best practice, it is acceptable to use 60-90% ethyl or isopropyl alcohol to disinfect the surgical site.
- c. Drying and irritation of the eye during anesthesia in rodents can be prevented by placing a lubricating ophthalmic ointment (e.g., Lacrilube) in the anesthetized animal's eyes.

## 3. Preparation of the Surgeon

The surgical procedure itself must be performed or directly supervised by a trained and experienced individual. Personnel unfamiliar with aseptic surgical procedures should contact the ARC veterinary staff for information or training.

- a. A facemask is required. Changing into a clean scrub shirt or donning a clean lab coat is recommended.
- b. Wash hands with a soap (minimal) or an antiseptic surgical scrub solution (optimal).
- c. Sterile surgical gloves must be donned prior to handling sterile instruments or touching surgically-exposed animal tissues. When performing surgeries on multiple animals during a single session, one pair of sterile gloves can be used provided that the gloves remain free of tears or punctures and are disinfected (by wiping with an appropriate disinfectant) between animals.
- d. Long hair of surgeons and assistants should be covered and/or restrained to keep it away from the surgical field.

## 4. Intraoperative Monitoring and Post-operative Care

The animal's physical condition and anesthetic depth must be continually monitored during the procedure and frequently assessed during the recovery period until animal is fully ambulatory. Animals should not be left alone during surgery. All needed materials, (instruments, drugs, etc.) should be easily accessible from the surgery location. The use of monitoring equipment to record clinical parameters (e.g., core body temperature, pulse oximetry, blood pressure, etc.) is recommended when it is appropriate and available. Written records on individual animals must be maintained for surgeries. Visual assessment of the following parameters is recommended for each animal (RECORD ANY DEVIATION FROM NORMAL):

- a. **Analgesia quality:** Assess withdrawal reflex via toe pinch a minimum of every 15 minutes. Similar

noxious but noninjurious stimuli may be substituted as needed for non-rodent species.

- b. **Mucous membrane color:** Mucous membranes should remain pink.
- c. **Breathing pattern:** Breathing should be regular and within normal range.

Drugs, including dosages, routes of administration, and times given should be recorded before and during surgery. Postoperative analgesia or other supportive care must be recorded as well.

During recovery, animals must be kept warm and dry in an environment that doesn't pose a risk of injury as they regain muscular control. Warm-blooded animals (or ectotherms subjected to hypothermia) should be provided with a heat source (preferable) or be placed on an insulating surface (minimally) until they have fully recovered to prevent post-procedural hypothermia. CAUTION: Use of heat lamps and electric heating pads can result in severe burns or hyperthermia in animals that are anesthetized or otherwise unable to escape from the heat. Heating devices should not come into direct contact with the animal and the animal must be monitored to ensure overheating does not take place. The use of safer equipment such as a circulating water blanket or isothermic pad is recommended whenever possible.

In some cases the animal may be returned to its home cage during recovery, but no food or water should be left in the cage until the animal is fully conscious. Separate recovered animals from those that are still under the influence of anesthesia to avoid wound licking or cagemate-induced trauma. Avoid placing rodents directly on wood chip bedding when recovering, because the altered breathing patterns and lack of respiratory reflexes may result in the inhalation of wood dust or fibers.

The drugs specified in the approved protocol for relief of pain and/or distress must be readily available for use as described in the approved animal use protocol. Fluids, analgesics, and antibiotics must be administered as indicated in the protocol or as directed by the attending veterinarian or designee.

Surgical wounds must be kept clean. If bandages or wound dressings are used, they must be changed as frequently as necessary to keep them clean and dry. Subsequent care must consist of daily monitoring of the animal to include daily body temperature (in species amenable to handling) and clinical observation for signs of pain, abnormal behavior, appetite and excretory functions.

After the immediate postoperative period, check the animal at least daily to ensure that there are no complications; record deviations from normal. Nonabsorbable sutures or wound clips should be removed in 7-10 days. If the animal appears ill postoperatively, or if the surgical wound appears abnormal, the veterinary group should be contacted for consultation.

## 5. Minimal Requirements for Surgery Records

Notebooks, checklists, computer logs or other records should contain the date of the procedure, the surgeon name, animal or cage/group number, type/dose of anesthesia utilized, type/dose of analgesic administered, vital parameters monitored, times of monitoring and a notation of any complications or abnormalities identified. NOTE: When DEA/DPS controlled substances are used, the date and drug usage volumes recorded in the controlled substance log and the dates and dosages recorded in the animal surgery records should match.

## 6. "Tips Only" Technique

"Tips only" aseptic practices are considered appropriate for survival surgeries in rodents, birds or cold-blooded vertebrates that involve only very small incisions, e.g., embryo transfer, gonadectomy, intracerebral placement of a pellet or transponder, etc. During these procedures it is not necessary for the surgeon to directly touch the tissues and the likelihood of inadvertent contact with exposed tissues is minimal. In this case, the emphasis of

aseptic surgical practices will be to keep the tips (which enter the body) of the instruments free of contamination during the procedure. In this situation, sterile gloves are not required, and instrument-working surfaces can be selectively sterilized by immersion in a hot bead sterilizer. Likewise, the instrument tips can be disinfected between animals by immersion in a hot bead sterilizer or an acceptable disinfectant as described above. If this method is chosen, the surgeon must be very cautious to avoid contact with the surgical site or the working surfaces of the instruments, and to keep the tips free of contamination by having a sterile location to place the instruments if they are put down during the procedure. Animal users can consult with the veterinary staff to determine if the "tips only" method is appropriate for their particular procedure.

### **Section D. Additional Sources of Information on Disinfectant, Sterilants, and Wound Closure Materials**

1. The NIH intramural animal research program has compiled tables listing appropriate antiseptics, disinfectants and wound closure materials at <http://oacu.od.nih.gov/ARAC/surguide.pdf>
2. National Research Council. 1996. *Guide for the Care and Use of Laboratory Animals*. National Academy Press, p78.

### **Section E. Acknowledgements**

This document contains content that was adapted from materials obtained from Stanford University.