The University of Pennsylvania’s Institutional Animal Care and Use Committee (IACUC) and the Attending Veterinarian (Director of ULAR) is charged with ensuring that all surgical facilities and procedures meet the criteria set by the federal regulations, including the Animal Welfare Act (AWA), the Animal Welfare Regulations (AWR), and the Public Health Service Policy (PHS). The PHS requires institutions to comply with the performance-based standards in the *Guide for the Care and Use of Laboratory Animals* (Guide).

The purpose of this guideline is to clarify the requirements of the Principal Investigator (PI) and the Institution with regards to survival surgical procedures performed on USDA-covered, non-rodent species. All investigators, laboratories, and facilities performing survival surgery on USDA-covered, non-rodent species are expected to adhere to the minimum standards addressed in this IACUC guideline. Species typically studied at Penn that are relevant to this guideline include (but are not limited to): rabbits, ferrets, cats, dogs, sheep, pigs, non-human primates, and other farm species. USDA-covered rodent species (e.g. hamsters, gerbils, guinea pigs) are addressed in the IACUC guideline on Rodent Survival Surgery.

This guideline offers direction on the following topics:
- Pre-surgical approval and assessment
- Requirements of a surgical suite
- Aseptic technique
- Anesthetics and analgesics
- Monitoring the anesthetized patient
- Anesthetic and postoperative recovery
- Recordkeeping for USDA-species

**PRE-SURGICAL APPROVAL AND ASSESSMENT**

Before performing surgery on any species, the PI must obtain IACUC approval for the research activity. Attaining approval to perform surgery is at least a three-step process:

1. The Institution, during the IACUC review process, evaluates the surgical training qualifications of all participants on protocols involving surgical procedures. The PI describes and endorses all personnel qualifications.
2. The IACUC provides approval of the protocol for the surgical project.
3. IACUC members evaluate the location and provide approval of the surgical location for USDA survival surgery.

**Surgical Training Qualifications**

Prior to IACUC approval of protocols and/or amendments to add personnel, the Knowledge Link training modules “Regulations and the IACUC,” “Species Specific Training,” and “Aseptic Technique” must be completed and documented for personnel listed on surgical procedures. As additional surgical training opportunities become available, they may be required by the institution. The PI is responsible for ensuring that personnel involved with anesthesia induction, monitoring of an anesthetized animal, direct surgical manipulation, post-operative care, and other advanced techniques receive additional training if necessary. If there are any questions about surgical training or scheduling, please contact ULAR Training (ULAR-tr@pobox.upenn.edu).

**Protocol Approval**

All surgical protocols of USDA-covered species are to be specifically pre-reviewed in consultation with a ULAR laboratory animal veterinarian. This ULAR veterinary review emphasizes the appropriateness of
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the species used as the surgical model, the surgical procedure itself, anesthesia and analgesia regimens, and completeness of the pre- and post-surgical care plans.

If a research protocol calls for an animal to undergo multiple survival surgeries, this must be specifically reviewed and approved by the IACUC (Guide, AWR 2.31 (d)(x)):

- If at least one animal on the protocol undergoes surgery, is recovered, and undergoes at least one other survival surgical procedure, then the PI should select “yes” for “Multiple survival surgery” in the associated question in ARIES. In addition, the PI should then provide a scientific justification as to why multiple survival surgeries are necessary in the space provided at the bottom of the specific “Survival Surgery” Procedures section. The description section should include details, including the timing between surgical procedures, duration of each procedure, and recovery parameters.
- If all animals assigned to the protocol will undergo just one survival surgery procedure, to be eventually followed by a terminal surgery or euthanasia, then select “no” for “Multiple survival surgery” in the associated question.

Surgical Location Approval
If the pre-, peri-, and post-surgical manipulation and care of the animal occur in a ULAR-operated facility, approval of the protocol is all that is required. If the survival surgery procedures are planned to be performed in a non-ULAR operated facility, then (a) the protocol should include a justification as to why a ULAR facility is unsuitable for the surgical procedure, and (b) prior to protocol approval, the location must be evaluated and approved by the IACUC. This site evaluation occurs independently of the IACUC protocol review process and must be specifically scheduled by the PI or senior research staff through contacting the Animal Welfare Compliance Staff at 215-746-6271 or the IACUC office at 215-898-2615. See below, “Requirements of a Surgical Suite,” for details on what is expected of both ULAR and non-ULAR managed surgical areas.

REQUIREMENTS OF A SURGICAL SUITE
Minimize Contamination
The entire surgical suite must be maintained in such a manner as to reduce risk to the animal patient, as “inadequate or improper technique may lead to subclinical infections [in animals] that can cause adverse physiologic and behavioral responses affecting surgical success, animal well-being, and research results” (Guide). Standard operating procedures (SOPs) should be written to describe how the surgical suite is routinely cleaned, sanitized and documentation maintained. ULAR SOP 8.45 “Cleaning of ULAR Surgical Suites” should be followed. Alternatively, you may submit your own SOP, but it must follow the minimum standards set by this ULAR SOP. Review of these SOPs will be required prior to IACUC approval of any location for USDA-species survival surgery.

Surgery performed on USDA-covered animals must be conducted in a dedicated facility that contains space for 1) Surgical Support, 2) Animal Preparation, 3) Surgeon’s Scrub Area, 4) Operating Room (OR), and 5) Post-Operative Recovery:

1) Surgical Support
“The surgical-support area should be designed for washing and sterilizing instruments and for storing instruments and supplies. Autoclaves are commonly placed in this area” (Guide). The surgical support area
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does not have to be immediately adjacent to the surgical suite, but there must be such an area available to the surgical laboratory. Sink areas should clean and free of rust. Instruments should be stored in suitable containers to keep them clean and in good condition. Laboratories must have the capability to sterilize surgical equipment, e.g. access to autoclaves, plasma sterilizers, etc.

2) Animal Preparation
In order to maintain a clean OR, the preparation of the animal should not be done in the operating room. To avoid contamination, there should be a separate but adjacent area (‘animal prep’) where the animal will be physically prepared to undergo a surgical procedure (e.g. clipping of fur, cleaning of gross debris, intubation, placing intravenous catheters, etc.). Initial monitoring and recording of anesthesia depth and vital signs should begin while the animal is in this area. There must be adequate electrical outlets in the animal prep area in order to power monitoring equipment, a heating source, and lighting as needed. This animal prep area may double as a recovery area after proper cleaning.

3) Surgeon’s Scrub Area
A specific area “equipped with foot, knee or electric eye surgical sinks” where the surgeon(s) can scrub before performing a surgical procedure must be part of the surgical suite. “To minimize the potential for contamination of the surgical site by aerosols generated during scrubbing, the scrub area should usually be outside the operating room and the animal preparation area” (Guide). Adequate space for gowning into sterile surgical garb within or near to the surgeon’s scrub area and the operating room will minimize risks of contamination of sterile status.

4) Operating Room (OR)
The following requirements should be considered for operating rooms (ORs):
- ORs must be sanitized prior to surgical procedures. When not in use, the OR should be maintained clean and clutter-free. Surfaces should be non-porous.
- In order to assure proper sanitation, it is recommended that the OR not be used for primary storage. If needed, items that are not easily sanitized (e.g. non-surgical equipment, card board boxes, general supplies, etc.) should be stored in drawers or cabinets which can be sanitized.
- Air pressure should be maintained at positive pressure in the OR relative to the surrounding facilities in order to prevent airborne contaminants from entering the OR.

5) Post-Operative Recovery
There must be an area available where the animal can safely recover from the effects of anesthesia and surgery. Like the animal prep space, the post-operative recovery area should be appropriately equipped with emergency equipment and supplies, electrical outlets for power supply to warming devices and support equipment, and proper lighting as needed. Ideally, the post-operative recovery should be separate from the animal prep area, but in facilities with limited space, the animal prep area may double as the post-operative recovery space. If the area will be used for both animal prep and recovery, the space should be cleaned between animals.

ASEPTIC TECHNIQUE

Patient
How the surgeon or assistants will prepare the animal should be described in the “description” field of the ARIES Survival Surgery procedure. Preparing the animal for surgery is at least a two-step process:

1. In the Animal Prep Area:
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- Remove the hair (e.g. with clippers or depilatory cream) in a wide area around the body site where surgery is to be performed. Close monitoring of the length of time that depilatory creams are applied to animals is encouraged to avoid skin irritation.
- Clean hair/fur and any other gross debris from the skin using an appropriate surgical scrub pattern (remove in a concentric pattern moving from the innermost to outermost areas) with a detergent-based surgical scrub (Betadine or Chlorhexidine) and alcohol or sterile water.
- If clippers are used, please ensure fur is removed from the blades and the clippers are cleaned after use.

2. In the Operating Room:
- To minimize excessive movement of the animal during the surgery and to avoid a break in sterility, the anesthetized animal should be properly secured to the operating table using suitable restraint; secure placement should avoid being excessively tight and thus permit blood flow to extremities.
- Disinfect the area with appropriate surgical scrub for as long as the product indicates prior to initiating the surgical procedure.
- Alcohol alone is NOT an appropriate disinfectant. Iodophors (e.g. Betadine) or chlorohexidines (e.g. Nolvasan) should be used with appropriate contact time.
- Drape the surgical site by placing and securing sterile drapes over the entire animal and the operating room table.

Surgeon and Surgical Assistant(s)
Surgeons and any person who will have contact with the sterile surgical field during the surgery must:

- Wear clean scrubs, appropriate facemask, head covering, close-toed shoes, shoe covers.
- Perform a surgical scrub in the surgeon’s scrub area. This includes scrubbing both hands, in between fingers, and both forearms with a designated surgical scrub brush with an antibacterial soap (e.g., chlorhexidine or iodophor) for as long as the product indicates prior to initiating the surgical procedure.
- Don sterile gown and gloves using appropriate technique.
- Limit excessive movement (e.g. foot traffic) to avoid contamination of the surgical locations and OR.

If it is necessary to leave the surgical suite during a procedure, rescrubbing and regowning is necessary before re-entering.

Non-surgeons (that is, surgical assistants) must wear appropriate personal protective equipment (PPE), which is at least a disposable cover gown, shoe covers, mask, gloves and head cap (exception: non-human primate surgeries require additional PPE). Non-surgeons are defined as those individuals that will NOT have contact with the sterile field, but they may assist the surgeon. For example: anesthetists and those operating equipment that will be used during the surgery but which while not come into direct contact with the sterile surgical field.

Instruments
It is extremely important to ensure that all instruments are appropriate for surgery, in particular:

- All instruments must be cleaned and sterilized prior to use on animals for all surgical procedures. Alcohol is NOT a sterilant. Examples of methods of sterilization include steam autoclave, gas (e.g. ethylene oxide), and plasma sterilization.
- Cold sterilization (e.g. Cidex) of surgical instruments must strictly follow manufacturer instructions. The CDC lists specific cold sterilants and the necessary conditions to be considered a
sterilant or a disinfectant. Rinse instruments free from the cold sterilants with sterile water or sterile saline before putting them in contact with animals.

- Best standard of practice is to use only sterile instruments for one animal at a time. Therefore, new autoclaved, gas sterilized, or plasma sterilization packs are recommended for each animal.
- Do not use dull or rusted surgical instruments or those not manufactured for surgical use, as these cannot be reliably sterilized.

When sterilizing by autoclave or plasma sterilizer, surgical packs should contain a sterilization indicator, the date of sterilization and expiration date for the pack. Equipment used for sterilization (e.g. steam autoclaves and plasma sterilizers) should be routinely monitored for efficacy. Appropriate storage of sterilized items is expected; integrity of sterile packaging should be examined prior to use.

ANESTHETICS AND ANALGESICS
When writing a procedure, one must consider how to provide the maximum relief of pain/distress possible, while maintaining the integrity of the research. Every surgical IACUC protocol must describe a clear plan for providing in-date, pharmaceutical-grade (if available) injectable or inhalation anesthetics and a description of how and when analgesics will be administered. In order to provide flexibility when performing the procedure, consider including relevant dose ranges, for the anesthetic and analgesic regimens.

In-Date, Pharmaceutical Grade Compounds
“Non-pharmaceutical grade chemical compounds should only be used in regulated animals after specific review and approval by the IACUC for reasons such as scientific necessity or non-availability of an acceptable veterinary or human pharmaceutical-grade product. Cost savings is not a justification for using non-pharmaceutical grade compounds in regulated animals (USDA Policy #3).” See the IACUC policy on the Use and Disposal of Expired Drugs and Materials for more details. Also see specific guidance from OLAW on this topic, which includes acceptable justifications for using non-pharmaceutical grade drugs.

Injectable and Inhalation Anesthetics
Animals should be weighed prior to surgery to calculate the appropriate dose of anesthetics for the intended route of administration. Contact a ULAR Veterinarian or consult the Large Animal Anesthesia and Analgesia Formulary for suggested drugs, doses, and route of administration for your species and procedure.

For delivery of inhalants, consideration should be given to adequately protect the airway by placing an endotracheal tube. Personnel should be properly trained in this technique for the specific species being utilized.

Commercially manufactured vaporizers must be used to deliver inhalant anesthetics to USDA-species. The use of a bell jar or any other homemade delivery device is not permitted. Anesthetics must be scavenged with appropriate devices or methods as approved by EHRS. Contact the ULAR Training Staff for assistance if personnel require training in using an anesthesia machine. Please refer to IACUC Guideline Anesthetic Vaporizers and Gas Scavenging for more information.

Analgesics
Surgery is considered a painful procedure; therefore administration of analgesics is required for any animal that undergoes surgery. Pre-emptive analgesia is expected unless there is scientific justification or
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it is against the clinical judgment of a ULAR veterinarian. The goal is to have every animal patient maintained post-surgically with minimal to no pain. Animals given pre-emptive analgesia often require less anesthetic to reach a surgical plane, and thus may be more stable anesthetic patients. The analgesic options available to investigators are quite varied and certain drugs may provide better pain relief for patients undergoing certain procedures. Contact a ULAR Veterinarian or consult the Large Animal Anesthesia and Analgesia Formulary for suggested drugs and doses that will best suit the surgical research model.

MONITORING THE ANESTHETIZED PATIENT
Careful monitoring includes confirmation of anesthetic depth, maintenance of anesthesia, and monitoring of vital signs.

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

- **Toe pinch (pedal response):** Brief clamping of the web of skin between toes or hooves with a hemostat or fingers. Firmly pinching multiple toes should not elicit a withdrawal response from an animal that is currently at a surgical depth of anesthesia.
- **Jaw tone:** The animal’s jaw should remain slack when gently extending the mandible (lower jaw). If the jaw is “tight” and clenched, then the animal’s anesthesia level may not be deep enough for surgery.
- **Pupil position:** For most species and under most anesthetic regimens, pupils will rotate downwards in an adequately anesthetized patient. This method must be combined with other forms of anesthetic monitoring as it is not reliable as a stand-alone option.
- **Palpebral (eyelid) reflex:** Touching the edge of the eyelid with a gauze sponge or cotton swab will produce a reflexive “blink” if the patient is too light. Movement of the eyelids is an indication that the depth of anesthesia may not be sufficient to conduct surgery.
- **Vital signs:** Heart rate and respiratory rate may increase if anesthetic depth becomes too light.

Maintenance of Anesthesia
Each animal responds slightly differently to anesthesia, therefore it may be necessary to modify the use of anesthetics during the procedure. *All routinely used anesthesia options must be described in the IACUC protocol.* Anesthetists must be highly skilled 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 reach an anesthetic level that is too light, stop the procedure and adjust the inhalant anesthetic level or give a supplemental dose of injectable anesthetics. Reassess the animal to ensure surgical depth of anesthesia 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 should NEVER be left alone.*
- In order to maintain sterility during complex surgical procedures and to properly monitor the animals, it is generally necessary to include at least a second person in the procedure—a surgical assistant or anesthetist.
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Monitoring Vital Signs
The anesthetist must continuously monitor the animal patient’s basic physiological function for the duration of the procedure—from induction through recovery. At a minimum, the respiratory and heart rate must be monitored and documented every 5-10 minutes, and body temperature every 15 minutes:

- **Respiratory Rate** (RR) can be assessed by watching the rising and falling of the chest, by monitoring end-tidal CO₂, or by artificial ventilation. Subjective changes should be addressed by altering the depth of anesthesia.
- **Heart rate** (HR) may be monitored manually or with more advanced techniques including ECG, SPO₂, or Doppler.
- **Body temperature** should be kept at physiologically normal temps during surgical procedures. Hypothermia often occurs due to anesthesia-induced vasodilation and from surgery via opened body cavities. Hypothermia may lead to hypotension and bradycardia, as well as prolonged recovery times for animals.
  - **Supplemental Warming of Patient** During any surgical procedure, the animal’s body temperature should be maintained by a heat lamp, a [covered] recirculating water heating pad, medical-grade thermal support, or forced-air warming device (Bair-Hugger).
  - **Over-the-counter heating pads and microwavable thermal devices are not permitted.**
  - **ULAR Veterinarians** may assist in choosing appropriate monitoring and supplemental warming methods for your species.

It is strongly recommended that additional monitoring techniques be employed such as pulse oximetry, blood pressure, ECG, arterial blood gas parameters, and end-tidal CO₂.

ANESTHETIC AND POSTOPERATIVE RECOVERY

Immediate Recovery Period
The anesthetic recovery period may last from minutes to hours. During this time:

- Animals should be placed in a clean recovery area with emergency drugs and equipment available.
- The animal should be maintained in sternal recumbency until it can position itself. Rubber mats should be placed if there is concern for the animal slipping during recovery.
- The animal’s body temperature should be supported with an appropriate, well-maintained heating device (e.g. recirculating water heating pad, or heat lamp). To avoid burning, be cautious that the heating device is not too hot or too close to the animal. There should always be a cooler location in the enclosure to which the animal can escape if they become too warm.
- Recovering animals should not be placed into an enclosure with other awake/conscious animals.
- Personnel monitoring recovery of animals must remain in the same room as the animals at all times. Only when animals have regained all postural reflexes and are ambulatory (can walk well on their own) should they be left alone in their regular, freshly-cleaned housing space. As done during the procedure, the body temperature, respiratory rate and heart rate should be monitored, evaluated, and documented during the recovery period at least every 15-30 minutes, with the exception of nonhuman primates. The last entry before the lab member leaves should reflect that the animal’s vital signs have returned to the acceptable range, or a veterinarian should be contacted.

Long Term Recovery Period
Depending on the surgical procedure, the postoperative recovery period may last from days to weeks.
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- During the post-surgical period, animals must be appropriately monitored for signs of pain and/or distress. In most species, signs of pain include decreased activity, abnormal posture, increased attention to surgical site, and gait abnormalities. The surgical site should be gently palpated following recovery from surgery, and in the days following the procedures, to assess painful responses.
- The cardinal signs of infection including heat, swelling, redness, pain, and/or exudation. Consult a ULAR veterinarian for further assessment of any abnormal medical condition(s).
- The frequency and length of observation may depend on the degree of invasiveness of the procedure and the individual animal. A written plan of observation must be outlined in the IACUC protocol for each procedure proposed. The IACUC recommends the PI or the lab staff observe animals at least once to twice daily for a minimum of five days following major procedures.
- The IACUC protocol must fully and clearly describe the clinical signs expected to be observed following the surgical procedure and the humane endpoints that may necessitate euthanasia or other removal from the study. Please refer to the IACUC guideline Monitoring and Humane Endpoints for Laboratory Animals for more information.

Table 1. Vital signs for commonly used laboratory animals. Values given are for awake, adult animals.

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>RESPIRATORY RATE (breaths/minute)</th>
<th>HEART RATE (beats/minute)</th>
<th>TEMPERATURE (°C)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit</td>
<td>32-60</td>
<td>200-300</td>
<td>38.5-39.5</td>
<td>The Laboratory Rabbit, Guinea Pig, Hamster, and Other Rodents</td>
</tr>
<tr>
<td>Ferret</td>
<td>33-36</td>
<td>200-400</td>
<td>37.8-40.0</td>
<td>Ferrets, Rabbits, and Rodents: Clinical Medicine and Surgery, 3rd ed</td>
</tr>
<tr>
<td>Dog</td>
<td>18-34</td>
<td>70-120</td>
<td>37.7-39.9</td>
<td>Merck Veterinary Manual</td>
</tr>
<tr>
<td>Pig</td>
<td>32-58</td>
<td>70 - 120</td>
<td>38.7-39.8</td>
<td>Merck Veterinary Manual</td>
</tr>
<tr>
<td>Sheep</td>
<td>16-34</td>
<td>70-80</td>
<td>38.3-39.9</td>
<td>Merck Veterinary Manual</td>
</tr>
<tr>
<td>Macaque</td>
<td>30-70</td>
<td>120-180</td>
<td>37-39.1</td>
<td>Association of Primate Veterinarians Nonhuman Primate Formulary</td>
</tr>
</tbody>
</table>

RECORDKEEPING FOR USDA-SPECIES

The USDA and PHS require proper documentation of animal care and use to assess compliance with research protocols and clinical care procedures. Please refer to IACUC guideline on USDA Species Recordkeeping for details on how to properly maintain surgical records.
REFERENCES


