The University of Pennsylvania’s Institutional Animal Care and Use Committee (IACUC) 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).

AWR 2.31 (d) (ix)
“Activities that involve surgery include appropriate provision for pre-operative and post-operative care of the animals in accordance with established veterinary medical and nursing practices. All survival surgery will be performed using aseptic procedures, including surgical gloves, masks, sterile instruments, and aseptic techniques. Major operative procedures on non-rats will be conducted only in facilities intended for that purpose which shall be operated and maintained under aseptic conditions...”

AWR 2.33 (b) (4) (5)
“Each research facility shall establish and maintain programs of adequate veterinary care that includes: (4) Guidance to principal investigators and other personnel involved in the care and use of animals regarding handling, immobilization, anesthesia, analgesia, tranquilization, and euthanasia; and (5) Adequate pre-procedural and post-procedural care in accordance with current established veterinary medical and nursing procedures.”

PHS Policy VI.C.1.
“...the IACUC shall confirm that the research project will be conducted in accordance with the Animal Welfare Act insofar as it applies to the research project, and that the research project is consistent with the Guide unless acceptable justification for a departure is presented.”

The purpose of this guideline is to clarify the requirements of the Principal Investigator (PI) and the Institution concerning surgical procedures performed on USDA-covered non-rat species. Some species typically studied at Penn that are relevant to this guideline include: ferrets, rabbits, cats, dogs, ruminants, pigs, nonhuman primates, and other USDA-covered species.

This guideline offers direction on the following topics:

- Pre-surgical approval and assessment
- Requirements of a surgical suite
- Preparation of the surgeon, animal, and surgical instruments
- Aseptic technique
- Anesthesia
- Monitoring the anesthetized patient
- Anesthetic and postoperative recovery
- Recordkeeping for USDA-species

PRE-SURGICAL APPROVAL AND ASSESSMENT
IACUC Guideline  
USDA-SPECIES SURGERY

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

1. The IACUC provides protocol approval of the surgical project
2. The Animal Welfare Compliance Staff evaluates the location and provides surgical location approval
3. The Institution, during the IACUC review process, evaluates the surgical training qualifications of all participants on protocols involving surgical procedures. The PI assures and describes personnel qualifications.

Protocol Approval
A surgical protocol involving USDA-species undergoes an identical review process to any other IACUC protocol, with one exception: A University Laboratory Animal Resources (ULAR) veterinarian is specifically assigned to perform a secondary review of surgical protocols, in addition to the primary IACUC-designated review. This “vet review” emphasizes the appropriateness of the species used as the surgical model, the surgical procedure itself, anesthesia and analgesia regimen, and completeness of pre- and post-surgical care plan. “The veterinarian must provide guidance or oversight to surgery programs and oversight of postsurgical care” (Guide).

Surgical Location Approval
Approval of the IACUC protocol covers the “act” of the surgery. 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 procedures are planned to be performed in a non-ULAR operated facility, then the location must first be evaluated and approved by the Animal Welfare Compliance Staff. This evaluation occurs independently of the IACUC protocol review process and must be specifically scheduled by the Principal Investigator through contacting the Animal Welfare Compliance Staff at 215-746-6271.

Adequate Surgical Training
“It is important that persons have had appropriate training to ensure that good surgical technique is practiced, that is, asepsis, gentle tissue handling, minimal dissection of tissue, appropriate use of instruments, effective hemostasis, and correct use of suture materials and patterns” (Guide). Prior to IACUC approval of protocols and/or amendments to add personnel, the “Regulations and the IACUC” and “Species Specific Training” must be completed and documented for personnel listed. As additional surgical training opportunities become available (e.g. “Aseptic Technique”), they may be voluntary or 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, or other advanced techniques receive additional training if necessary.

No surgical procedures may be performed on an animal until the IACUC protocol and the surgical location have been approved. All personnel must be properly trained and listed on the IACUC protocol.

REQUIREMENTS OF A SURGICAL SUITE

“Major operative procedures on non-rodents will be conducted only in facilities intended for that purpose [dedicated facilities] which shall be operated and maintained under aseptic conditions” (AWR 2.31). “For most surgical programs, functional components of aseptic surgery include surgical support, animal preparation, surgeon’s scrub, operating room, and postoperative recovery. The areas that support those
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functions should be designed to minimize traffic flow and separate the related, nonsurgical activities from the surgical procedure in the operating room. The separation is best achieved by physical barriers but might also be achieved by distance between areas or by the timing of appropriate cleaning and disinfection between activities” (Guide).

Dedicated Facilities
“AWA regulations require that survival surgeries be performed using aseptic techniques and that major operative procedures on non-rodents be performed only in dedicated surgical facilities” (USDA Policy 3), i.e. operating rooms for USDA-species may only be used for operating on USDA-species. “The interior surfaces should be constructed of materials that are monolithic and impervious to moisture. Ventilation systems supplying filtered air at positive pressure can reduce the risk of postoperative infection. Careful location of air supply and exhaust ducts and appropriate room-ventilation rates are also recommended to minimize contamination. To facilitate cleaning, the operating rooms should have as little fixed equipment as possible” (Guide).

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 does not have to be immediately adjacent to the surgical suite, but there must be such an area available to the surgical laboratory.

Animal Preparation
There must be an area available where the animal will be physically prepared to undergo a surgical procedure. Induction of anesthesia and intubation will be performed in this area. In addition, removing hair/fur and the initial cleaning of gross debris from the surgical site should be performed here. To avoid contamination, this room should be separate from the surgeon’s scrub area and the operating room. Secondary procedures should be performed in this area, e.g. placing intravenous catheters, minor procedures (cut-down) and the initial monitoring/recording of vital signs. There must be adequate electrical outlets in the animal preparation area in order to power monitoring equipment, a heating source, and lighting as needed.

Surgeon’s Scrub
A specific area “equipped with foot, knee or electric eye surgical sinks” where the surgeon can scrub before performing a surgical procedure must be part of the surgical suite. To avoid contamination with aerosols released during scrubbing, this room should be separated from the operating room (Guide). Adequate space for gowning in sterile surgical garb in or near the surgeon’s scrub and the operating room will minimize risk of contamination.

Operating Room (OR)
Must be kept clean; items that are not easily disinfected (i.e. un laminated papers on the wall, rusty equipment, etc.) should not be in this room. Air pressure should be monitored to maintain positive pressure in the OR relative to the surrounding facilities in order to prevent airborne contaminants from entering the OR. During a procedure, all personnel in the OR should be wearing proper surgical attire (e.g. scrubs or disposable cover gown) and appropriate personal protective equipment (PPE)—at least shoe covers, mask, and head cap. Nonhuman primate users must also wear eye protection. The area should be fully sanitized between animals.

Post-Operative Recovery
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In facilities with limited space, this space may double as the animal prep area. It should be sanitized between animals and, like the animal preparation space, should be appropriately equipped with electrical outlets for warming devices and other equipment.

Minimize Contamination
The surgical suite must be maintained in such a manner as to reduce risk to the animal patient. Cleanliness is an essential and integral part of the surgical process. Standard operating procedures should be developed and used to ensure the surgical suite is routinely cleaned and sanitized. The appropriateness and efficacy of the cleaning procedures should be routinely assessed (e.g. Replicate Organism Detection and Counting (RODAC) monitoring). Refer to ULAR surgery and post-operative care standard operating procedures (SOPs) for guidance on how the OR and associated areas are maintained will be required prior to IACUC approval of any location for USDA-species surgery.

Surgery must be performed using aseptic technique in an approved dedicated surgery suite containing space for animal prep, surgeon’s scrub, the operating room, and post-operative recovery. This suite must be kept clean before, during, and after surgeries.

ASEPTIC TECHNIQUE

“Aseptic technique is used to reduce microbial contamination to the lowest possible practical level. No procedure, piece of equipment, or germicide alone can achieve that objective... Aseptic technique includes preparation of the patient... preparation of the surgeon... and sterilization of instruments” (Guide). “All survival surgery will be performed using aseptic procedures, including surgical gloves, masks, sterile instruments, and aseptic techniques” (AWR 2.31).

Patient
Preparing the animal for surgery is at least a two-step process:
1. In the Animal Preparation Area
   - Remove the hair (clip, depilatory cream) in a wide area around the intended surgical site. Clean the hair and any other gross debris from the skin with a surgical scrub (Betadine or Chlorhexidine) and alcohol.
2. In the Operating Room
   - The animal must be properly secured to the operating table e.g. tying with gauze, umbilical tape, or other suitable restraint.
   - Disinfect the area with appropriate surgical scrub. Alcohol is NOT an appropriate disinfectant. Iodophors (i.e. Betadine) or Chlorhexidines (i.e. Nolvasan) should be used.
   - Place and secure sterile drapes over the animal and the OR table.

Surgeon and Surgical Assistant(s)
All personnel taking part in the surgery must:
- Wear clean scrubs.
- Wear appropriate face mask and head covering.
- Wear close-toed shoes and 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 for approximately five minutes.
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- Wear a sterile gown and gloves.
- Move carefully to avoid contamination of the surgical location.
- If it is necessary to leave the surgical suite during a procedure, one must perform the above steps again before re-entering.

“It is important that persons have had appropriate training to ensure that good surgical technique is practiced, that is, asepsis, gentle tissue handling, minimal dissection of tissue, appropriate use of instruments, effective hemostasis, and correct use of suture materials and patterns” (Guide). It is the PI’s responsibility to assure that all his/her personnel are properly trained in order to perform the procedures described in the IACUC protocol. The Institution (Penn) is responsible for providing training. Arrangements for specialized training may be made with ULAR Training.

**Instruments**

It is extremely important to ensure that all instruments are appropriate for surgery:

- All instruments must be cleaned and sterilized prior to use on animals. Alcohol is NOT a sterilant. Examples of methods of sterilization include: steam autoclave, gas (e.g. ethylene oxide), and plasma sterilization. Surgical packs should be marked with sterilization indicators and the date of sterilization to confirm proper sterility. Packs are generally considered non-sterile one year after initial sterilization. Equipment used for sterilization (e.g. steam autoclaves) should be routinely monitored for efficacy.
- Cold sterilization of surgical instruments must strictly follow manufacturer instructions. The FDA lists specific cold sterilants and the necessary conditions to be considered a sterilant or a disinfectant.
- Ensure that the tools are appropriate for surgery. Do not use dull or rusted surgical instruments or those not manufactured for surgical use.
- If instruments are to be used for multiple surgeries on a single day they must be sterilized between animals.

_For any surgical procedure to be successful, steps must be taken to ensure that the animal is properly prepared for surgery, the surgeons are outfitted appropriately, and the surgical instruments are sterilized._

**ANESTHETICS**

**USDA Policy #11** defines a painful procedure as “…any procedure that would reasonably be expected to cause more than slight or momentary pain and/or distress in a human being to which that procedure is applied.” “Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals” (PHS).

Therefore, when writing a procedure, one must 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 injectable or inhalation anesthetics and a description of how and when analgesics will be administered.

**In-Date, Pharmaceutical Grade Compounds**
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**USDA Policy #3** states that “drugs administered to relieve pain or distress and emergency drugs must not be used beyond their expiration date...” and “...investigators are expected to use pharmaceutical-grade medications whenever they are available, even in acute procedures.” See IACUC Policy on the Use of Expired Drugs and Materials in Laboratory Animals for more details.

**Injectable 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 for suggested drugs, doses, and route of administration for your species and procedure.

**Inhalation Anesthetics**
Vaporizers must be used to deliver inhalant anesthetics to USDA-species. Anesthetics must be scavenged with appropriate devices or methods. Contact the ULAR Training Staff for assistance if personnel require training in using an anesthesia machine.

**Analgesics**
Surgery is considered a painful procedure; therefore administration of analgesics is required for any animal that undergoes a surgical procedure (Surgical Definitions and Biopsy Guidelines). Analgesics must be administered before an animal is expected to be painful, not after significant signs of pain worsen. The goal is to have every animal patient maintained post-surgically in a pain-free state. Animals given pre-operative analgesia often require less anesthetic to reach a surgical plane, and thus may be more stable anesthetic patients. Pre-emptive analgesia is expected with the exceptions of a specific scientific justification or clinical judgment of a ULAR veterinarian. The analgesic options available to investigators are very varied and certain drugs may provide better pain relief for patients undergoing certain procedures. Contact a ULAR Veterinarian for suggested drugs and doses that will best suit the surgical research model.

*Animals must be anesthetized during any surgical procedure and be provided appropriate analgesics to be maintained in a pain-free state.*

**MONITORING THE ANESTHETIZED PATIENT**

“Careful surgical monitoring and timely attention to problems increase the likelihood of a successful surgical outcome” (Guide). Careful surgical monitoring includes confirmation of anesthetic depth, maintenance of anesthesia, and monitoring of vital signs.

**Confirmation of Anesthesia Depth**
The animal must be maintained on a plane of anesthesia appropriate to the surgical intervention from immediately before a surgical procedure begins until the procedure is finished. This includes the time after post-operative analgesics have been administered before they are expected to be effective. For most species, the following techniques can be used to ascertain that the animal is appropriately anesthetized.

- **Toe pinch.** Brief clamping of the web of skin between toes or claws with a hemostat or fingers. Firmly pinching multiple toes should not elicit a withdrawal response from an animal at a surgical depth of anesthesia.
- **Palpebral reflex.** Gently tapping the medial canthus of the animal’s eye should not elicit a blink or eye flutter. This technique is not always reliable in all animals (e.g. swine).
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- Jaw tone. The animal’s jaw should remain slack when gently extending the mandible. If the jaw is “tight” and clenched, then the animal’s anesthesia depth may not be deep enough for surgery.
- Corneal reflex. Touching the edge of the cornea with a gauge sponge or cotton swab will produce a good reflex if the patient is too light on anesthesia. Movement of the eyelids is an indication that the depth of anesthesia is not sufficient to do surgery.

Maintenance of Anesthesia
Each animal responds slightly 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 IACUC protocol as well as a plan of how and by whom anesthetic emergencies will be addressed.** Anesthetists must be highly skilled in not only delivering the anesthetic to the patient, but also in identifying anesthetic related problems.

- An animal that experiences a sudden increase in respiratory rate and effort or heart rate might be too light on anesthesia and may require supplemental dosing or an increase of the anesthetic being administered.
- If the animal’s respiratory rate and effort or heart rate greatly decreases, the animal could be too deep, thereby requiring a decreased anesthetic dose.
- A drop in arterial oxygen saturation (SaO2) may indicate an easily corrected improperly placed endotracheal tube or potentially life-threatening respiratory problem (e.g. pulmonary edema).
- 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 should NEVER be left alone.**

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 following vital signs are required to be monitored with written documentation every 5-10 minutes (see **ULAR Anesthesia Sheet** for a suggested template):

- **Respiratory Rate** (RR) can be assessed by watching the rising and falling of the chest, by monitoring end-tidal CO2, or by artificial ventilation.
- **Heart rate** (HR) may be monitored manually (stethoscope) or with ECG or Doppler.
- **Body temperature** should be measured using a digital thermometer or rectal probe
  - Hypothermia often occurs due to anesthesia-induced vasodilation and from surgery via opened body cavities.
  - During any surgical procedure, the animal’s body temperature should be maintained by a heat lamp, a [covered] recirculating water heating pad, forced-air warming (Bair-Hugger) or by covering the animal with warm drapes/towels.
  - **ULAR Veterinarians** may assist in choosing an appropriate method for your species.

For best practices, 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 also be monitored when appropriate for the animal model and procedure.

**Vital signs for common USDA-covered laboratory animal species.**
IACUC Guideline
USDA-SPECIES SURGERY

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>RESPIRATORY RATE (breaths/minute)</th>
<th>HEART RATE (beats/minute)</th>
<th>TEMPERATURE (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit</td>
<td>32-60</td>
<td>200-300</td>
<td>38.5-39.5</td>
</tr>
<tr>
<td>Laboratory Animal Medicine, 2002</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferret</td>
<td>33-36</td>
<td>200-400</td>
<td>37.8-40.0</td>
</tr>
<tr>
<td>Laboratory Animal Medicine, 2002</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>20-40</td>
<td>70-80</td>
<td>37.5-39.2</td>
</tr>
<tr>
<td>Laboratory Animal Medicine, 2002</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cat</td>
<td>20-40</td>
<td>110-140</td>
<td>38.0-39.5</td>
</tr>
<tr>
<td>(Martin, 1997)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pig</td>
<td>10-20</td>
<td>70-100</td>
<td>39.0</td>
</tr>
<tr>
<td>Sheep</td>
<td>30-70 (young)</td>
<td>120-160 (young)</td>
<td>39.0-40.5</td>
</tr>
<tr>
<td>Laboratory Animal Medicine, 2002</td>
<td>12-72 (adult)</td>
<td>60-120 (adult)</td>
<td></td>
</tr>
<tr>
<td>Macaque</td>
<td>32-50</td>
<td>120-180</td>
<td>37.0-39.1</td>
</tr>
</tbody>
</table>

A well-trained anesthetist with experience with the species under investigation must continuously monitor the animal’s vital signs and maintain the surgical patient at an appropriate plane of anesthesia throughout the surgical procedure.

ANESTHETIC AND POSTOPERATIVE RECOVERY

“The investigator and veterinarian share responsibility for ensuring that postsurgical care is appropriate. An important component of postsurgical care is observation of the animal and intervention as required during recovery from anesthesia and surgery. The intensity of monitoring necessary will vary with the species and the procedure and might be greater during the immediate anesthetic recovery period than later in postoperative recovery” (Guide).

Anesthetic Recovery
“Particular attention should be given to thermoregulation, cardiovascular and respiratory function, and postoperative pain or discomfort during recovery from anesthesia” (Guide). The anesthetic recovery period may last from minutes to hours.

- Animals should be placed into a clean recovery area in sternal or lateral recumbency. Sternal recumbency is preferred.
- Emergency drugs and equipment must be available in the recovery area.
- During anesthetic recovery, the animal’s body temperature should be supported with an approved, 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.
- As during the procedure, temperature, respiratory rate and heart rate should be monitored, evaluated, and documented during the recovery period.
- Recovering animals should not be in the enclosure with awake animals.
- Animals in the recovery area should be continuously monitored by at least one staff member. Only when animals have regained all postural reflexes and are ambulatory (can walk well on their
own) should be left alone in their regular, freshly-cleaned housing. Animals should NOT be left unattended until they have completely recovered from anesthesia.

Postoperative Recovery
“After anesthetic recovery, monitoring is often less intense but should include attention to basic biologic functions of intake and elimination and behavioral signs of postoperative pain” (Guide). Depending on the surgical procedure, the postoperative recovery period may last from days to weeks.

- 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 cardinal signs of infection including heat, swelling, redness, pain, and/or exudation. Consult a ULAR Veterinarian for any abnormal medical condition.
- 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 daily for a minimum of seven days following major procedures.
- If the health of the animal is questionable, a ULAR veterinarian should be notified for treatment recommendations, or to help assist in determining clinical endpoints for the animal (i.e. euthanasia).
- “The attending veterinarian [or designee] retains the authority to change post-operative care as necessary to ensure the comfort of the animal” (USDA Policy #3). The IACUC supports the professional judgment of ULAR veterinarians. The Attending Veterinarian has direct or delegated program authority and responsibility for activities involving all animals at the institution as defined under the Animal Welfare Act and PHS policy. At Penn, the Attending Veterinarian is the Director of ULAR.
- The IACUC requires that peri-/preoperative and postoperative analgesics are used for all major invasive procedures, and for procedures that have the potential for causing pain in laboratory animals (USDA Policy 11). Consult a ULAR Veterinarian for recommended analgesic agents.

After surgery, animals must be monitored and given appropriate veterinary treatment during anesthetic and postoperative recovery.

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 20 - Record Keeping for USDA Covered Species for details on how to properly maintain operative/perioperative records.

REFERENCES
IACUC Guideline
USDA-SPECIES SURGERY


http://www.nap.edu/readingroom/books/labrats/chaps.html


