

SURGICAL PROCEDURES

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Background

In accordance with the *Guide for the Care and Use of Laboratory Animals (Guide)* and the US Department of Agriculture (USDA) Animal Welfare Act and Regulations (AWA/AWR), all survival surgical procedures on vertebrate animals must be performed using aseptic procedures (e.g., use of sterile instruments) to prevent postoperative infections.

All surgeries and associated details (e.g., anesthesia, pre- and post-operative care, and recordkeeping) must be described in the Animal Use Protocol (AUP) and approved by the Animal Care and Use Committee (ACUC).

These guidelines outline the general training and techniques required to perform surgeries at UC Berkeley (UCB).

Classification of Surgical Procedures

Surgical procedures are classified as major or minor; and, survival or non-survival (terminal).

- Minor surgery does not expose a body cavity and causes little or no physical impairment. Examples include suturing superficial wounds, superficial cut-down for vascular cannulation, or subcutaneous implants.
- Major surgery penetrates and exposes a body cavity (e.g., calvarium, abdomen, thorax) or includes procedures with the potential for significant impairment (e.g., orthopedic surgeries, stroke models, brain cannulation).
- Survival surgery is one from which animals are expected to recover
- Non-survival or terminal surgeries are procedures where the animals are euthanized prior to anesthetic recovery as described in the AUP. This includes terminal vascular perfusion procedures unless euthanasia is performed prior to starting perfusion.

Surgery Suites and Designated Surgical Areas

Asepsis and/or sterility are required for all survival surgeries and those non-survival surgeries that exceed 6 hours in duration.

Facilities used for survival surgery or non-survival surgery lasting longer than 6 hours must be designed and maintained in such a way that they help prevent the development of intra- and post-procedural infections. Design features include:

- Separation of the preparation and surgery areas
- Location that minimizes personnel traffic through the surgery area
- Room surfaces should be non-porous and easily sanitized
- Rooms are usually positively pressured relative to surrounding areas
- Surgery rooms should be cleaned and disinfected on regular schedules, daily after surgery for work surfaces, weekly to monthly cleaning for floors, walls and cabinets
- The surgery area should be free of all equipment and materials not necessary for the procedure. Any stored items should be in cabinets or drawers.

Multiple Survival Surgeries

The use of multiple survival surgeries, either major or minor, must be described and scientifically justified in the PI's AUP and approved by the ACUC. Surgery details (anesthesia, pre-, intra- and post-operative monitoring, etc.), the amount of recovery time between surgeries, the number and type of survival surgeries that a single animal may go through, and a reason for why the surgeries cannot be accomplished during one anesthetic episode must be included in the description. Animals transferred between research protocols that have undergone major survival surgery cannot go through an additional major survival surgery unless an exception is granted by the ACUC.

Training

All personnel who perform anesthesia and surgery must be appropriately trained. The Principal Investigator (PI) is responsible for assuring that research personnel receive appropriate training and certification prior to performing any procedure. New surgeons are trained and supervised by the PI, or appropriate designated personnel, until they are competent to perform the procedure independently. All new surgeons, including PIs, must be observed by OLAC veterinary staff and their competency certified to the ACUC prior to working independently. The Office of Laboratory Animal Care (OLAC) veterinary and training staff are available to provide assistance with or training in aseptic technique, surgery and the proper administration of anesthesia, analgesia, and euthanasia. Please contact OLAC at 510-642-9232 for more information.

Survival Surgery and Monitoring

a) Patient Preparation

- i. While it is common in larger species to withhold food prior to surgery to prevent the possibility of aspiration pneumonia after regurgitation, this practice is not necessary in rodents or rabbits. Water should never be withheld.
- ii. All animals must be weighed prior to surgery. Pre-surgical weights must be recorded on the blue postsurgical cage card and/or full surgical record as applicable.
- iii. In most cases, sterile ophthalmic ointment should be applied to each eye to prevent corneal damage.
- iv. Hair or feathers must be removed (via shaving, depilatory agent, plucking, etc.) from the surgical site. This step should be performed in a location separate from where the surgery is to be conducted (i.e., a separate lab bench).

- v. The skin is scrubbed with surgical disinfectants, such as dilute chlorhexidine or betadine scrub. Scrubbing starts at the center of the surgical site and radiates outward with disinfectants subsequently wiped off with alcohol or sterile water. Repeat at least three times or until the site is free of visible debris.

Note:

- a. Surgical scrubbing of the skin is recommended for scaled vertebrates but not for fish and amphibians, as it removes the protective slime layer. A sterile saline rinse of the incision site is appropriate for fish and amphibians.
 - b. Alcohol by itself is not an appropriate skin disinfectant for surgery.
 - c. Do not overly wet the animal as rodents are especially subject to hypothermia.
- vi. Sterile surgical drapes are appropriate for most surgeries. Drapes should be secured so as to remain in place over the animal yet allow for monitoring of the anesthetic depth of the animal. Sterile paper, cloth, clear plastic adhesive drapes or, for smaller species, the product Press'n Seal may be used.
- vii. Ancillary heat should be provided for most species undergoing procedures lasting longer than 5 minutes.
- Accepted heat sources: circulating warm water or warm air blankets, infrared warming pads, chemical warming pads, thermal gel packs.
 - If an electric heating pad is used, it must have an attached thermostat that specifies the temperature in Fahrenheit or Celsius. Heating pads with settings that only indicate temperature levels, such as high, medium, and low, are not acceptable.
 - Electric heating pads must never be folded, which can create cracks and hot spots within the heating pad.
 - A barrier (paper towel, towel, etc.) must be in place between the animal and the heating pad.
 - Anesthetized animals must be completely dry before being placed on a heat source to prevent formation of steam and potential burns.

b) Surgeon Preparation

For non-rodent mammal major survival surgeries, hands are thoroughly scrubbed with an antiseptic surgical soap and sterile surgical gloves are to be worn for all survival surgeries.

- i. For all rodent surgeries and all non-rodent mammal minor survival surgeries, sterile gloves are required if you will be touching the animal or the tips of your sterile instruments with your hands. If you will only be touching the handles of

your instruments, then clean, non-sterile gloves (i.e. examination gloves) are acceptable.

- ii. Sterile gowns must be worn for major surgeries on non-rodent mammal species and are recommended for all surgical procedures. If non-sterile garments are worn for rodent or ectotherm surgery, they must be clean; garment sleeves must not come into contact with sterile surfaces.
- iii. A head cover, face mask, and shoe covers are worn for non-rodent mammal major survival surgeries, and face shields are required for surgery involving nonhuman primates. A head cover and surgical mask must be worn for survival surgery on rodents and ectotherms. A surgical mask is recommended for non-survival surgeries to protect the sterile field from accidental contamination.
- iv. Minimizing traffic flow and conversation in the operating room significantly reduces the risk of contamination.

c) Instrument Preparation

- i. Surgical instruments must be sterilized prior to use in animals using heat (e.g., autoclave or bead sterilizer), gas (i.e., ethylene oxide, hydrogen peroxide), or chemical methods (limited to 2% glutaraldehyde; e.g., Cidex, according to manufacturer's recommendations for use as a sterilant) and rinsed free of chemical using sterile water or saline. For guidance on autoclave maintenance and validation, see [OLAC's Autoclave Validation Testing SOP](#).
- ii. Sterile instruments must be used for each non-rodent mammal. When performing survival surgeries on multiple rodents or ectothermic animals within a related group, surgical instruments may be reused if they are sterilized between animals using a glass bead sterilizer (20 seconds at a minimum of 240-270°C) or ACUC-approved alternative sterilization method. Make sure instruments are cool to the touch prior to using on the animal. Hot instruments are extremely damaging to tissue.
- iii. Gloves must be changed between non-rodent mammals. It is acceptable to use the same gloves between surgeries on rodents or ectotherms in a related group if non-sterile surfaces are not touched. However, gloves should be changed if soiled.
- iv. For rodent, avian, and ectotherm surgeries, using clean exam gloves and a "tips-only" technique restricts you to using only the sterile working ends of the surgical instruments to manipulate the surgical field. The gloved, but not sterile, hand must never touch the working end of the instruments, the suture, suture needle, or any part of the surgical field. This technique is useful when working alone and manipulation of non-sterile objects (e.g., anesthesia machines, microscopes, lighting) is required.

d) Surgical Technique

- i. Surgical technique is a major influence on the surgical outcome, including postoperative infection and pain. Proper technique can minimize postoperative complications.
- ii. Be aware of instrument and hand position at all times. If an instrument or hand touches something outside of the sterile field the instrument or glove must be replaced immediately.
- iii. Be gentle when handling tissues. Hold the cut edge rather than grasping in the middle of a tissue layer. Use electrocautery or electroscalpels sparingly as they cause significant tissue damage.
- iv. Close incisions using appropriate techniques and materials. Any suture buried in tissues should be absorbable. Sutures should be placed evenly and as close to the tissue edge as possible to prevent obstruction of blood flow. Sutures should only be tightened enough to oppose the tissue edges, but not so tight as to obstruct blood supply. If using wound clips, these should be evenly spaced and incorporate enough tissue to prevent inadvertent failure of the clips. Wound clips must be removed 10-14 days after placement.
- v. Cyanoacrylate tissue adhesives can be used in any species to help approximate skin edges. These materials hold tissues in approximation to encourage healing; however, cells cannot penetrate the adhesive material. It is best used as a sealant on top of sutures. Medical grade cyanoacrylate is biologically inert and causes minimal tissue reaction. Commercial grades (e.g., SuperGlue) contain substances that are toxic to tissues and are not permitted.

e) Species-Specific Considerations

- i. In amphibians, epidermal suture tension can cause dehiscence. An everting pattern or simple interrupted pattern should be used.
- ii. In scaled vertebrate animals, it is imperative that the epidermis be closed in an everting suture pattern (mattress patterns) to facilitate approximation of the vascular component of the skin.
- iii. In avian species, the technique of suture placement is very important as most avian tissue has low tensile strength and tears easily. Sutures used should be the smallest effective size and placed gently with a minimum amount of intrinsic tension.

f) Intraoperative Monitoring

- i. The animal must be monitored closely during the surgical procedure.

- ii. The surgical team should know the correct initial responses to the most common emergencies associated with the type of procedure they are performing.
- iii. A surgical record must be kept for each surgical patient. The record should document monitoring of appropriate parameters (e.g. body temperature, respiratory pattern, heart rate), drugs administered, dosages, routes of administration and all complications that arise from the procedure as applicable. Please refer to ACUC Guidelines on [Recordkeeping for Surgical Procedures on Laboratory Animals](#) for additional information.

g) Immediate Post-operative Care

- i. Animals in the immediate postsurgical period must be closely monitored until they demonstrate basic postural responses. During this time, analgesics are administered as required by the ACUC approved protocol and appropriate medical records completed.
- ii. Hypothermia should be prevented by placing the animals in a warm room or a cage warmed with a supplemental heat source. Ideally, rodents should be placed in an empty cage or one lined with paper towels to prevent accidental inhalation of bedding substrate. Half of the cage should be placed on a supplemental heat source to provide the animal with the opportunity to regulate its body temperature and avoid hyperthermia.
- iii. Supplemental subcutaneous or intravenous fluid therapy during surgery and recovery may be indicated to prevent dehydration.
- iv. Recovering non-aquatic animals should be rotated from side to side every 15-30 minutes to minimize congestion of the lungs. Aquatic frogs should be kept in shallow water or wrapped in wet paper towels until recovered to prevent drowning and desiccation.
- v. To prevent cannibalism or suffocation, animals should be monitored continuously or housed individually until they are fully ambulatory.
- vi. Cages containing animals recovering from anesthesia should be free of any live food sources (e.g., crickets for ectotherms).
- vii. For surgeries which may inhibit an animal from reaching food and water in the post-operative period (e.g., thoracotomy, laparotomy, limb paralysis), it is strongly recommended that palatable food (e.g., wet chow, Nutrical gel) be placed on the floor of the cage until the animal has fully recovered. Small trays are available from OLAC for this purpose.

h) Subsequent Post-operative Care

- i. Post-surgical animals must be assessed at least daily by laboratory or OLAC staff as appropriate until the animal has fully recovered from the surgery (usually 5-7 days).
- ii. Post-operative analgesic administration must comply with the ACUC-approved AUP and any additional veterinary orders.
- iii. Veterinarians must be contacted if the animals lose 10% of their pre-surgical weight. Animals must be euthanized if weight loss exceeds 15% unless otherwise approved in the AUP or authorized by an OLAC veterinarian based on clinical presentation (e.g., the etiology of the weight loss is clinically transient and immediately treatable). The weights must be recorded on the blue postsurgical cage card and/or full surgical record as applicable.
- iv. Non-absorbable skin sutures or wound clips must generally be removed:
 - a. 10-14 days after surgery in mammals and birds
 - b. 2-4 weeks after surgery in amphibians and fish
 - c. 4-6 weeks after surgery in reptiles
- v. In the event of post-operative infections or complications, the OLAC veterinary staff must be notified. The veterinary staff will assist the research team to develop a treatment plan and, if necessary, to review the entire surgical procedure (facility, preparation of the animal and instruments, expertise of the surgeon, and postsurgical care).
- vi. Animals with chronic implants must be monitored at least 3 times a week for incision health and weighed in accordance with the animal use protocol.

Concurrent Survival Surgeries and Monitoring

a) Surgeon Preparation

All lab members performing surgery simultaneously must be familiar with and adhere to the following guidelines:

i. [Guidelines for Anesthesia and Analgesia](#)

ii. [Recordkeeping](#)

b) Instrument Preparation

A sterile pack of instruments is required for each animal scheduled for surgical sessions occurring simultaneously.

c) Surgical Space

Spacing must be adequate between surgical and patient preparation areas, allowing surgeons to move about in designated areas as necessary without posing a risk for potential safety and/or occupational health hazards (i.e., contamination of surgical sites, accidental needlesticks, scalpel blades, etc.). For more information on safety in the workplace please visit: <https://ehs.berkeley.edu/>.

d) Scheduling of Surgeries and Patient Monitoring

Scheduling and planning of concurrent surgeries must be done such that surgeons will be able to manage the surgical session involving multiple animals with ease.

This includes:

- i. pre-surgical preparation
- ii. anesthesia monitoring and documentation (adequate personnel must always be available to ensure all animals are monitored as outlined in the surgical procedures within the approved AUP)
- iii. post-op recovery

Non-Survival Surgery and Monitoring

Prolonged (> 6 hours) Non-survival Surgery:

a) Patient Preparation

- i. While it is common in larger species to withhold food prior to surgery to prevent the possibility of aspiration pneumonia after regurgitation, this practice is not necessary in rodents or rabbits. Water should never be withheld.
- ii. All animals must be weighed prior to surgery. Pre-surgical weights must be recorded on the full surgical record as applicable.
- iii. In most cases, sterile ophthalmic ointment should be applied to each eye to prevent corneal damage.
- iv. If a non-survival surgery goes more than 6 hours, antibiotics may be indicated even though the animal will not be revived. Consult with an OLAC Veterinarian.
- v. Hair or feathers must be removed (via shaving, depilatory agent, etc.) from the surgical site. This step should be performed with the patient in a location separate from where the surgery is to be conducted (i.e., a separate lab bench).
- vi. The skin is scrubbed with surgical disinfectants, such as dilute chlorhexidine or betadine scrub. Scrubbing starts at the center of the surgical site and radiates

outward with disinfectants subsequently wiped off with alcohol or sterile water. Repeat at least three times or until the site is free of visible debris. Note:

- a) Surgical scrubbing of the skin is recommended for scaled vertebrates but not for fish and amphibians, as it removes the protective slime layer. A sterile saline rinse of the incision site is appropriate for fish and amphibians.
 - b) Alcohol by itself is not an appropriate skin disinfectant for surgery.
 - c) Do not overly wet the animal as rodents are especially subject to hypothermia.
- vii. Sterile surgical drapes are appropriate for most surgeries. Drapes should be secured so as to remain in place over the animal, yet allow for monitoring of the anesthetic depth of the animal. Sterile paper, cloth, clear plastic adhesive drapes or, for smaller species, Press 'n Seal may be used.
- viii. Ancillary heat should be provided is preferable for most species undergoing procedures lasting longer than 5 minutes.
- Accepted heat sources: circulating warm water or warm air blankets, infrared warming pads, chemical warming pads, thermal gel packs.
 - If an electric heating pad is used, it must have an attached thermostat that specifies the temperature in Fahrenheit or Celsius. Heating pads with settings that only indicate temperature levels, such as high, medium, and low, are not acceptable.
 - Electric heating pads must never be folded or rolled during use or storage. Both actions can create cracks and hot spots within the heating pad.
 - A barrier (paper towel, towel, etc.) must be in place between the animal and the heating pad.
 - Anesthetized animals must be completely dry before being placed on a heat source to prevent formation of steam and potential burns.

b) Surgeon Preparation

- i. For non-rodent mammal major survival surgeries, hands are thoroughly scrubbed with an antiseptic surgical soap and sterile surgical gloves are to be worn for all survival surgeries.
- ii. For all rodent surgeries and all non-rodent mammal minor survival surgeries, sterile gloves are required if you will be touching the animal or the tips of your sterile instruments with your hands. If you will only be touching the handles of your instruments, then clean, non-sterile gloves (i.e., examination gloves) are acceptable.

- iii. Sterile gowns must be worn for major surgeries on non-rodent mammal species and are recommended for all surgical procedures. If non-sterile garments are worn for rodent or ectotherm surgery, they must be clean; garment sleeves must not come into contact with sterile surfaces.
- iv. A head cover, face mask, and shoe covers are worn for non-rodent mammal major survival surgeries, and face shields are required for surgery involving nonhuman primates. Exceptions for using a face shield during nonhuman primate surgeries (e.g., when performing microscopy) must be approved on the lab's BUA. A head cover is recommended and a surgical mask must be worn for survival surgery on rodents and ectotherms. A surgical mask is recommended for non-survival surgeries to protect the sterile field from accidental contamination and personnel from allergens.
- v. Minimizing traffic flow and conversation in the operating room significantly reduces the risk of contamination.

c) Instrument Preparation

- i. Surgical instruments must be sterilized prior to use in animals using heat (e.g. autoclave or bead sterilizer), gas (i.e. ethylene oxide), or chemical methods (limited to 2% glutaraldehyde; e.g., Cidex, according to manufacturer's recommendations for use as a sterilant) and rinsed free of chemical using sterile water or saline. For guidance on autoclave maintenance and validation, see [OLAC's Autoclave Validation Testing SOP](#).
- ii. Sterile instruments must be used for each non-rodent mammal. When performing survival surgeries on multiple rodents or ectothermic animals within a related group, surgical instruments may be reused if they are sterilized between animals using a glass bead sterilizer (20 seconds at a minimum of 240-270°C) or ACUC-approved alternative sterilization method. Make sure instruments are cool to the touch prior to using on the animal. Hot instruments are extremely damaging to tissue.
- iii. Gloves must be changed between non-rodent mammals. It is acceptable to use the same gloves between surgeries on rodents or ectotherms in a related group if non-sterile surfaces are not touched. However, gloves should be changed if soiled.
- iv. For rodent, avian, and ectotherm surgeries, using clean exam gloves and a "tips-only" technique restricts you to using only the sterile working ends of the surgical instruments to manipulate the surgical field. The gloved, but not sterile, hand must never touch the working end of the instruments, the suture, suture needle, or any part of the surgical field. This technique is useful when working alone and

manipulation of non-sterile objects (e.g., anesthesia machines, microscopes, lighting) is required.

Non-survival Surgery (< 6 hours)

Non-survival surgeries not performed aseptically or in a dedicated facility must at least be performed in a clean area that is free of clutter. Personnel present in the area must observe reasonable cleanliness practices for both themselves and the animals.

No expired drugs or fluids are allowed. Pharmaceutical-grade agents (USP) must be used unless an exemption is approved by the ACUC.

a) Surgeon Preparation

Clean gloves and a clean lab coat or disposable gown are required.

A surgical mask is required to protect the surgical field from accidental contamination.

b) Surgical Technique

- i. Surgical technique is a major influence on the surgical outcome, including postoperative infection and pain. Proper technique can minimize postoperative complications.
- ii. Surgical instruments must be clean and free of debris
- iii. Be aware of instrument and hand position at all times. If an instrument or hand touches something outside of the sterile field the instrument or glove must be replaced immediately.
- iv. Be gentle when handling tissues. Hold the cut edge rather than grasping in the middle of a tissue layer. Use electrocautery or electroscalpels sparingly as they cause significant tissue damage.
- v. Use appropriate suture techniques. Sutures should be placed evenly and as close to the tissue edge as possible without compromising suture integrity to prevent obstruction of blood flow. Sutures should only be tightened enough to oppose the tissue edges, but not so tight as to obstruct blood supply.
- vi. Cyanoacrylate tissue adhesives can be used in any species to help approximate skin edges. These materials hold tissues in approximation to encourage healing; however, cells cannot penetrate the adhesive material. It is best used as a sealant on top of sutures. Medical grade cyanoacrylate is biologically inert and causes minimal tissue reaction. Commercial grades (e.g., SuperGlue) contain substances that are toxic to tissues and are not permitted.

c) Intraoperative Monitoring

- i. The animal must be monitored carefully during the surgical procedure.
- ii. The surgical team should know the correct initial responses to the most common emergencies associated with the type of procedure they are performing.
- iii. A surgical record must be kept for each surgical patient. The record should document monitoring of body temperature, respiratory pattern, heart rate, drugs administered, dosages, routes of administration and all complications that arise from the procedure as applicable. Please refer to ACUC Guidelines on [Recordkeeping for Surgical Procedures on Laboratory Animals](#) for additional information.

Conducting Surgery Under a Pandemic Event

As applicable, [campus health and safety guidelines](#) must be followed.

References

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Call 3-VETS if there is an animal emergency

(510-643-8387)