



BROWN

Institutional Animal Care and Use Committee (IACUC): Standard Operating Procedure (SOP) on Surgery in Non- USDA-Regulated Species

1.0 SOP Purpose

The purpose of this SOP is to provide guidance to personnel conducting survival surgical procedures on species not covered by the USDA Animal Welfare Act.

2.0 Survival Surgery

All survival surgical procedures performed on non-USDA-covered species must follow the general principles of asepsis. Aseptic technique is used to reduce microbial contamination to the lowest practical level; includes preparation of the patient, surgeon, and instruments; and requires adherence to certain practices throughout the procedure.

2.1 Surgical Space

Surgery on animal species not covered by the USDA does not have to be performed in a dedicated surgical suite. A procedure room within the animal facility or a dedicated space within the lab may be used. If the space is within the lab, it must be done in an area dedicated to surgery.

The space should be chosen based on space, ease of sanitization, and traffic flow. The space should be large enough to designate separate areas for animal preparation, surgery, and recovery. The area should also be uncluttered and clean. It should have hard, impermeable surfaces that may be easily sanitized before and after surgery. Because the main source of intraoperative infection is airborne bacteria, the area should have minimal traffic to decrease the risk of infection. This surgical space must be described in the IACUC protocol and requires IACUC approval and semi-annual IACUC inspections.

Remove any extraneous materials from the surgical space and clean the area with a disinfectant. Prior to surgery and collecting the animals, gather and aseptically lay out all of the necessary supplies and equipment, including sterilized instruments/implants, drapes, suture material, anesthetics, and analgesics. Set up the surgical location with a warming device and proper lighting.

2.2 Preparation of the Animal

Animal preparation must occur in an area separate from the operating space and after the animal is sedated/anesthetized. Preparation must include the following:

- Placing ophthalmic ointment in both eyes to prevent drying of the cornea during surgery.
- Removal of hair/feathers from the surgical site using clippers or depilatory cream. Feathers may be manually removed. The shaved/plucked area should be wide enough to avoid contamination from the surrounding skin and hair/feathers during surgery but should be the minimum compatible with achieving an appropriate sterile field. Removing more hair/feathers than is necessary predisposes the animal to hypothermia during the surgical procedure.

- Hair/feathers that have been removed, along with hair removal cream if used, should be cleaned away using water and/or alcohol. If using a depilatory cream, it is important to be sure to thoroughly remove all of it prior to surgery to avoid chemical burns.

Following completion of the above procedures, the animal should then be moved to the surgical field. Once the animal has been moved, preparation of the animal is continued.

- The skin must be disinfected using a concentric surgical scrub pattern moving from the innermost to outermost portions of the shaved area. Scrub the area no less than three alternating scrubs with antiseptic followed by alcohol or water. Dilute chlorhexidine or povidone-iodine may be used as an antiseptic.
- Drape the animal so that the sterile surgical site is the only part of the animal not covered with a sterile covering. Sterile paper drapes, reusable fabric drapes, or Glad Press 'N' Seal® may be used.

2.3 Preparation of the Surgeon and Surgical Assistant(s)

Surgeons and surgical assistants who will have contact with the sterile surgical field during the surgery must prepare in the following manner:

- Surgeons must don appropriate personal protective equipment (PPE) for the species and Animal Biosafety Level (ABSL). The minimum PPE includes clean lab coat or disposable gown, an appropriate face mask, a hair bonnet, closed-toe shoes, and shoe covers. The face mask and hair bonnet must be donned prior to surgical gloves.
- The surgeon should thoroughly wash their hands and sterile surgical gloves of the correct size are then donned using the appropriate technique so as to not contaminate the outside of the gloves.
- Once the surgeon has donned sterile gloves, care must be taken to avoid touching nonsterile items such as the table, anesthetic equipment, lights, or scopes. Sterile gauze or autoclaved squares of tin foil can be placed over items that may need to be adjusted during the surgery (i.e. lights, microscopes, stereotaxic equipment).
 - If a non-sterile item/surface is accidentally touched, the surgeon must change their contaminated gloves for a new sterile pair.

Non-surgeons that will not have contact with the sterile field must wear appropriate PPE, including a disposable cover gown, a mask, a hair bonnet, shoe covers, and gloves. Surgical assistants that have not scrubbed in must not touch sterile instruments, drapes, or consumables.

2.4 Preparation and Handling of Surgical Instruments, Equipment, or Implanted Material, and Consumables

All surgical instruments must be cleaned and autoclaved prior to use on animals for all survival surgical procedures.

- Autoclaved surgical packs should contain a sterilization indicator. The date of sterilization and the expiration date should also be listed on the pack.
- Prior to surgery, the indicator and expiration must be checked. The outer packaging should also be inspected to ensure it has not been breached, which would compromise sterility.

Equipment or implants that will come into contact with the sterile field that are unable to withstand the conditions of autoclaving must be sterilized in another way.

- Ethylene oxide sterilization is available through the Center for Animal Resources and Education (CARE) for a fee. It is important to plan ahead for the time it takes for processing and subsequent off-gassing.
- Cold sterilization should only be used as a last resort when other methods are not appropriate. A specific cold sterilant must be used and the manufacturer's instructions must be strictly followed. The instruments must be rinsed with sterile water or sterile saline before putting them in contact with animals.

If surgeries will be completed on multiple animals in the same session, a hot bead sterilizer can be used to sterilize the instruments between animals. It is important to use the hot bead sterilizer correctly to ensure sterilization.

- It must be turned on and given sufficient time to heat up to a working temperature.
- All blood and tissue must be cleaned from the instruments. Volatile compounds (i.e. alcohol) should not be used to clean the instruments.
- The tips of the instruments should be placed at least 1.5 inches into the beads and left in place for at least 15 seconds.
- Only 1-2 instruments should be sterilized at a time.
- It is critical that the instruments are allowed to cool off before coming into contact with an animal to avoid severe, life-threatening burns.

All consumables that will be used during the procedure (e.g. gauze, swabs, needles, suture materials) must be sterile and should be of an appropriate size and packaged in suitable quantities.

Instruments should be placed on a sterile surface (e.g. sterile drape) when not being used to avoid accidental contamination of the surgical site.

- Where it is suspected that instruments may have been accidentally contaminated, these must be replaced with sterile ones before continuing.

2.5 Intraoperative Monitoring

Animals must be monitored throughout the duration of the surgery to ensure an adequate plane of anesthesia. This must be done and documented every 15 minutes.

- Before beginning surgery, a firm toe pinch using either two fingers or atraumatic forceps must be performed. Any reaction is an indication that the animal is not at a surgical plane of anesthesia and requires additional anesthesia prior to surgery.
 - A toe pinch needs to be performed at least every 15 minutes to ensure that the animal is being maintained at the appropriate anesthetic plane.
- It is the responsibility of the surgeon to monitor respirations. A sudden noticeable increase in respiration rate may indicate that the animal is not deep enough and requires additional anesthesia, while a sudden noticeable decrease in respiratory rate may indicate that an animal is too deeply anesthetized.
- Observation of the animal's color is another indication of anesthetic depth. The animal's ears, muzzle, and mucous membranes should be pink. If they are cyanotic (blue), there is poor oxygenation and this may indicate a respiratory issue. If they are pale, this indicates poor perfusion or blood loss and may indicate a heart-related issue.

2.6 Recovery and Post-Operative Care

Surgical wounds should be closed using appropriate techniques and materials. Proper wound closure is essential to avoid wound dehiscence.

Move the animal to a warm, clean cage. The animal's face should be placed on a paper towel, or something similar, to avoid the nose becoming buried in the bedding, as this can lead to asphyxiation. The cage should be placed half on and half off of a warming device.

- Avoid placing recovering rodents in a cage with awake animals to avoid cannibalistic behavior and injury to the anesthetized animal.

The animal must be continuously monitored until fully recovered from anesthesia. Recovery is indicated by normal ambulation and behaviors. Only when an animal is fully recovered can it be returned to its normal housing location. Complete and place a Surgical Care card on the cage. These cards should be available in all rodent rooms.

Analgesics and post-operative monitoring must be provided as stated in the approved IACUC protocol.

- For survival surgeries, typical post-operative monitoring is twice a day (AM and PM) for at least three days following surgery.
- Skin sutures and wound clips are typically removed 10-14 days post-surgery once the incision has completely healed.

2.7 Recordkeeping

Records documenting any survival surgery and post-operative monitoring must be retained by the lab and be readily available for review by the IACUC, the veterinary staff, and representatives of regulatory and accrediting organizations. All non-USDA-regulated animal anesthesia and surgery records must be retained for a minimum of one (1) year.

2.7.1 Surgical Recordkeeping

The following information must be included in the surgical records.

- Principal investigator name and protocol number
- Title or brief description of procedure performed
- Species, identification, and the total number of animals
- Name of the surgeon
- Date of the surgery
- Name and dose/dosage of all agents administered before, during, and after anesthesia and/or surgery, including anesthetics, analgesics, therapeutics, and any experimental agents delivered
- Monitoring of vital signs
- Any complications (e.g. respiratory distress, bleeding, prolonged recovery, unanticipated mortality) that occurred during or after the procedure

See [Rodent Anesthesia Monitoring Record \(Survival Surgery\)](#) as an example form that can be used as-is or modified for use with specific surgical procedures

2.7.2 Post-Operative Recordkeeping

The following information must be included in the post-operative monitoring records.

- Monitoring the integrity of any surgical incisions, ensuring that they are clean, dry, and intact.
- The animal's general posture and activity.
- Hydration status and appetite – urine and feces should be present in the cage and indicate that hydration and appetite are normal. A dehydrated mouse has sunken eyes and may have pale ears.
- Additional comments for any variations from the normal and expected events during the recovery period.
- The animal's weight must be recorded if it's included in the IACUC protocol (this is generally the most objective measure of well-being).
- Analgesics used including the time, dosage, and route.
- The initials of the person doing the monitoring.

The frequency of monitoring must adhere to what is described in your approved IACUC protocol.

- The length of monitoring noted in the protocol is a minimum. If there is any indication that the animal is not doing well, the record must extend beyond this period.

See [Rodent Post-Surgical Monitoring Record](#) as an example form that can be used as-is or modified for use with specific surgical procedures

2.8 Nonsurvival Surgery Guidelines

For non-survival surgeries, it may not be necessary to follow all the requirements outlined for survival surgeries. However, for procedures of extended duration, attention to aseptic technique may be more important to ensure stability of the model and a successful outcome. The following guidelines describe the minimum requirements for nonsurvival surgery on non-USDA-regulated species.

2.9 Surgical Space

Surgery on animal species not covered by the USDA does not have to be performed in a dedicated surgical suite. A procedure room within the animal facility or a dedicated space within the lab may be used. If the space is within the lab, it must be done in an area dedicated to surgery.

The space should be chosen based on space, ease of sanitization, and traffic flow. The space should be large enough to designate separate areas for animal preparation, surgery, and recovery. The area should also be uncluttered and clean. It should have hard, impermeable surfaces that may be easily sanitized before and after surgery. Because the main source of intraoperative infection is airborne bacteria, the area should have minimal traffic to decrease the risk of infection. This surgical space needs to be described in the IACUC protocol and requires IACUC approval and semi-annual IACUC inspections.

2.10 Preparation of the Animal

The hair/feathers around the surgical site should be removed and the area should be clean and free of gross debris. Removal may be achieved with clippers, depilatory cream, or by manual plucking for feathers.

2.11 Preparation of the Surgeon and Surgical Assistants

Don appropriate personal protective equipment (PPE) for the species and Animal Biosafety Level (ABSL). The minimum PPE includes a clean lab coat or disposable gown, an appropriate face mask, a hair bonnet, closed-toe shoes, and shoe covers. The surgeon does not have to don additional sterile attire but needs to wear clean gloves.

2.12 Preparation and Handling of Surgical Instruments, Equipment, or Implanted Material, and Consumables

Instruments should be clean and free of gross debris. Nonsterile instruments and supplies are acceptable. It is not acceptable to use any expired goods or materials. If multiple animals are being done on a single day, the instruments need to be cleaned between animals.

2.13 Intraoperative Monitoring

Animals must be monitored throughout the duration of the surgery to ensure an adequate plane of anesthesia. This must be done and documented every 15 minutes. Non-survival surgeries lasting 15 minutes or less in duration do not require further documentation of anesthetic monitoring.

- Before beginning surgery, a firm toe pinch using either 2 fingers or atraumatic forceps must be performed. Any reaction is an indication that the animal is not at a surgical plane of anesthesia and requires additional anesthesia prior to surgery.
 - A toe pinch needs to be performed at least every 15 minutes to ensure that the animal is being maintained at the appropriate anesthetic plane.
- It is the responsibility of the surgeon to monitor respirations. A sudden noticeable increase in respiration rate may indicate that the animal is not deep enough and requires additional anesthesia, while a sudden noticeable decrease in respiratory rate may indicate that an animal is too deeply anesthetized.
- Observation of the animal's color is another indication of anesthetic depth. The animal's ears, muzzle, and mucous membranes should be pink. If they are cyanotic (blue), there is poor oxygenation and this may indicate a respiratory issue. If they are pale, this indicates poor perfusion or blood loss and may indicate a heart-related issue.

2.14 Recordkeeping

2.14.1 Surgical Recordkeeping

The following information must be included in the surgical records:

- Principal investigator name and protocol number
- Title or brief description of procedure performed
- Species, identification, and the total number of animals
- Name of the surgeon
- Date of the surgery
- Name and dose/dosage of all agents administered before and during anesthesia and/or surgery, including anesthetics, analgesics, therapeutics, and any experimental agents delivered
- Monitoring of vital signs
- Any complications (e.g. respiratory distress, bleeding, or unanticipated mortality) that occurred during the procedure
- How the animal was euthanized at the end of the procedure, including primary and secondary methods

See [Rodent Anesthesia Monitoring Record \(Non-Survival Surgery\)](#) as an example form that can be used as-is or modified for use with specific surgical procedures.

3.0 Definitions: N/A

4.0 Responsibilities

All individuals to whom this SOP applies are responsible for becoming familiar with and following this SOP. University supervisors are responsible for promoting the understanding of this SOP and for taking appropriate steps to help ensure compliance with it.

5.0 Related Information

The following information compliments and supplements this document. The information is intended to help explain this SOP and is not an all-inclusive list of policies, procedures, laws and requirements.

5.1 Related University Policies: N/A

5.2 Related SOPs: N/A

5.3 Related Forms:

- USDA Anesthesia Monitoring Form

5.4 Frequently Asked Questions (FAQs): N/A

5.5 Other Related Information: References:

- Fossum, Theresa W. Small Animal Surgery. 4th ed., Elsevier Mosby, 2013.
- Lilley, E, and M Berdoy, editors. Guiding Principles for Preparing for and Undertaking Aseptic Surgery. 2nd ed., Laboratory Animal Science Association, 2017.
- National Research Council. Guide for the Care and Use of Laboratory Animals. 8th ed. Washington, D.C.: The National Academies Press, 2011.
- Office of Laboratory Animal Welfare. Public Health Service Policy on Humane Care and Use of Laboratory Animals. National Institutes of Health, 2015.
- United States Department of Agriculture. Animal Welfare Act and Regulations. USDA-APHIS, 2020.

6.0 SOP Owner and Contact(s)

6.1 SOP Owner: IACUC

6.2 SOP Approved by: IACUC

6.3 Subject Matter Contact: Brown University Animal Research Compliance (ARC)

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7.0 SOP History

7.1 SOP Issue Date: October 7, 2022

7.2 SOP Effective Date: October 7, 2022

7.3 SOP Update/Review Summary: The SOP was updated to include both survival and non-survival surgery information.