

IACUC Guidance: **TAMU-G-013** Title: **Guidelines for Survival Surgical Procedures in Rodents**

Location	Effective Date	Review By
College Station/Dallas/Galveston/Kingsville	02/01/2024	01/31/2027
Houston	03/01/2024	01/31/2027

1. PURPOSE

1.1. This document provides information to be used when planning and performing survival surgical procedures on rodents used for research, teaching, or other purposes at Texas A&M University.

2. SCOPE

- 2.1. Applies to rodents
- 2.2. Anesthesia/analgesia will not be discussed here, see TAMU-G-002
- 2.3. For non-survival surgery, see TAMU-G-022

3. **RESPONSIBILITY**

- 3.1. The **PI** is responsible for:
 - 3.1.1. Following these guidelines for approval of protocols which include surgery.
 - 3.1.2. Listing all locations where surgery will be performed in the AUP, including areas used for preparation and recovery of the animal; and amending the AUP to add/remove locations to ensure the list of surgical locations remain up to date.
 - 3.1.3. Listing participants, with their qualifications to perform the activities or procedures selected, on the AUP when initially submitted.
 - 3.1.4. Ensuring that AUP personnel complete all training and BOHP enrollment activities as outlined in TAMU-G-029 and maintaining documentation of training.
- 3.2. The IACUC must inspect and approve all locations where surgery will be performed prior to use.
- 3.3. The IACUC and the AV are responsible for:
 - 3.3.1. determining the categorization of surgery as major or minor and the impact on the animal's well-being; and
 - 3.3.2. reviewing the stated experience and qualification of protocol participants and identifying any needed additional training requirements.
- 3.4. The **PI** and the **AV** share responsibility for ensuring that postsurgical care is appropriate.

4. DEFINITIONS AND/OR ACRONYMS

- 4.1. **Aseptic Surgical Techniques**: Well-established methods used to avoid the introduction of microbial contamination into tissues exposed and/or manipulated during surgery.
- 4.2. Aseptic Tip Technique (Or "Tips-Only" Technique): A modified combination of techniques wherein the surgeon only uses the sterile working ends of the surgical instruments (or "tips") to manipulate the surgical field in order to achieve asepsis. The gloved, but non-sterile, hand never contacts the instrument tips, suture, suture needle, or any other part of the surgical field during use.
- 4.3. **AUP:** Animal Use Protocol. Document submitted by the PI indicating the housing and procedures involving animals.
- 4.4. **AV**: Attending Veterinarian. Individual designated by Texas A&M University to fulfil the regulatory role of AV. May also describe veterinary staff who report directly to, and have delegated authority from, the AV.
- 4.5. Disinfection: Reduces or eliminates unacceptable concentrations of microorganisms.
- 4.6. **Drape**: A sterile drape is a porous (cloth, paper) or non-porous (plastic) sheet that is used to provide a temporary sterile barrier.
- 4.7. **Glass Bead Sterilizer**: Glass bead "sterilization" uses ~1.5mm glass beads and high temperature (217-232°C) for brief exposure times to inactivate microorganisms. The instruments must be placed about halfway into the

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beads for at least 45 seconds, or per manufacturer's instructions. Only the portion of the instrument placed in contact with the beads will be sterilized. All instruments must be cleaned of blood and tissue debris prior to being placed in the bead sterilizer. Remove and place instruments on the sterile instrument drape to cool before touching the animal again.

- 4.8. **Major Surgery:** Usually penetrates and exposes a body cavity, and includes the potential for significant impairment of physical or physiologic functions, or involves extensive tissue dissection or transection. Examples: laparotomy, thoracotomy, joint replacement, and limb amputation.
- 4.9. **Minor Surgery:** Minor surgery usually does not expose a body cavity and causes little or no physical impairment; Examples include suturing superficial wounds, peripheral vessel cannulation, percutaneous biopsy, intracranial injection, subcutaneous osmotic pump implant, routine agricultural animal procedures such as castration.
- 4.10. **Multiple Survival Surgery:** Multiple anesthetic events or more than one survival surgery (major or minor) on a single animal.
- 4.11. **Non-survival surgery:** A surgery in which animals are euthanized under general anesthesia prior to anesthetic recovery.
- 4.12. Post-Operative Period: The 7-10 days following the initial day of surgery.
- 4.13. Rodent: Of the order Rodentia
- 4.14. Sterile: Free of all forms of life and biological agents, such as bacteria, viruses, and fungi.
- 4.15. **Surgery**: Cutting into the body through the use of a tool such as a scalpel blade, surgical scissors, laser, or other suitable device. May also refer to an invasive measurement under anesthesia.
- 4.16. Surgical Session: Set of distinct surgical procedures performed during a defined, congruent period of time
- 4.17. Survival surgery: A surgery in which animals are expected to recover from anesthesia following the procedure.

5. GUIDELINES OR PROCEDURE

5.1. Preparation of the Surgical Area

- 5.1.1. A dedicated surgical facility is not required for rodents.
- 5.1.2. The surgical area should be a room (or a portion of a room) that is disinfected and uncluttered.
- 5.1.3. Access to the area by personnel not directly involved in the surgery must be limited when surgery is being performed.
- 5.1.4. Animal housing locations are not ideal surgical locations

5.2. Preparation of Surgical Supplies

- 5.2.1. Surgical Instruments
 - 5.2.1.1. Clean instruments of organic material prior to sterilization, or use prepackaged aseptic supplies.
 - 5.2.1.2. Methods used for sterilization may vary, but all must conform to established medical standards for complete sterilization. Options include:
 - 5.2.1.2.1. Steam sterilization at proper pressures and exposure times.
 - 5.2.1.2.2. Ethylene oxide gas (ETO) used in a specialty chamber.
 - 5.2.1.2.3. Gas plasma sterilization
 - 5.2.1.2.4. Dry heat sterilization at proper temperature and exposure time.
 - 5.2.1.2.5. Prolonged immersion in a hospital-grade formaldehyde- or glutaraldehyde-based cold sterilant following label directions (alcohol immersion is NOT acceptable). Chemical sterilants are irritating to tissues and thus instruments must be rinsed with sterile water/saline before use on tissues.
 - 5.2.1.3. If sterile packs are stored for later use, they are considered sterile until some event (e.g. tear in packaging, packaging becomes wet, seal is broken) causes the item to become contaminated.
 - 5.2.1.4. "Tips only" aseptic practices
 - 5.2.1.4.1. Must be indicated in the approved AUP.
 - 5.2.1.4.2. For surgeries that involve only very small incisions, as indicated in the approved AUP.



- 5.2.1.4.3. The surgeon does not directly touch the tissues.
- 5.2.1.4.4. Sterile gloves are not required.
- 5.2.1.4.5. Aseptic surgical practices focus on the tips (which enter the body) of the instruments.
- 5.2.1.4.6. Instruments are initially sterilized at the start of the surgical session by a method outlined in 5.2.1.2.
- 5.2.1.4.7. Instrument-working surfaces can be selectively sterilized by immersion in a glass bead sterilizer, or other acceptable disinfectant.
- 5.2.1.4.8. Surgical setup must ensure maintenance of instrument tip sterility.
 - 5.2.1.4.8.1. The use of a sterile instrument stand (e.g. wooden dowel) helps maintain instrument tips at an elevated position. Alternatively, the use of a sterile cloth instrument holder with pockets can reduce the potential for contamination.
 - 5.2.1.4.8.2. Arrange the sterile instruments so that the tips are within a sterile field and the handles are outside the sterile field.
- 5.2.1.4.9. Maintain sterile suture material within the sterile field at all times
- 5.2.1.4.10. Avoid pulling suture across non-sterile areas (e.g., across animal's body, areas surrounding the sterile field)
- 5.2.2. Drapes
 - 5.2.2.1. Drapes are highly recommended.
 - 5.2.2.2. Drapes can be cloth, paper, sterile stockinettes, 3M[™] Steri-Drape[™] Incise Drapes, or new and unused boxes of GLAD Press'n Seal[®] wrap.

5.3. Preparation of the Animal

- 5.3.1. Recently shipped animals should be given a period to acclimate to their new surroundings. General practice is to allow a 3-7 day acclimation period, when possible.
- 5.3.2. Perform a pre-surgical evaluation to ensure the rodent is not overtly ill. Animals displaying clinical signs of illness such as dehydration, diarrhea, ocular or nasal discharge should be passed over as surgical candidates.
- 5.3.3. Withholding food/water
 - 5.3.3.1. Water should NOT be withheld unless required by the protocol.
 - 5.3.3.2. Withholding food is not necessary in rodents unless specifically mandated by the protocol.
- 5.3.4. Administer antibiotics and analgesics (preemptive analgesia) as appropriate and approved in the protocol.
- 5.3.5. Remove hair from the surgical site(s) at a location removed from the surgery area.
 - 5.3.5.1. Use clippers, a razor, or the application <u>and removal</u> of depilatory cream.
- 5.3.6. Prepare the surgical site(s) with an appropriate skin disinfectant and allow sufficient contact time.
 - 5.3.6.1. The skin should be cleaned in a manner than moves hair and debris from the cleanest location (incision site) to the dirtiest area (periphery) of the clipped site. A gradually enlarging circular pattern works well.
 - 5.3.6.2. The scrubbed area is rinsed with 70% alcohol or warmed sterile saline/water and the scrub/rinse cycle is repeated 3 times with a new pad/swab each time
 - 5.3.6.3. The incision site is wiped/painted with an antiseptic solution (not scrub) that is compatible with the surgical scrub solution (e.g. chlorhexidine solution with chlorhexidine scrub) or end with final application of 70% alcohol, or sterile saline/water
 - 5.3.6.4. To prevent hypothermia, avoid excess wetting and minimize the use of alcohol.
- 5.3.7. Use a sterile drape to lay out instruments.
- 5.3.8. Apply sterile, non-medicated ophthalmic ointment to eyes to prevent corneal drying (e.g., Lacrilube).
- 5.3.9. Initiate thermoregulatory supportive care (unless otherwise approved) and fluid administration, per protocol.



- 5.3.9.1. **CAUTION**: Use of heat lamps and non-thermoregulating electric heating pads can result in severe burns or hyperthermia in animals. The use of safer equipment such as a circulating water blanket or isothermic pad is recommended, whenever possible.
- 5.3.10. Anesthetize the animal, in accordance with the approved AUP.

5.4. Preparation of the Surgeon

- 5.4.1. A surgical cap and face mask is required and surgeons must change into a clean scrub shirt or wear a clean lab coat.
- 5.4.2. When opening sterile gloves, consider using interior portion of packaging as a sterile field.
- 5.4.3. When opening sterile materials, do so without touching contents and place in sterile field (without touching sterile field).
- 5.4.4. Wash hands with a soap (minimal) or an antiseptic surgical scrub solution (optimal) prior to donning sterile gloves, or clean examination gloves (for tips only aseptic practices).
- 5.4.5. When donning sterile gloves, do so without touching the exterior of the glove surface.
- 5.4.6. Assistants working in the immediate vicinity must wear a clean garment, head cap, mask and gloves.

5.5. Performing Multiple Rodent Surgeries in Series in individual animals

- 5.5.1. Begin with at least one set of sterile instruments (tips only)
 - 5.5.1.1. Between animals, clean the instruments followed by disinfection with a glass bead sterilizer.
- 5.5.2. Begin with at least two sets of sterile instruments to allow for disinfection of instruments between animals.
 - 5.5.2.1. Between animals, clean the instruments and soak in an appropriate disinfectant solution (such as appropriately diluted Cidex[®] for 15 minutes).
- 5.5.3. Use a new drape for each animal, if using drapes.
- 5.5.4. When performing surgeries on multiple animals during a single session, one pair of sterile gloves can be used provided that the gloves remain free of tears or punctures and are disinfected (by wiping with an appropriate disinfectant) between animals.
- 5.5.5. The surgical area should be cleaned with an appropriate disinfectant between animals. 5.5.5.1. See TAMU-G-026 for guidelines on evaluating sanitation practices.
- 5.5.6. Even with the use of aseptic techniques, disinfection between animals, and the use of a sterile field, the instrument pack should be replaced with a new sterile instrument pack after being used on 4-5 animals.

5.6. Intra-operative

- 5.6.1. Establish and maintain a surgical plane of anesthesia.
 - 5.6.1.1. Anesthetic depth may be monitored in a number of ways and may vary depending upon the species and anesthetic agent used.
- 5.6.2. Handle tissue gently and prevent tissue from drying.
- 5.6.3. Close surgical wounds with appropriate techniques and materials.
- 5.6.4. Maintain sterility of gloves and instruments throughout the surgery
- 5.6.5. Maintain sterile suture material within the sterile field at all times
- 5.6.6. Avoid pulling sterile suture across non-sterile areas (e.g., across animal's body, areas surrounding the sterile field)

5.7. Post-Operative Monitoring

- 5.7.1. Ensure uneventful recovery from anesthesia.
- 5.7.2. Keep animal warm and dry in an environment that doesn't pose a risk of injury.
 - 5.7.2.1. Do not place recovering rodents directly on bedding as there is risk of aspiration.
 - 5.7.2.2. Animals recovering in cages, should have the cage placed half on /half off the heat source.
- 5.7.3. Provide analgesics, fluids, antibiotics etc., as specified in the protocol or as directed by the AV/designee.
 - 5.7.3.1. For example, 1-2 mL of warmed fluids (0.9% NaCl, or equivalent) per 100g body weight by subcutaneous or intraperitoneal injection to maintain hydration. If blood loss occurred during the procedure, or the animal is slow to recover, additional fluids should be provided.
- 5.7.4. Rodents must be continuously monitored (i.e. present in the room with ability to see the animal) until maintaining upright posture and walking normally about the cage. It is recommended that the animals



be monitored for an additional 30 minutes (after regaining consciousness) before being returned to the animal housing room.

- 5.7.5. Once returned to the housing area place appropriate signage on the cage indicating they have been anesthetized.
- 5.7.6. Post-surgical animals should be seen by a member of the investigator's staff or designee at least daily or as described in the approved animal use protocol.
- 5.7.7. See TAMU-G-023 for signs of pain and distress that may indicate the need for clinical care and additional analgesia.

5.8. Post-Operative Care

- 5.8.1. Keep surgical wounds clean.
- 5.8.2. Monitor the animal daily to ensure that there are no surgical complications.
- 5.8.3. Remove non-absorbable sutures or wound clips in 7-14 days.
- 5.8.4. Contact the AV/designee if the animal appears ill, or if the surgical wound appears abnormal.
- 5.9. **Surgical Records.** Records are required for every rodent that has undergone surgery. Research records, including surgical records, must be maintained consistent with TAMU SAP 15.99.03.M1.03., and should contain:
 - 5.9.1. Date of procedure
 - 5.9.2. Protocol number
 - 5.9.3. Identification of the type of surgery performed; e.g. "laparotomy"
 - 5.9.4. Species and animal or cage identifier
 - 5.9.5. The name of the surgeon and any assistants
 - 5.9.6. Pre-surgical assessment
 - 5.9.7. Pre-op preparation, as applicable
 - 5.9.7.1. Ophthalmic ointment applied
 - 5.9.7.2. Hair/fur removal
 - 5.9.7.3. Surgical scrub or site preparation
 - 5.9.8. Event times or total time under anesthesia
 - 5.9.9. Vital parameters monitored
 - 5.9.10. Times of monitoring
 - 5.9.11. A notation of any complication or abnormality identified
 - 5.9.12. Drugs administered: dose, route and frequency of administration
 - 5.9.13. When DEA controlled substances are used, the date and drug usage volumes recorded in the controlled substance log and the dates and amounts recorded in the animal surgery records should match.
 - 5.9.14. Surgical records should be kept with the animal in the housing location for the immediate postoperative period (7-10 days)
 - 5.9.15. Records for survival surgery should include recovery and post-operative monitoring performed until wound closure removal (if applicable), or timeframe established in the AUP, including:
 - 5.9.16. Date and time of monitoring
 - 5.9.17. General observations which may include assessment of wound closure(s), signs of pain/discomfort, complications, or abnormalities including need for early performance of euthanasia
 - 5.9.18. Drugs administered: dose, route, date and time of administration
 - 5.9.19. Date of wound closure removal (if applicable)

5.10. Multiple Survival Surgery (in same animal)

- 5.10.1. The PI must describe in the AUP when multiple survival surgical procedures are performed on the same animal to meet the objectives of the study, project, or class, including the interval between surgeries
- 5.10.2. The performance of multiple <u>major</u> survival surgery (MMSS) on a single animal is acceptable when included on a single project and scientifically justified by the PI in the AUP

5.10.2.1. Cost savings alone is not an adequate reason for performing MMSS



- 5.10.2.2. The conduct of MMSS on a single animal used in separate projects or AUPs is discouraged and must be clearly indicated in the AUPs impacted
- 5.10.2.3. Some procedures characterized as minor may induce substantial postprocedural pain or impairment and should similarly be scientifically justified if performed more than once in a single animal

6. EXCEPTIONS

- 6.1. The PI may request an exception to the above standards by describing the departure in the AUP
- 6.2. For programmatic exceptions, the facility director or manager may submit a request for the exception using TAMU-F-013

7. REFERENCES, MATERIALS, AND/OR ADDITIONAL INFORMATION

- 7.1. References
 - 7.1.1. Emmer et al. Evaluation of the Sterility of Press'n Seal Cling Film for Use in Rodent Surgery. J Am Assoc Lab Anim Sci. 2019 Mar 1;58(2):235-239.
 - 7.1.2. National Research Council. 2011. <u>Guide for the Care and Use of Laboratory Animals</u>. National Academies Press, Washington, D.C. 220p.
 - 7.1.3. American College of Laboratory Animal Medicine, Position Statement <u>Medical Records for Animals Use</u> <u>in Research, Teaching and Testing</u>
 - 7.1.4. ACLAM Position Statement Medical Records for Animals Used in Research, Teaching, and Testing
 - 7.1.5. Centers for Disease Control <u>Sterilization Practices</u>
 - 7.1.6. AAALAC International <u>Alcohol as a disinfectant</u>
 - 7.1.7. TAMU SAP 15.99.03.M1.03 The Responsible Stewardship of Research Data
- 7.2. Resources
 - 7.2.1. For more information concerning the planning and performance of surgical procedures, please contact:
 - 7.2.1.1. <u>CMP</u> at 979-845-7433
 - 7.2.1.2. ARU: at (214) 828-8149
 - 7.2.1.3. PAR: at (713) 677-7471
 - 7.2.1.4. PRF: at (361) 221-0770
 - 7.2.2. AWO: (requires TAMU NetID authentication)
 - 7.2.2.1. TAMU-G-002 Guidelines on the Use of Anesthesia and Analgesia
 - 7.2.2.2. TAMU-G-022 Guidelines for Non-Survival Surgery
 - 7.2.2.3. TAMU-G-023 Guidelines on Recognizing Pain and Distress in Research Animals
 - 7.2.2.4. TAMU-G-026 Guidelines for the Evaluation of Sanitation Practices
- 7.3. Acknowledgements
 - 7.3.1. This document contains content that was adapted from materials obtained from University of Texas at Austin and the University of Michigan.

8. HISTORY

Effective Date	Version #	Description
07/18/2019	000	College Station/Galveston: New format and updated content; replaced unnumbered SOP
		("Vertebrate Animal Surgery")
11/18/2019	001	Houston/Kingsville: New format and updated content; replaced IBT-200
11/19/2019	002	Dallas: New format and updated content; replace CD-200
05/03/2021	003	College Station/Galveston: Renewal document; updated definitions and references;
		updated content congruent with CMP's asepsis course
06/01/2021	004	Houston/Kingsville: Renewal document; updated definitions and references
06/15/2021	005	Dallas: Renewal document; updated definitions and references



03/24/2022	006	College Station/Dallas/Galveston: Merging of Dallas animal care and use program with
		College Station/Galveston
10/20/2022	007	College Station/Dallas/Galveston/Kingsville: Merging of Kingsville animal care and use
		program with College Station/Dallas/Galveston.
02/01/2024	008	College Station/Dallas/Galveston/Kingsville: Renewal document; updated scope,
		responsibility, definition and reference sections; clarified surgical incision prep
		procedure, thermoregulatory support, post-op monitoring and record keeping
		expectations; addressed multiple survival surgery in a single animal; added exception
		request section. Reviewed and approved via TEAMs
03/01/2024	009	Houston: Renewal document; updated scope, responsibility, definition and reference
		sections; clarified surgical incision prep procedure, thermoregulatory support, post-op
		monitoring and record keeping expectations; addressed multiple survival surgery in a
		single animal; added exception request section. Reviewed and approved via TEAMs