

Title:

Asepsis for Rodent Survival Surgery**SOP.ACS.818.Asepsis for Rodent Survival Surgery****Approval Date: November 10, 2023 (effective May 1, 2024)****Revision Date:**

1. **Purpose:** To provide instructions for performing aseptic surgical procedures during survival procedures on rodents and to meet or exceed the standards as set out in the CCAC Guide to the Care and Use of Experimental Animals.
2. **Responsibilities:** Animal care staff, veterinarians, and trained individuals listed on an approved Animal Utilization Protocols (AUPs). All animal users performing procedures in animals must have successfully completed Mouse/Rat A/B training courses and any relevant surgery/anesthesia training. Anyone performing anesthesia independently must have been certified by an Animal Care Services veterinarian.
3. **Introduction:** Survival surgical procedures on rodents must be performed using aseptic procedures. This includes the use of sterile instruments and the aseptic preparation of the surgery suite, surgical site, and surgeon to prevent post-operative infections. Infections can be observable (clinical) or hidden (sub-clinical) and both can impact animal welfare and data quality.

Any anesthesia/surgery in rodents must be outlined in an approved Animal Utilization Protocol (AUP). Any deviations from this policy must be detailed in the protocol and approved by the Animal Care Committee.

For detailed instructions on set-up of the inhalant anesthetic machine and performing anesthesia, see CAF.342 Isoflurane Anesthetic Machine Set up and Operation and SOP.ACS.817 Rodent survival anesthesia - inhalant, respectively. A list of potential sources for described equipment is available on request from ACS Veterinarians or the Central Animal Facility.

4. Procedures

A checklist for the major steps in preparing the surgical space, sterile field, animal, and surgeon can be found in Appendix 1.

SURGICAL SPACE:

- Aseptic rodent surgery should be conducted in dedicated facilities or spaces that are temperature controlled with no drafts. This space must be booked with the facility manager or technician.
- Traffic and activities should be minimized in the room or area at the time of the procedure.
- Conduct surgery on a table that is impervious to liquids.
- Mist/wipe the work surface and equipment (including knobs of the anesthetic machine) with a high-level disinfectant (e.g., Peroxigard) before beginning set-up.
- Ensure the area where surgery will be conducted is separate from where hair is removed during animal preparation (see Figure 1).
- Prepare a heat pad for the surgical area and elevate it so that the animal can lie flat with a neutral spine once in the face mask/nose cone.
- Prepare additional heat pads for under the induction chamber, recovery chamber, and to warm prep soaps and solutions.



Figure 1. Separation of the surgery table and prep area. Note that the surgeon is wearing mask, bonnet, and EXAM gloves for prep, but not disinfected arm sleeves and surgical gloves. A

Title:

Asepsis for Rodent Survival Surgery

SOP.ACS.818.Asepsis for Rodent Survival Surgery

Approval Date: November 10, 2023 (effective May 1, 2024)

Revision Date:

different gown or lab coat should be designated for clipping hair vs. surgery. The prep area and surgical area can be separate areas of the same table if only one anesthetic machine is available.

INSTRUMENT PREPARATION

Surgical instruments must be cleaned (removal of organic debris), and sterilized (removal of all viable micro-organisms) prior to surgery. The first surgery must begin with an autoclaved instrument pack.

Cleaning dirty instruments (see Figure 2):

1. Open all “clamping” instruments (hemostats, needle drivers) so that all surfaces of the instrument are exposed.
2. Soak in hot water mixed with an enzymatic solution and scrub to remove all organic debris
3. Rinse with hot water.
4. Allow to dry on a clean absorbent surface.
5. Lubrication with a surgical grade lubricant extends the life of the instrument: apply and allow to dry on.



Figure 2. Instrument cleaning and lubrication

Sterilizing clean instruments:

- Autoclaving is the sterilization method of choice – clean, dry instruments can be wrapped in a clean drape or sealed in an autoclave pouch.
- Use a method to verify sterilization such as a steam chemical indicator tucked into the package or autoclave tape wrapped around the drape (see Figure 3).

Title:

Asepsis for Rodent Survival Surgery

SOP.ACS.818.Asepsis for Rodent Survival Surgery

Approval Date: November 10, 2023 (effective May 1, 2024)

Revision Date:

- Delicate materials may be “cold” sterilized with chemicals such as glutaraldehyde – be sure to follow label directions for preparation and exposure times.
- *ALCOHOL IS NEITHER A STERILANT NOR A HIGH-LEVEL DISINFECTANT*

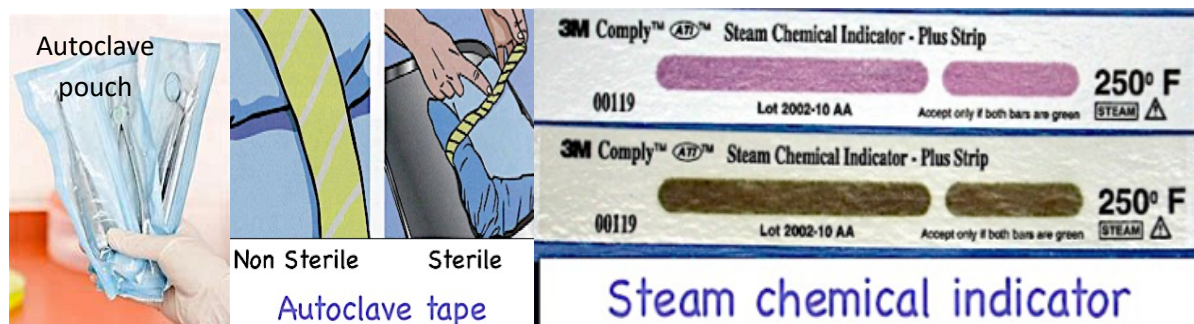


Figure 3. Verifying sterility

For subsequent surgeries (up to 5 per instrument pack), metal instrument tips can be sterilized with a glass bead sterilizer:

1. Pre-heat the glass bead sterilizer prior to surgery – it may take up to 30 minutes to reach sterilization temperature (250°C).
2. After use, clean instrument tips grooves and hinges with a brush and alcohol to remove excess debris.
3. Place in bead sterilizer for 60 seconds at 250°C; do not crowd, as too many instruments will cool down the beads to a non-sterilizing temperature (see Figure 4).
4. Allow to cool prior to use. Instruments that have just come out of the sterilizer should be placed with their tips on a clean gauze in the sterile field or allowed to cool for 30 seconds in the air to avoid melting the Press'n Seal.
5. No more than five successive surgeries are allowed to be conducted per instrument pack without autoclaving.

Reuse of suture material for serial rodent surgeries (in one sitting) is permitted if...

- The suture is soaked between animals in a 10% povidone iodine or 70% alcohol solution then rinsed in sterile saline.
- Some suture can also be re-sterilized in an autoclave for future procedures:
 - Monofilament (Prolene, PDS, Monocryl, or Nylon/ Ethilon) or 'coated' sutures (look at the package) can be sterilized e.g. some Vicryl or Ethibond.
 - Multi-filament or braided fibres, such as Chromic, Silk, Mersilene and non-coated Ethibond or Vicryl must be discarded after use.

Title:

Asepsis for Rodent Survival Surgery

SOP.ACS.818.Asepsis for Rodent Survival Surgery

Approval Date: November 10, 2023 (effective May 1, 2024)

Revision Date:



Figure 4. Proper use of the bead sterilizer

Preparation of the sterile field:

1. Cover table where surgical instruments will be kept with a sterile drape (autoclaved fabric or GLAD Press'n Seal), ensuring that only the edges are handled with non-sterile gloves (Appendix 2, [video 1](#)).
2. Remove instruments from autoclave packaging in an aseptic manner on to the sterile drape (i.e., only touching the outer packaging). Remove scalpel blade and suture from packaging in the same way (see Figure 5 and Appendix 2, [video 3](#)).
3. Drape all surfaces that the surgeon will need to touch with Press'n Seal or autoclaved tinfoil (equipment knobs only), ensuring there is sufficient space for instruments (see Figure 5 and Appendix 2, [video 1](#)).
4. Ensure that no contact occurs with the sterile field until the surgeon is wearing the required PPE. An additional drape can be placed on top of the instruments to prevent accidental contact.
5. For subsequent surgeries where the bead sterilizer is used, the instruments should only be handled by the surgeon wearing sterile gloves. The surgeon places instruments in the bead sterilizer before removing gloves and does not touch them again until the next set of gloves is donned, to prevent contamination of the handles which cannot be re-sterilized.



Figure 5. Maintaining asepsis during surgery: ideally, the bead sterilizer and sharps bin should be more distant from the sterile surgical instruments.

Title:

Asepsis for Rodent Survival Surgery**SOP.ACS.818.Asepsis for Rodent Survival Surgery****Approval Date: November 10, 2023 (effective May 1, 2024)****Revision Date:**PREPARATION OF THE ANIMAL

Immediate steps after anesthetic induction and placement of animal in the nose cone for all procedures:

- Apply ophthalmic ointment to eyes; non-ophthalmic lubricants such as mineral oil, Vaseline, etc. are not appropriate.
- Administer analgesics (local and systemic) & antibiotics if indicated in protocol.
- Administer WARMED SQ or IP fluids (saline or lactated Ringer's solution, syringe or bag placed on a heating pad) at 10-20 mL/kg (fluids can also be given post-operatively or split between a pre-operative and post-operative dose).
 - Adult rat: generally 5 -10 mL
 - Adult mouse: generally 0.5 -1 mL
- Place the animal on a homeothermic heat pad and begin monitoring body temperature with a rectal thermometer (see Figure 5, can be signed out from the Central Animal Facility).

Preparation of the surgical site is performed wearing a lab coat, exam gloves, a mask, and bonnet and is completed once the animal has received eye lube, analgesics, and fluids:

1. Remove hair from the surgical site with hair clippers (#40 blade); depilatory cream causes skin irritation (leading to self-trauma) and is not recommended.
2. Remove loose hair with a small handheld vacuum (preferred) or a piece of gauze dampened with alcohol. Remove lab coat to reveal clean scrubs or change lab coat and transfer animal to the surgical area.
3. Prepare the skin with a 3-step solution: chlorhexidine (soap/scrub) → alcohol → chlorhexidine solution (see Appendix 2, [video 4](#)).
 - a. Apply with gauze or Q-tips (for mice or small surgical sites) in a concentric pattern starting at the center of the incision site and moving outwards – once you touch hair, discard the gauze/Q-tip.
 - b. Alternate between the soap and alcohol three times.
 - c. After the last alcohol, paint on the chlorhexidine solution and allow to dry.
4. Confirm the heat pad is working and that the patient's temperature is not dropping.
5. Once the surgeon is aseptically prepared (see below), the surgeon drapes the patient with GLAD Press'n Seal (see Figure 6, Appendix 2, [videos 2](#) and [5](#)) or, if the surgical site is small, with a Tegaderm transparent dressing.
 - a. If the surgeon is solo (i.e., does not have someone to hold the box once sterile gloves are on), pull out a larger piece of Press'n Seal BEFORE aseptic preparation and only touch the very edges, tucking these edges over the side of the table (see Appendix 2, [video 1](#)).
6. Once aseptically prepared, with sterile instruments, cut a window in the dressing prior to the first incision (see Appendix 2, [video 5](#)), or incise through the dressing.

Title:

Asepsis for Rodent Survival Surgery**SOP.ACS.818.Asepsis for Rodent Survival Surgery****Approval Date: November 10, 2023 (effective May 1, 2024)****Revision Date:**

Figure 6. Equipment to maintain body temperature and sterility. If there is a separate surgeon and anesthetist, the Press'n Seal drape can be placed so that the left hind foot is exposed for adjusting the pulse oximeter and testing the withdrawal reflex.

PREPARATION OF THE SURGEON

Ideally, the surgeon prepares for surgery while the anesthetist monitors and preps the animal so that the animal is always being monitored closely and is never waiting for the surgeon. If there is an assistant, then the surgeon should drape the patient after donning clean/sterile personal protective equipment (PPE), as the assistant can hold the Press'n Seal box. If the surgeon is solo, then the surgeon should don PPE after draping the patient and only touch the edges of the drape (see above), as the box of Press'n seal is NOT sterile. Full PPE can be seen in Figure 7.

1. You should already be wearing a mask and bonnet – you do not need to change these.
2. Wash your hands with antiseptic or surgical soap for five minutes, paying attention to the backs of the hands, between the fingers, and fingernails.
3. Don a clean lab coat (NOT the one used for hair clipping) or surgical gown, or remove a lab coat to reveal clean scrubs.
4. Don disposable arm sleeves and mist with Peroxigard.
5. Aseptically don a pair of sterile surgical gloves using the “open gloving” technique (see Appendix 2, [video 6](#)).
6. Once gloves are on, asepsis must be maintained until the end of surgery: the surgeon may only touch sterile surfaces (instrument field, instruments, non-sterile items that are covered with Press'n Seal or Autoclaved foil).
7. Having an assistant is important for consecutive surgeries: if the surgeon does not break sterility, the same surgical gloves can be used for 5 consecutive animals.
 - a. The assistant should spray the surgeon's gloves with alcohol between animals.
 - b. If the surgeon enters the gastrointestinal tract or gloves become soiled with blood (i.e., beyond the fingertips), gloves must be changed for the next animal.
8. Without an assistant, the surgeon must remove the clean lab coat/gown or cover the clean scrubs to anesthetize and prepare the animal, and then start from step 2 again once the patient is prepped.

Title:

Asepsis for Rodent Survival Surgery

SOP.ACS.818.Asepsis for Rodent Survival Surgery

Approval Date: November 10, 2023 (effective May 1, 2024)

Revision Date:



Figure 7. Required surgeon personal protective equipment.

POST-OPERATIVE CARE

Post-operative care is described more thoroughly in SOP.ACS.817 – Rodent Survival Anesthesia. However, guiding principles of post-operative care to decrease the risk of post-operative infections include:

- Allowing the animal to recover in a clean cage on a soft surface such as a towel with supplemental heat (heating pad placed under half of the cage or facility incubator)
- Returning the animal to a cage with clean bedding but with nesting material from the previous cage (to retain familiar scents) once it is fully recovered.
- Careful and regular monitoring for 3-7+ days, depending on the level of invasiveness of the procedure and whether sutures/staples remain.
- Managing other sources of stress: post-operative pain and dehydration, noise and light intensity, thermal comfort, social stress (social housing is the default).
- Nutritional supplementation with food that is energy-dense and highly palatable (e.g., Clear H₂O gel cups, strawberry Ensure, Bio-Serv Bacon Softies). Any novel item should be introduced prior to surgery.
- Providing supportive care and contacting ACS veterinarians promptly if evidence of infection or illness is observed.



Animal Care Services

Title:

Asepsis for Rodent Survival Surgery

SOP.ACS.818.Asepsis for Rodent Survival Surgery

Approval Date: November 10, 2023 (effective May 1, 2024)

Revision Date:

5. References

Advanced Anesthesia courses [MOOC]. Research animal training.

<https://researchanimaltraining.com/courses/>

University of Texas San Antonio: Rodent Survival Surgery Policy

https://research.utsa.edu/_files/pdfs/compliance-integrity-pdf-folder/iacuc-forms/IACP-Policy-004-Rodent-Survival-Surgery-Review-approved-11-12-2021.pdf

Title:

Asepsis for Rodent Survival Surgery**SOP.ACS.818.Asepsis for Rodent Survival Surgery****Approval Date: November 10, 2023 (effective May 1, 2024)****Revision Date:**

Appendix 1 – Checklist to Prepare for Aseptic Rodent Surgery

- Dedicated room/area for the surgical procedure, where clutter, noise, drafts and activity are minimized
- Surfaces (table and touch points on equipment) disinfected with Peroxigard
- Area for skin prep is separate from the location where the surgery will be conducted
- Instruments pre-cleaned & lubricated prior to sterilization
- Instruments and implants sterilized (as confirmed with tape or a steam strip, or recommended contact time with cold sterile) and expiration of the sterilized materials within a year
- Surgery area prepared (surgeon touch surfaces draped with Press'n Seal, heat pad warmed, dedicated sterile field covered with a sterile drape for instruments, scalpel blade, and suture)
- Heat pads warmed for induction, recovery, and for fluids/prep solutions
- Mask, bonnet, and exam gloves donned before starting animal prep
- Ophthalmic ointment, analgesics, and fluids and temperature monitoring initiated upon induction
- Dedicated lab coat worn for clipping and hair removed before bringing the animal to the surgical area
- Surgical site prepped 3 times with surgical soap and alcohol, followed by a last application of surgical solution
- If solo surgeon, animal is draped (only touching the edges) prior to donning PPE
- Surgeon hands washed for five minutes
- Clean lab coat/gown and disposable arm sleeves (misted with Peroxigard) donned before gloving
- Surgical gloves donned without contaminating them using an open gloving technique
- If there is an assistant, patient is draped by the surgeon after donning surgical gloves (assistant holds the box)
- Window made in drape and toe pinch reflex checked prior to making an incision

For consecutive surgeries:

- If there is an assistant, alcohol has been applied to sterile gloves if minimally soiled (if no assistant, then surgeon must remove PPE and don again once animal is prepped)
- Instruments wiped before placing them in the bead sterilizer for 1 minute at 250°C
- Instruments cooled after removal from the bead sterilizer
- Instrument pack/sterile gloves are not used for more than five consecutive surgeries, and new scalpel blade and animal drape is used for each surgery

Title:

Asepsis for Rodent Survival Surgery

SOP.ACS.818.Asepsis for Rodent Survival Surgery

Approval Date: November 10, 2023 (effective May 1, 2024)

Revision Date:

Appendix 2 – Video links for steps in surgical asepsis

Video 1: Preparing a surgical field with a sterile drape to minimize cross-contamination using NON-STERILE gloves - <https://www.youtube.com/watch?v=vgl3nn4DOIE>

Video 2: Removing a drape piece from an already opened Press'n Seal box - <https://www.youtube.com/watch?v=3WEgxlfxh74>

Video 3: Placing sterile instruments and supplies on sterile field - <https://www.youtube.com/watch?v=2U895NomieA>

Video 4: Surgical skin prep - https://www.youtube.com/watch?v=BZ_ZCORTaCU

Video 5: Draping skin with sterile Press'n Seal drape with STERILE gloves - <https://www.youtube.com/watch?v=09nMBxINra4>

Video 6: Donning surgical gloves - <https://www.youtube.com/watch?v=oncd1q5ztOc>