

UNIVERSITY OF TEXAS AT ARLINGTON

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

SURVIVAL SURGERY, ASEPTIC TECHNIQUE, AND POST-SURGICAL CARE SOP

I. SURVIVAL SURGERY AND POST SURGICAL CARE

A. Definitions:

1. Aseptic technique:
 - a. Surgical technique conducted under conditions that prevent exposure of the patient to pathogenic organisms, including wearing of sterile surgical gloves, gowns, caps and face masks; use of sterile instruments; and aseptic preparation of the surgical field.
 - b. For rats and mice, the use of a surgical cap and gown is optional.
2. Survival surgery: Surgery performed on a live animal under general anesthesia, from which the animal is expected to recover.
3. Non-survival surgery: the animal is euthanized at the end of the surgical procedure before recovering from anesthesia.
4. Major operative procedure or major survival surgery: Surgical intervention that penetrates a body cavity or could potentially produce a permanent handicap in an animal that is expected to recover.
5. Minor surgical procedure: Surgical procedure restricted to the management of minor problems and injuries (e.g., wound suturing)

B. Legal Requirements:

1. Surgery must be performed or directly supervised by trained, experienced personnel.
2. Procedures that will cause more than momentary or slight pain or distress must be performed with appropriate sedatives, analgesics, and/or anesthetics, unless withholding such agents is justified for scientific reasons and that justification is provided to the UTA IACUC in writing by the principal investigator.
3. Pre- and post-surgical care must be provided in accordance with established veterinary medical and nursing practices.
4. Survival surgery:
 - a. Aseptic surgical techniques must be used on all animals. Major surgical procedures must be conducted only in facilities that are intended for that purpose and are maintained under aseptic conditions. Non-major operative procedures do not require a dedicated facility but must be performed using aseptic procedures.
 - b. Surgery on rats and mice does not require a dedicated facility but must be performed using aseptic procedures.
5. Multiple major surgical procedures on one animal may not be performed unless the procedures are justified for scientific reasons, have been approved by the UTA

IACUC, and the justification stated in writing by the principal investigator. Multiple surgical procedures may be performed as necessary to protect the health or well-being of the animal, as determined by the attending veterinarian.

Attachments: **UTA Rodent Surgery Guidelines – Application of Aseptic Technique and Perioperative Care**, pp.3-21 of this document.

UTA Rodent Surgery Guidelines

Application of Aseptic Technique and Perioperative Care

Regulations and Guidelines

- The **Guide for the Care and Use of Laboratory Animals** (National Research Council) can be downloaded from http://aaalac.org/resources/Guide_2011.pdf. The 2011 **Guide** states, “Inadequate or improper technique may lead to subclinical infections that can cause adverse physiologic and behavioral responses (Beamer 1972; Bradfield et al. 1992; Cunliffe-Beamer 1990; Waynforth 1980, 1987) affecting surgical success, animal well-being, and research results (Cooper et al. 2000). General principles of aseptic technique should be followed for all survival surgical procedures (ACLAM 2001).”
- According to OLAW guidelines for rodents and the *Guide*, a dedicated facility is not required solely for rodent survival surgery, except for when the surgical procedure is conducted.

Definitions:

- **Sterile**: Free from all living microorganisms and their spores.
- **Asepsis**: Very insignificant numbers of microorganisms. A condition in which living pathogenic organisms are absent; a state of sterility.
- **Aseptic surgery**: The performance of an operation with sterile gloves, instruments, etc., and utilizing precautions against the introduction of infectious microorganisms from the outside environment.
- **Contaminated/Colonized**: Bacteria/microorganisms present ($<10^6$ /gram tissue on average animal). Clinical signs may or may not be present. Immune response may be able to ward off infection.
- **Infected/Sepsis**: Bacteria/microorganisms present ($>10^6$ /gram tissue on average animal). Clinical infection is evident and cultures are positive.



- **Sterilization**: The process whereby all viable microorganisms are eliminated or destroyed. The criterion for adequate sterilization is the failure of organisms to grow if a growth-supporting medium is supplied.
- **Disinfection**: The chemical or physical process that involves the destruction of pathogenic organisms. All disinfectants are effective against vegetative forms of organisms, but not necessarily spores.
- **Major Survival Surgery** (Guide 2011 definition): As a general guideline, major survival surgery (e.g., laparotomy, thoracotomy, joint replacement, and limb amputation) penetrates and exposes a body cavity, produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection.
- **Minor Survival Surgery** (Guide 2011 definition): Minor survival surgery does not expose a body cavity and causes little or no physical impairment; this category includes wound suturing, peripheral vessel cannulation, percutaneous biopsy, routine agricultural animal procedures such as castration, and most procedures routinely done on an “outpatient” basis in veterinary clinical practice. Animals recovering from these minor procedures

typically do not show significant signs of postoperative pain, have minimal complications, and return to normal function in a relatively short time.

Special Considerations

- Rats and mice have a high surface area to body volume ratio and rapid metabolism. Therefore, rodents have a high metabolic rate and limited fat storage. Energy depletion can be stressful.
 - Rodents dehydrate faster per unit of time.
 - Rodents lose body heat rapidly. **Hypothermia during surgery is a frequent cause of intraoperative mortality or prolonged and stressful recovery.**
- Surgical Stress:
 - The purpose of a survival surgical procedure is to produce an animal model that is defined and that has the smallest degree of controllable variables. Minimizing tissue trauma, preventing infection, controlling postsurgical pain and discomfort, and supporting the animal's nutritional needs will reduce the magnitude of the metabolic response to surgery. An important objective is to return the animal to physiological normality as rapidly as possible. Supportive care during the first 4-6 hours post-op is key to the animal's recovery.

Tissue Trauma & Contamination

- Trauma and infection negatively impact the animal and also serve as confounding variables for experimental data. Diminish tissue trauma and infection by adhering to the following **four principles**:
 1. **Surgery is gentle**: Rough tissue handling results in increased pain, infection (clinical & subclinical) and recovery time.
 2. **Time is trauma**: Organ exposure to room environment is toxic to tissues. The longer the exposure the greater the trauma. Find the right balance between speed and fine surgical technique. Incidence of infection increases three times when surgery is longer than 90 minutes.
 3. **Wet tissues are happy tissues**: Avoid desiccation (drying) of exposed tissues by maintaining tissues moist at all times with warm saline or lactated ringer's solution (LRS).



The solution for pollution is dilution: Infection occurs when the number (generally $\sim 10^6$ infectious particles/gram [IP/gr] of tissue in immunocompetent animals) of infectious particles overwhelms the animal's immune system. Adhere as close as possible to the aseptic principles outlined in these notes to diminish the number of microorganisms in the wound site. If contamination occurs during the surgical procedure, dilute the contaminant with use of copious amounts of warm rinse solution (sterile saline or lactated Ringer's solution).

Dealing with the Risk

- There is no such thing as a 100% guarantee of “sterility” or a risk-free environment, therefore bringing the number of infectious particles to low and practical risk levels should be the goal.
- The level of acceptable risk depends on:
 - Type and length of procedure.
 - Complexity of the procedure.
 - Species, physiological status and immune status.
 - Surgeon’s training/skills/experience.
 - Animal preparation.
 - Surgical instrument/supplies preparation.
 - Aseptic technique.

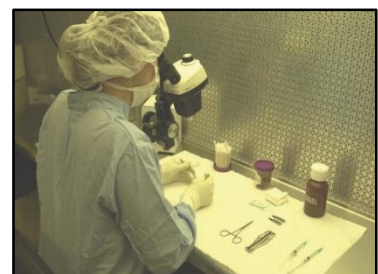
Preoperative Preparation of the Animal

- Assess health status. Recommendations:
 - Allow a minimum of a 3-day acclimation to the new environment to overcome the stress of transportation.
 - Should be free of clinical signs of disease:
 - Appearance should include normal posture and movement, glossy coat, bright eyes and good body condition.
 - Assess the character of respiration (no unusual respiratory sounds or pattern) and the cardiovascular status (bright pink coloration of ears and mucous membranes).
- Fasting rats and mice is generally unnecessary. Because rats and mice do not vomit, they do not have the risk of intra/post-op vomiting as in other species. If you will perform a surgery on the gastrointestinal tract, consult your veterinarian.
- Animal positioning
 - If limbs must be positioned for control of the surgical field, avoid placing excessive tension on the limbs, which may cause neural damage and shut off circulation and in some cases, respiratory compromise.
 - Secure limb(s) that need to be positioned.
 - If limbs must be secured, apply strips of tape around the carpal area and forelimbs. You can also apply a length of tape over the back, from carpus to carpus, to stabilize the forelimbs and torso.

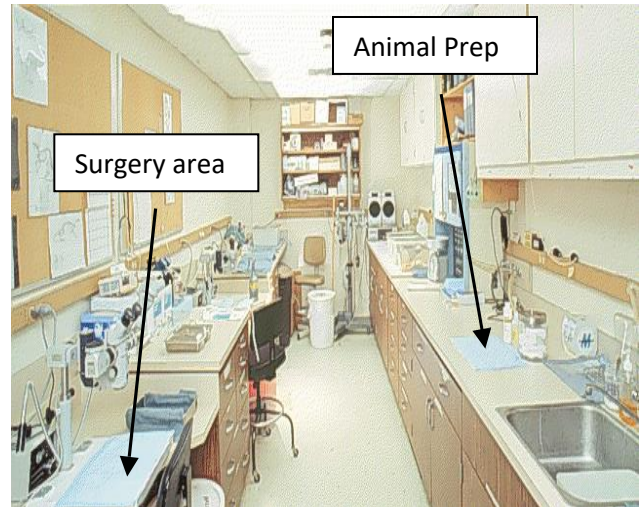
General Preparations for Surgery

Location

- What is necessary and required for survival surgery in these species is:

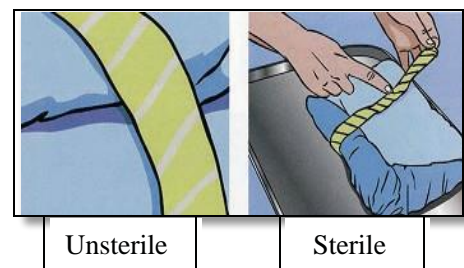


- 1) A clean, neat (uncluttered), disinfected area dedicated to rodent surgery for the duration of the procedure.
- 2) Free of debris and equipment not related to surgery.
- 3) A separation of functions of animal prep, operating field and animal recovery. These may be adjoining areas on a long bench top or better yet, animal prep is best done when performed in a room separate from the room where surgery is to be performed. The rationale is to avoid contaminating the operating field with loose animal fur, splashes from incision site scrubbing, and bedding dust and fur from nearby cages.
- 4) Avoid locations that are beneath supply ducts to minimize contamination from dust and air currents that may contribute to hypothermia in the animal.
- 5) Avoid high traffic areas such as those near doorways to prevent unnecessary interruptions and creation of air turbulence.



Instruments

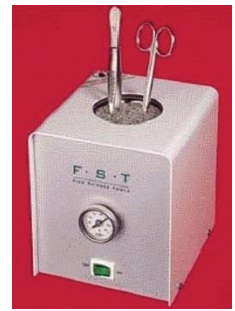
- Surgical instruments must be autoclaved. Be sure to use an indicator to test that the instruments are sterile, e.g., the strip test or chemical color indicator shown in these pictures. Temp stripes turn black indicating proper sterilization has occurred and chemical color indicator turns brown.



All Instruments should be double wrapped in linen or special commercially available autoclave peel pouches. Expiration dates should be written on all equipment packs. At UTA packs are considered expired one year after sterilization, if a pack has ripped (exposing contents), or if it has become wet. Autoclave settings should be as follows:

Autoclave Settings	Temp (F)	Pressure (PSI)	Time (min)
General Wrapped Items	250	20	30
Bottled Solutions	250	20	30

- If performing batch surgeries, i.e., using the same instruments on a series of animals, wipe them clean with alcohol or sterile saline, brush debris in instrument grooves with a toothbrush and re-sterilize instrument tips (e.g. in a hot bead sterilizer – see next section below) between animals. You may need two sets of instruments to alternate use between animals.



Hot Bead Sterilizer

- Hot bead sterilizer
 - Hot bead sterilizers are used for sterilizing instruments between surgeries. **This method sterilizes only the tips of the instruments.**
 - Beads must be pre-heated to the recommended temperature and the instruments exposed for the recommended time (generally tips of instruments are exposed for 60 sec or longer).
 - Gross debris must be removed from the instrument prior to sterilization. A sterile hard-bristled brush is recommended to brush debris away from grooves.
 - Allow instrument to cool before touching tissues.
 - If you are doing a full day of batch surgeries, then use a fresh set of autoclaved instruments for the morning and the afternoon series.

- No more than **five** rodent surgeries should be done using this sterilization method. A new set of autoclaved instruments must be used for the next group of animals. **TIP:** The use of two hot bead sterilizers will help distribute instruments between two systems, avoid overcrowding of instruments and reduce sterilization time.



- Liquid sterilants (e.g., Sporklenz)
 - If using cold sterilant solutions make sure instruments are exposed for the proper length of time specified by the manufacturer. Adhere to expiration dates of solutions.
 - Instruments must be removed from solution and rinsed with sterile water, saline, or alcohol to remove sterilant chemical residue.
 - Place rinsed instruments on a sterile field.
- Delicate instruments
 - Delicate instruments, materials for implantation or items that otherwise may melt or become damaged when heated can be sterilized using ethylene oxide.
 - The packs must be sufficiently aerated to prevent toxic side effects from residual gas.
 - This may require 24 to 72 hours.
- Instrument packs
 - Once packs are opened all other sterile equipment must be placed on the sterile field. These items must be opened in a way as to prevent contamination of the item or the surgical pack



- Organize the instruments in your surgical pack
 - Point all tips in one direction.
 - It is helpful to place them in the order used.
 - Between surgeries cover the tips of the instruments with sterile material (e.g., gauze).
 - Note that the space between the pack and the draped animal is not sterile; do not lay instruments in this space.

Animal Preparation

Preventing hypothermia

- Support normal body temperature during anesthesia.
 - Rodents have a high surface area and lose body heat rapidly.
 - A major cause of surgical mortality is not typically the surgery or the effects of anesthesia but **hypothermia**. Body temperature drops precipitously under sedation or anesthesia, which can cause irreversible shock and death.
 - Animals should be provided with a heat source pre-, intra- and post-operatively
 - Water circulating blankets, slide warmers, and Delta phase pads are preferred over electric heating pads. Delta phase pads only heat to a certain temperature set point, and therefore will not cause thermal burns.
 - If an electric pad is used, the setting should be placed on LOW only, and significant padding should be placed between the animal and the pad to insulate it. Rodents should never be placed directly on a heating pad.
 - The tail is an important thermoregulatory organ in rodents. When heating the animal, when possible, place the tail over the heating pad and under covers as illustrated in the picture to the right.
 - Place the animal on insulating materials (e.g. bubble wrap or folded drape).
 - It is easier to maintain normal body temperature than to reheat a chilled animal. If the animal is allowed to chill, there will be a reduction in circulation and organ function. Therefore, heating should be started immediately upon anesthesia induction.
 - Supplemental heat is especially important when using chemical fume hoods or biosafety cabinets because the high currents generated in these hoods will tend to further cool the animal.
 - Use warm fluids on tissue within an open cavity.
 - Check body temperature throughout the procedure.
 - Avoid keeping ambient temperature too cold if possible.



Animal Preparation

- Animals waiting for surgery should not be kept at a visual and olfactory distance from those animals undergoing surgery.
- Anesthesia
 - Isoflurane gas anesthesia administration through a precision vaporizer is generally considered the preferred method of anesthesia in rodents; however injectable anesthetics may also be used.
 - Gas anesthesia may be induced in a pre-charged (with gas anesthetic) induction chamber or it may be preceded by an injectable anesthetic cocktail.
 - For maintenance of anesthesia, a face mask or endotracheal tube may be used to deliver the anesthetic
- Protect the eyes: Anesthetized animals must have their corneas protected with an ophthalmic **ointment** (not solution). Avoid touching the eye with the tip of the ointment dispenser as it may scratch the cornea. The use of petroleum-based products such as mineral oil and Vaseline are not acceptable.



- Hair Removal
 - Remove fur along the incision site with small clippers. Clip surgical area so at least 1 cm of hair is removed from where incision where be placed. Use the sticky side of white tape to remove the loose fur or a handheld vacuum cleaner.
 - Depilatory creams may also be used. Strict adherence exposure time (up to 45-60 seconds) is important, as prolonged exposure to these creams may lead to chemical burns and localized inflammatory response of the skin.

- Antiseptic preparation of the surgical site:
 - The use of alcohol alone is not considered adequate as it is not a sterilant or high level disinfectant.
 - **Standard surgical prep consists of three alternating scrubs of a chlorhexidine scrub OR iodophor (povidone iodine) and 70% alcohol.**
 - Using a gauze sponge or cotton tipped applicator, cleansing should be done in a circular motion.
 - Begin at the center of the hairless area and work toward the periphery.
 - Never go back to the center with the same sponge.
 - Be careful not to excessively wet the animal as this can exacerbate hypothermia.
 - The following step-by-step procedure is recommended and will serve as a guide for a proper surgical preparation:
 1. Remove hair.
 2. Apply 70% alcohol to degrease the area.
 3. Apply chlorhexidine **scrub** (NOT solution) OR iodophor from center to periphery.
 4. Apply 70% alcohol from center to periphery.
 5. Repeat steps 3 & 4 two more times (or more).
 6. Allow the alcohol to dry before making the incision.

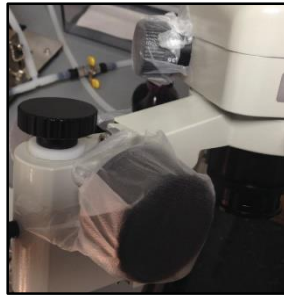


- Sterile draping is necessary to prevent viscera or sterile instruments from coming in contact with non-sterile areas like skin and fur. Types of drapes that may be used are:
 - Surgical impermeable paper drapes:
 - Inexpensive and autoclavable.
 - It may be precut or one in which you cut a hole.
 - A disadvantage to paper drapes is that they are not see-through and usually cover the animal making monitoring difficult.
 - Plastic drapes offer the advantage of more visibility.
 - Transparent, self-adhesive drapes, provided that the animal's body is dry (use sterile gauze to daub dry prepped skin).

Glad's **Press'n Seal** provides a sterile, inexpensive and effective method to cover the surgical field. Although this is a food/grocery item, it is manufactured sterilely.



The sticky part is placed on the animal, which allows easy monitoring due to the see-through nature of this material. Make sure the nose is not covered to avoid suffocation if a gas mask is not used. Press'n Seal may also be used to cover areas outside the surgical field that may need to be manipulated by the surgeon (e.g., gas anesthesia dials, knobs of the microscope or stereotaxic apparatus) and the surgical table.

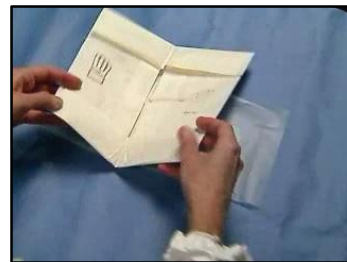


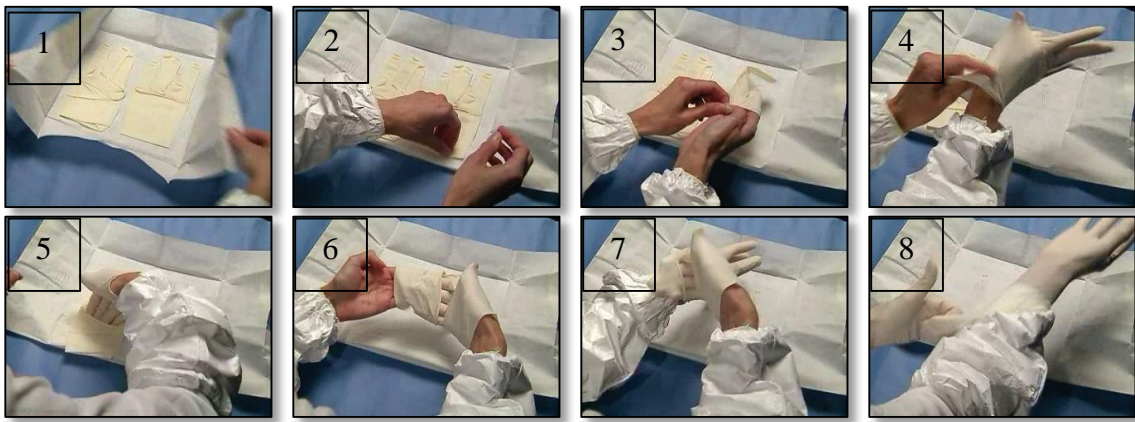
Press'n Seal – sticky side down

The sticky side of Press'n Seal should be down or it may stick to instruments and gloves. Permeable paper drapes are not recommended as they may allow wicking of bacteria into the wound.

Surgeon Preparation

- At minimum, the surgeon must wear a surgical facemask and a clean surgical gown, lab coat or scrub top.
- Before donning gloves, the surgeon should wash his/her hands.
- Sterile gloves or standard latex/nitrile gloves can be used. If standard gloves are to be used they must be disinfected using SporKlenz prior to starting surgery, after touching a non-sterile surface, and in-between surgeries. Allow time for gloves to dry before use or wipe-down with a sterile surgical towel prior to handling exposed tissue.
- Donning surgical gloves:
 - Open the package of gloves observing sterile technique.
 - Remember, the inside of the package is STERILE – exam gloves are not the same as sterile gloves.





- Donning sterile surgical gloves procedure:
 1. Don gloves in a way that prevents contamination of the outer surface of the gloves.
 2. One glove is lifted from the opened glove package by its turned down cuff.
 3. & 4. The glove is pulled on the hand with a rotating motion.
 5. Place the gloved fingers beneath the cuff of the other glove.
 6. With the gloved fingers under the cuff, the glove is placed on the ungloved hand. The folded cuff protects the gloved hand from contamination.
 7. Pull the cuff of the glove over the lab coat following insertion of the hand.
 8. The fingers are then slipped under the cuff of the first glove to pull it over the lab coat cuff.

Maintaining Asepsis

Once the surgeon, surgical areas, and animal are prepared, one must remain conscious throughout the procedure not to break the aseptic barrier that has been created.

- Gloved hands should be held elevated above the waist and table and should touch only the surgical incision and sterile objects, i.e. sterile instrument tray, sterile drape, and sterile coverings.
- Once gloved, do not touch or lean over a non-sterile area. Do not drop your hands to your sides. Do not touch gloves to your skin or clothes.
- Always lift an instrument from a sterile pouch or sterile surface. Do not drag instruments over the pack/drape edges because they can become contaminated.
- Do not allow surgical instruments to fall below the edge of the table. If an instrument does fall, the instrument is no longer considered sterile and should not be picked up and reused until re-sterilized.

ANESTHESIA AND ADMINISTRATION OF ANALGESICS

Anesthesia is a state where all perceived sensations are absent. Because drug effect can vary, you must assess the depth of anesthesia prior to beginning a painful procedure such as surgery.

The depth of anesthesia and the level of analgesia must be adequate to prevent the animal from feeling any pain in response to a surgical stimulus. **Before making an incision, vigorously squeeze both rear toes firmly (toe pinch reflex) 3-4 times, to test the animal's perception of sensation and pain. If the animal withdraws its leg or if respiration rate increases, then the anesthesia is too light.** The front toe pinch reflex may not be reliable as the pain perception may be present in the absence of a front toe pinch reflex.

Preemptive analgesia is the prevention of pain before it occurs or before the painful insult takes place. As an adjunct to general anesthesia, a preemptive local anesthetic(s) is used to desensitize a body area before making an incision. This reduces the pain of the surgical wound postoperatively. Preemptive analgesia is also accomplished by administering systemic analgesics before the pain insult occurs (e.g. before the surgical incision is made). **In general, analgesics are more effective when administered prior to surgery and should be used in most cases unless there is a justifiable reason not to.**

Much of the post-surgical pain is the result of sensations produced in the skin and body wall of the incision area. Anesthesia of the local nerves prior to incising these tissues will greatly reduce post-op pain and distress.

If a systemic analgesic is administered and/or a local anesthetic is infiltrated prior to the incision, such approach will block or diminish the sensory neuroexcitation caused by cutting the tissues even in the unconscious animal. When the animal wakes up, it will have a reduction in sensory stimuli from the incision area, and pain of the surgical wound will be greatly decreased both initially and throughout the period of wound repair.

Inject a local anesthetic subcutaneously to infiltrate it in the vicinity where the incision will be made. Allow a few moments for it to diffuse and take effect before beginning the surgery.

An effective and simple analgesic consideration (that does not require the use of DEA controlled drugs) would be to prepare a 50/50 mix of lidocaine 1-2% with 0.5% bupivacaine. This is relatively inexpensive and easy to administer. The surgeon can infiltrate the incision area immediately after closure or better yet, prior to making the incision while the animal is anesthetized. Lidocaine provides almost immediate pain control for 20-40 minutes and bupivacaine provides longer pain control for up to 4-6 hours. Lidocaine and bupivacaine doses should not exceed 10 and 6 mg/kg respectively. Higher doses may lead to heart arrhythmias.

METHODS OF DELIVERY OF INHALANT AGENTS TO RODENTS

The best method for the delivery of volatile agents to rodents involves the use of a precision vaporizer and an anesthesia chamber alone or in combination with a facemask appropriately sized for rodents. The **ACF has the equipment to safely and effectively administer inhalant anesthetics (isoflurane) to rodents using a precision vaporizer. Please contact ACF for details regarding use of this equipment.**

The rodent is placed within the chamber for induction at 4-5% isoflurane concentration. Once anesthetized, the animal is removed from the chamber with anesthesia maintained by delivery through a facemask at 1-3% concentration. Both chamber and mask delivery incorporate the use of a precision vaporizer for precise control of the concentration of anesthetic gas delivered to the patient. Because oxygen flow is required to volatilize the liquid anesthetic placed within the vaporizer, oxygen is also delivered to the patient and helps to maintain the blood oxygen saturation. Oxygen should be delivered at 0.5 L/min in mice and rats. Adequate scavenging of waste anesthetic gases (WAG) is necessary to avoid exposure to personnel. In general, isoflurane or sevoflurane anesthesia is superior to injectable anesthesia. Animals are induced and recover more quickly and the depth of anesthesia is easily controlled with the vaporizer dial. This allows for greater control of the anesthetic depth and minimizes experimental variables.

Precision vaporizers must be recertified at the manufacturer's recommended interval. Certification of isoflurane machines at UTA is required on an annual basis.

Calculating Vaporizer Oxygen Volumes:

Oxygen flow is calculated at 200-300 ml/kg/min but should never be kept at less than 500 ml/min, so for rats and mice maintain oxygen flows at 500 ml/min (0.5 L/min). 500 ml/min is an ideal oxygen flow rate.

- Gas Anesthesia Scavenging
 - o Waste anesthetic gas (WAG) from anesthetic gasses must be scavenged to minimize human exposure. Acceptable methods are:
 - Downdraft tables. These are usually only effective up to a height of 6-8 inches from the surface. Do not use induction chambers taller than this for induction of anesthesia.
 - Chemical fume hoods.
 - Type IIB biosafety cabinets that are vented to the outside.
 - Charcoal canisters. Charcoal canisters must be weighed before it is used for the first time, and after each use. Most canisters must be replaced after an increase in the recommended weight stated by the manufacturer. Depending on the size of the canister and the manufacturer's recommendations, the canister should also be weighed during especially long procedures to assure its continued effectiveness.

ANESTHETIC MONITORING OF RODENTS

Parameters that can be used to assess the depth of anesthesia in rodents include:

- Recumbency and loss of purposeful movements.
- Muscle relaxation.
- Lack of vocalization and limb movement.
- Loss of response to aversive stimulation (e.g. pinching the rear toes or tail pinch).

Because the ratio of body surface area to body mass is greater in rodents than in larger species, thermal support is critical to the successful recovery of rodents from anesthesia. Body heat may be dissipated from the tail, soles of the feet and ears with a resultant profound decline in the core and surface body temperature. Hypothermia may, in turn, lead to a decline in both anesthetic metabolism and any urinary excretion of the anesthetic agent. When possible, ensure the tail (a major thermoregulatory organ in rodents) is heated during surgery.

In most instances, cardiovascular and respiratory assessments are limited to observations of chest wall movement to determine respiratory rate and palpation of the apical pulse through the chest wall. For complex and long procedures physiological monitoring is recommended. Physiological monitoring systems are also available for purchase.

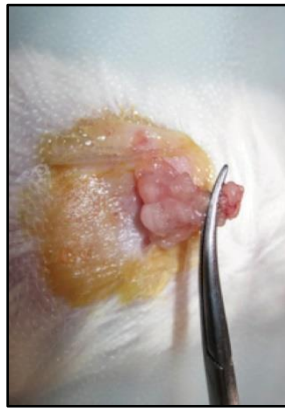
INTRAOPERATIVE CARE

- **Monitoring:**
 - Anesthetized animals must be monitored during the procedure to assure they stay in the proper anesthetic plane.
 - The anesthetic plane can be assessed by pinching the rear toes or tail for reflex response or by response to surgical painful stimuli.
 - Any reaction of the animal indicates the animal is too light and should be given more anesthetic.
 - The color of exposed tissues such as the pink soles of the feet are easy to monitor. Bright pink and red as opposed to pale, dusky grey or blue indicates tissue perfusion and oxygenation.
 - Respiratory pattern and frequency will also give an indication of anesthetic depth.
 - Core body temperature can also be monitored
 - Respiration – animal turns “blue” (hairless areas) if hypoxic.
 - Cardiovascular function – the animal’s hairless areas (normally pink) turn “pale” if tissue perfusion is poor.
 - Assess the cause of cardiac impairment:
 - *Anesthetic overdose* – if appropriate, use an antagonist or an anticholinergic (e.g. atropine or glycopyrrolate).
 - *Hypothermia* – perhaps the greatest cause of rodent surgical mortality.
 - *Hemorrhage* of 3-4 ml loss in a 200 g rat will cause irreversible shock.
 - Surgical technique to minimize blood loss.
 - Consider the use of fluid therapy – to support cardiovascular function or prevent dehydration.

SURGICAL TECHNIQUE

Good Technique includes:

- Asepsis.
- Gentle tissue handling.
- Minimal dissection of tissue.
- Appropriate use of instruments.
- Effective hemostasis.

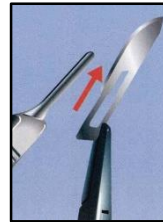


INSTRUMENT HANDLING: Generally scissors and hemostats are held with the thumb and the ring finger. Thumb forceps are held using a pencil grip technique.

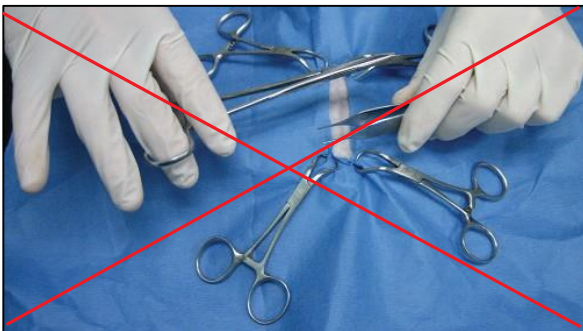
The following images illustrate proper (and improper) instrument handling:



Safe surgical blade loading with the needle driver on to the blade handle



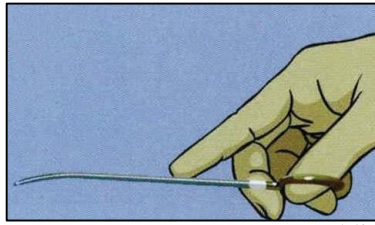
Safe surgical blade unloading



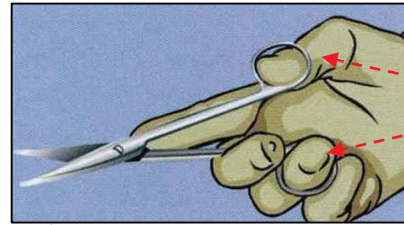
Wrong instrument holding



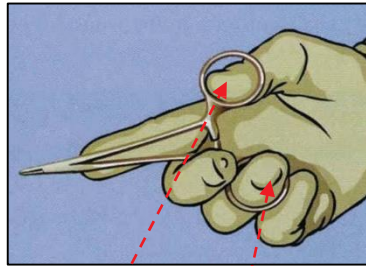
Correct holding: Pencil grip technique for holding thumb forceps



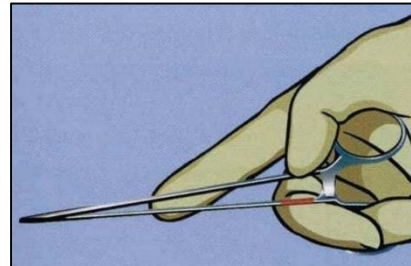
Holding hemostats or scissors
Thumb & Ring finger



Thumb & Ring finger



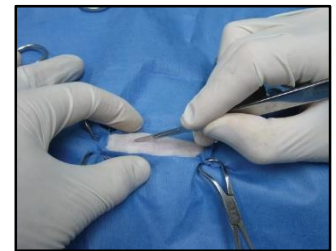
Holding the needle driver
Thumb & Ring finger



Palming the needle driver

18

SKIN INCISION: Placing tension on the sides of the incision with the non- dominant index finger and thumb while holding the scalpel handle with the dominant hand.



EZ WOUND CLIPS

- EZ Clip Applicators provide an excellent, fast and easy to apply method of skin wound closure. It is particularly helpful on animals with tendencies to chew their external sutures.
- Clips are easily removed with removing forceps.
- Place them approximately 5-8 mm apart.



TISSUE ADHESIVES

Wound closure should be simple, rapid and painless. The ideal surgical adhesive would be safe for topical application, easy to apply, polymerize rapidly, support the approximated skin edges and maintain the skin edge securely for maximum wound healing and eliminate the need for suture removal. Tissue adhesives offer many of the advantages of the ideal wound closure devices. They are painless and have antimicrobial activity against gram-positive organisms. They have a low rate of dehiscence and a low infection rate, and provide excellent cosmetic results. The use of Dermabond and Nexaband Topical Octyl Cyanoacrylate) as well as 3M Vetbond (n-butyl cyanoacrylate), which polymerize in seconds after contact with



tissue, significantly decrease the time for wound closure and eliminate the need for postoperative suture removal where appropriate. Additional benefits of these products include ease of use and formation of a protective barrier. Tissue adhesives are contraindicated when the wound is under tension.

Guide for Using Tissue Adhesive

1. Use subcutaneous or subcuticular sutures as needed to eliminate wound tension. Tissue adhesive should not be used as a replacement for proper subcutaneous closure.
2. Be sure wound edges and surrounding skin are dry, to assure direct tissue contact and prevent premature polymerization of adhesive.
3. Manually approximate the wound edges with forceps or gloved fingers.
4. Use gentle brushing strokes to apply a thin film of liquid to the approximated wound edges, and maintain proper eversion of skin edges as you apply adhesive.
5. Gradually build up 2-3 thin layers of adhesive. Ensure the adhesive is evenly distributed over the wound. The adhesive should extend at least ½ cm on each side of the apposed wound edges.
6. Avoid seepage into the wound as it may delay wound epithelialization and healing.
7. Avoid contact with eyes.

POSTOPERATIVE CARE

- Continue providing an external source of heat until the animal is conscious. Animals are considered conscious enough when the righting reflex has returned. The righting reflex is tested by placing the animal on its side or back. If the animal places itself on its four feet, then the righting reflex has returned.
- Provide the post-surgical animal a new cage that contains clean bedding to minimize wound contamination. The cage should also have a wipe all/paper towel placed for the animal to be laid on to prevent aspiration of bedding.
- Assess food and water intake for several days.
 - Animals may not drink for one or more days post-op and may therefore dehydrate. Recommended fluid replacement for mice is 17–33 ml/kg SC and 33 ml/kg IP; and for rats is 25 ml/kg SC or IP.
 - Test for dehydration by pinching and pulling the skin just cranial to the shoulder blades into a tent and then releasing it (“tenting the skin”). If normally hydrated, the skin will snap back towards the body. If dehydrated, the skin will fall slowly into place.



Recovery cage set-up



Skin Tenting

- Daily weighing is a sensitive method of monitoring the animal. While subtle changes in activity or appetite may not be observed, changes in weight will be quickly detected. Some analgesics depress appetite and must be differentiated from that which occurs if an animal is not feeling well. Weighing animals may be stressful to rodents and in place of this, a Body Scoring System (BCS) can also be used to assess the animal's condition. BCS can be

seen in the following pages.

- Observe the animals for signs of pain or distress postoperatively.
 - Remember that rats and mice are nocturnal and are less active during the day, making it difficult to assess their behavior at times of less than peak activity.
 - Unless proven otherwise, if a procedure is likely to produce pain in humans, it should be assumed to be painful in animals and should be treated with analgesics.
 - Compare posture and activity with normal animals.
 - Rats and mice are able to mask pain. Therefore, pain may be evident in altered behavior or it may not. Most evident will be a reduction in food and water consumption. Because of their ability to mask pain, it is my opinion that when pain is evident, it is likely to be at the moderate to severe levels. Therefore, a good and timely post-op analgesic regimen is paramount not only to protect against moderate/severe pain but for mild pain as well.
 - Abnormal signs of pain are: hunched posture, ruffled fur, red staining of eyes and nares, vocalization, greater or less tissue coloration, greater or less activity, resenting being handled, reluctance to be moved, abnormal gait, abdominal presses, aggressiveness, low water or food intake, and subtle weight changes.
 - **The first 24 hours are critical for pain management.**
- **In general the following guidelines should be used during the immediate post-op period:**
 - Never leave unconscious animal unattended (monitor continuously until return of righting reflex, i.e. sternal).
 - Administer analgesics as stated in the protocol.
 - Provide supportive therapy as needed (e.g. supplemental heat, warmed SC or IP fluids).
 - Monitor/document animal and incision daily for at least three days.
 - Notify your veterinary group if any complications appear.

Document post-op findings and activities. The following is an ACF suggested cage card that may be used for post-op monitoring:

RODENT POST-OPERATIVE DOCUMENTATION CARD

Surgical Procedure: _____

Date and Time of Procedure: ____/____/____ :____:

Anesthetic: _____ Analgesic: _____

Document administration of analgesia and monitoring by initialing in the table below. Additional information regarding monitoring is provided on the back of the card.

Time Post-Op	Post-Op	~ 12 hrs	~ 24 hrs	~ 36 hrs	~ 48 hrs	~ 72 hrs
Analgesia Administered						
Animals Monitored						
Comments						

Lab Contact _____

Contact Phone # _____

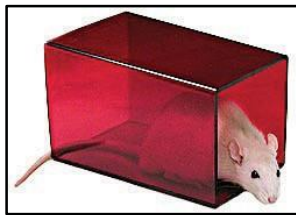
NON-PHARMACOLOGICAL METHODS OF PAIN CONTROL

Often ignored, non-pharmaceutical methods of pain control constitute an important adjunct to controlling post-operative pain.

Examples of non-pharmacological methods of pain control in rodents are:

- Keeping **low ambient lights** in a quiet room.
- Maintaining the **animal's body temperature**.
- Supporting **hydration** (supplemental fluids).

Providing **energy dense, highly palatable, and easily accessible food** (moist chow in dish on cage floor) or **supplements** (Diet Gel, sunflower seeds) will help reverse the immediate post-op catabolic state and speed up recovery.



- **Social housing during the post-op recovery period:** Single housing has been used as the norm during the post-op period in rodents with the traditional belief that pair or group housing after surgery may result in animals disturbing each others' wounds. Evidence has shown that post-op social housing for mice and rats has definite benefits in terms of increased survival and faster recovery rates. **Animals should be housed with cagemates once fully recovered from anesthesia.**
- **Enrichment:** Species-specific enrichment that promote species-specific behaviors such as hiding places, nesting and burrowing materials have been shown to decrease stress in the post-operative period and decrease blood pressure in hypertensive strains. Minimization of post-op stress is a critical non- pharmaceutical method of pain control.

BCS 1

Animal is emaciated



- Skeletal structure extremely prominent; little or no flesh cover
- Vertebrae distinctly segmented

BCS 2

Animal is under conditioned



- Segmentation of vertebral column evident
- Dorsal pelvic bones are readily palpable

BCS 3

Animal is well conditioned



- Vertebrae and dorsal pelvis not prominent; palpable with slight pressure

BCS 4

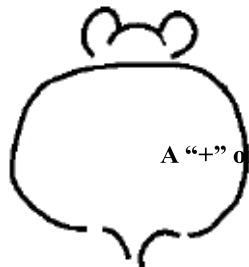
Animal is well over conditioned



- Spine is a continuous column
- Vertebrae palpable only with firm pressure

BCS 5

Animal is obese



- Animal is smooth and bulky
- Bone structure disappears under flesh & SC fat

A "+" or a "-" can be added to the body condition score if additional increments are necessary (i.e. ...2+, 2, 2-)