

Guidelines for Rodent Survival Surgery



∞ **Central Michigan University**
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Purpose

The Guidelines have been approved by the Institutional Animal Use and Care Committee (IACUC) and apply to all survival surgical procedures performed on rodents at CMU.

These guidelines provide information on aseptic surgical techniques in rodents. They are designed for experienced investigators and technicians, and serve as a teaching tool for individuals new to experimental surgery.

Prior to performing ANY surgery techniques on rodents an approved protocol must be in place with appropriately trained personal and procedures.

Survival surgery on rodents should be performed using aseptic technique (sterile instruments, surgical gloves, masks, lab coats, scrubs or sterile gown,) to reduce microbial contamination. Minor surgical procedures, such as wound suturing and peripheral vessel cannulation, should be performed in accordance with standard veterinary practices. As with all new techniques, patience and practice are required to harvest full benefits from the use of aseptic surgical techniques in rodents.

There is a common notion that rats are resistant to postoperative wound infection “This is False!” Relatively low-level bacterial contamination of surgical wounds may alter a rat’s physiology and behavior and confound the experimental measures, even though no clinical sepsis is evident. Published research has documented that post-surgical infections in rodents are subtle. The rat appears to eat and act normally, but will not respond appropriately to research stimuli.

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Regulatory and Policy Background

Animal Welfare Act (AWA)

- Requires aseptic technique for rodent surgery (hamsters, guinea pigs, gerbils and wild rodents).

Public Health Service (PHS), Policy on Humane Care and Use of Laboratory Animals

- Applies to animals involved in research conducted or supported by any component of PHS.
- Requires adherence to the “*Guide*” and the AWA

Guide for Care and Use of Laboratory Animals (“*Guide*”)

- Applies to all live vertebrate animals.
- Includes guidelines on facilities and procedures.
- Includes survival surgery, pre surgical planning, training and qualifications, aseptic techniques, surgical monitoring, post surgical care, and assessment of outcome.

U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, Teaching, and Training

VIII. Investigators and other personnel shall be appropriately qualified and experienced for conducting procedures on living animals. Adequate arrangements shall be made for their in-service training, including the proper and humane care and use of laboratory animals.

- Association for the Assessment and Accreditation of Laboratory Animal Care, International (AAALAC)
 1. Non-profit organization which promotes high-quality animal care, use, and well-being and enhances life-sciences research and education through a voluntary accreditation program;
 2. Uses guidelines set forth in the “*Guide*” for Care and Use of Laboratory Animals.

Definitions

Analgesia	The relief of pain without loss of consciousness.
Antiseptics	Chemical agents that either kill pathogenic microorganisms or inhibit their growth as long as the agent and microbe remain in contact.
Asepsis	The prevention of contact with microorganisms.
Aseptic Surgical Procedures	Surgery performed using procedures that limit microbial contamination so that significant infection does not occur.
Disinfectant	Kills 100% of vegetative bacteria (of certain species) under conditions specified by the Environmental Protection Agency, but are not efficacious against fungi, viruses, Mycobacterium tuberculosis or bacterial spores. These agents are only effective if used according the manufacturers instruction and may be inactivated by organic matter such as blood.

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.
Hemostasis	To stop bleeding.
Hypothermia	A body temperature below the average normal temperature.
Major Surgery	Any surgical intervention that penetrates and exposes a body cavity; any procedure that has the potential for producing permanent physical or physiological impairment (laparotomy, thoracotomy, craniotomy); and /or any procedure associated with orthopedics or extensive tissue dissection or transection.
Minor Surgery	Any surgical intervention that neither penetrates and exposes a body cavity, nor produces permanent impairment of physical or physiologic function (e.g. wound suturing, superficial vascular cutdowns, and percutaneous biopsy).
Sanitize	To make sanitary by cleaning (remove gross debris first).
Sterilant	Essentially the same as sporocides. They kill all microorganisms including bacterial endospores. A sporocidal product kills all microorganisms including bacterial endospores.
Sterile Zone	Area in front of the body, between the shoulder and the waist.
Sterilization	The process whereby all viable microorganisms are eliminated or destroyed. Sterilants are essentially the same as sporocides. They kill all microorganisms including bacterial endospores. The criteria of sterilization is the failure of organisms to grow if a growth supporting median is supplied.
Surgical Drape	Cloth or material used to cover parts of the body other than those to be operated on.

Surgical Facilities *(Also see Asepsis and Maintenance Log for Surgical Areas appendices)*

1. A Rodent Surgical area can be any room or portion of a room that is easily sanitized.
2. It should be an uncluttered area which promotes asepsis.
3. Survival surgery on rodents does not require a special facility;...however a room used primarily for aseptic procedures on rodents is desirable.
4. A laboratory setting is acceptable provided the procedures are performed on a clean, uncluttered table, lab bench; in a laminar flow HEPA filtered hood, or other type of isolator.
5. The surface area on which surgery will be performed should be cleaned using soap and water, rinsed thoroughly, and followed with an appropriate surface disinfectant (see Table) prior to and between surgeries. (Surface area must be impervious, sealed, durable and sanitizable).
6. Other activities should not occur in the surgery area when rodent surgery is in progress.
7. Access should be limited to people performing the procedure.
8. Areas close to corridors and doors should be avoided because air currents can cause dust to contaminate surgical fields.

RECOMMENDED HARD SURFACE DISINFECTANTS (e.g., table tops, equipment) Disinfectants must be applied following manufacturer's recommendations including <u>contact time</u>.		
Name	Examples¹	Comments
Alcohols	70% ethyl alcohol 85% isopropyl alcohol	Contact time required is 15 minutes. Contaminated surfaces take longer to disinfect. Absence of organic matter is necessary; remove gross contamination before using. Inexpensive.
Quaternary Ammonium	Roccal [®] , Cetylcide [®] Quatricide	Rapidly inactivated by organic matter. Compounds may support growth of gram negative bacteria.
Chlorine	Sodium hypochlorite (Clorox [®] 10% solution) Chlorine dioxide (Clidox [®] , Alcide [®] , MB-10)	Corrosive. Activity reduced by presence of organic matter. Chlorine dioxide must be made fresh. Kills vegetative organisms within 3 minutes of contact.
Aldehydes	Glutaraldehyde (Cidex [®] , Cide Wipes [®] , Cetylcide)	Rapidly disinfects surfaces. Toxic. Exposure limits have been set by OSHA.
Phenolics	Lysol [®] , TBQ [®]	Less affected by organic material than other disinfectants.
Chlorhexidine	Nolvasan [®] , Hibiciens [®]	Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses.

¹The use of common brand names as examples does not indicate a product endorsement.

General Guidelines

In general a rodent surgery should have the following components:

1. **A place for cages of rodents awaiting surgery.**
2. **An animal preparation area** for hair removal and initial skin preparation. This area should be separate from the surgery table to minimize the potential for contamination of the surgery area by aerosols generated during animal preparation.
3. **A surgery area.**
4. **A holding and recovery area** should be a quiet, undisturbed location where the animals can be observed.
- 5a. **A proper method of anesthesia** (consistent with approved protocol) should be selected. If gas anesthetics are used, appropriate methods for gas scavenging must be in place to avoid personnel exposure. The animal must be maintained in a surgical plane of anesthesia throughout the procedure. Vital signs (heart rate, respiratory rate, body temperature, pulse rate, skin color and hydration) of the animal must be monitored throughout the procedure. *(Also see Anesthetic Machine Log and Anesthetic Record appendices)*

5b. Request CMUs attending veterinarian look at Analgesic section and make recommendations

Special considerations along with general anesthesia:

- 1) Thoracotomy-systemic analgesia + local infiltration with bupivacaine along surgery site;
- 2) Stereotaxic procedures-3% lidocaine gel on ear bars and infiltrate incision line with local anesthetic (Examples: Lidocaine, Bupivacaine, and Liposomal Bupivacaine)

Preparation of Surgical Instruments (Also see Sterilization appendix)

1. Instruments, implantable devices, (catheters, trocars, osmotic pumps, telemetry) supplies and wound closure material used must be sterilized prior to surgery using any of the methods listed below.
2. Basic supplies should include sterile instrument pack, sterile supplies (drapes, gauze, gloves, instrument tray etc.), autoclave and /or glass bead sterilizer and a hot water blanket or some heat source for maintaining the animal’s body temperature.
3. The method of sterilization selected will depend upon the composition of the materials and the equipment available.
4. Proper sterilization techniques (including the use of sterilization monitoring devices, if applicable) must be followed to assure that consistent results are obtained. Sterilization indicators e.g. autoclave tapes or test cultures should always be included. Note that autoclave tapes only indicate that the surface reached the required temperature.
 - *Shelf-Life*: “The shelf life of a packaged sterile item is event-related and depends on the quality of the wrapper material, the storage conditions, conditions during transport, and the amount of handling”. Storing packs in sealed plastic bags will prolong shelf life.
 - *Expiration Date*: Each sterile item must be labeled with PD name, date sterilized and a control date for stock rotation. (HP rotates-repackages and sterilizes every 6 months.)
 - The following statement should be posted “Product is not sterile if packaging is open, damaged, or wet. Please check before using and monitor rotation dates”.

RECOMMENDED INSTRUMENT DISINFECTANTS		
Always follow manufacturers’ instructions (i.e. dilution, exposure times, and expiration periods)		
Agents	Examples ¹	Comments
Alcohols	70% ethyl Alcohol, 70-99% isopropyl alcohol	Contact time required is fifteen minutes. Not a high level disinfectant. <u>Not a sterilant</u> . Flammable.
Chlorhexidine	Nolvasan®, Hibiclens®	Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses.
¹ The use of common brand names as examples does not indicate a product endorsement. Instruments must be thoroughly rinsed with sterile water or saline to remove chemical disinfectants before being used.		

RECOMMENDED INSTRUMENT STERILANTS

Always follow manufacturers' instructions
(i.e. dilution, exposure times, and expiration periods)

Agents	Examples ¹	Comments
Physical: Steam Sterilization (moist heat)	Autoclave	Effectiveness dependent upon temperature, steam pressure, and time (e.g., 121 C for 15 min. vs. 131 C for 3 min.). Autoclaves should not be used for temperature sensitive instruments. Some corrosion may occur and some instruments may dull. Packs should not be removed from the autoclave until they are completely dried.
Dry Heat	Hot Bead sterilizer Dry Chamber	Rapid sterilization. Instruments must be cooled before contacting tissue. Only the tips of the instrument that have come in contact with the beads are sterile. Non-corrosive, penetrates most materials.
Ionizing Radiation	Gamma radiation	Requires special equipment.
Chemical (Gas sterilization)	Ethylene Oxide	Requires 30% or greater relative humidity for effectiveness against spores. Requires safe airing time.
Chlorine	Chlorine Dioxide	A minimum of 6 hours required for sterilization. Presence of organic matter reduces activity. Must be freshly made.
Aldehydes	Formaldehyde (6%)	For all aldehydes: requires many hours of contact time for sterilization. Corrosive and irritating. Consult safety representative on proper use. Glutaraldehyde is less irritating and less corrosive than formaldehyde.
Glutaraldehydes	Cidex [®] , Cetylicide [®] , Metricide [®]	Several hours required to sterilize corrosive irritating instruments must be rinsed with sterile saline or sterile water before use. Check with manufacture to determine contact time and if the chemical eliminates spores and which categories.
Hydrogen Peroxide	Acetic Acid Actril [®] , Spor-Klenz [®]	Several hours required to sterilize corrosive irritating instruments must be rinsed with sterile saline or sterile water before use. Check with manufacture to determine contact time and if the chemical eliminates spores and which categories.

¹The use of common brand names as examples does not indicate a product endorsement. Instruments must be thoroughly rinsed with sterile water or saline to remove chemical disinfectants before being used.

Sterilization Failure *(Also see Sterilization appendix)*

- Packs are wrapped too tight
- Improperly loaded autoclave
- Improper autoclave cycle selected
- Insufficient temperature and pressure
- Exposure time too short

Validation Methods of Sterilization

- Physical methods- thermocouples placed with load
- Chemical methods- packed within load or autoclave tape
- Biological methods- bacterial spores
- Bacillus Stearothermophilus for steam autoclaves

Maintaining Aseptic Technique (a Sterile Field)

1. The surgeon and assistants should restrict his/her contact to the surgical site and previously sterilized equipment until the incision is closed.
2. Use only sterile solutions and disinfect the tops/ports of bottles, vials, etc. before use.
3. Do not let catheters or implants become contaminated. Use a sterilized area (surgical tray, sterile towel or drape, or sterile gauze) to rest sterile materials on when not in use.
4. When possible, the ends of sterilized instruments should be used to manipulate and handle tissues. Minimize exteriorizing of organs, but if required, should be placed on the sterile drape and kept moist with sterile saline.
5. Instruments must be placed on a sterile surface when not in use.
6. Gloves must be changed if they come in contact with a non-sterile surface.
7. For “**major surgeries**” it is highly recommended to change sterile gloves between animals.
8. “**Minor surgeries**” on a single animal, require new sterile gloves.
9. Minor surgery on multiple animals housed in the same cage during the same sitting; one pair of sterile gloves can be used as long as they are disinfected (by wiping with an appropriate disinfectant and wiped with sterile saline) between animals and as long as asepsis has been maintained.

Instruments

1. Often rodent surgeries are done on multiple animals in a single session for major recovery surgeries; instruments must be sterilized between animals. More than one set of sterile instruments facilitates aseptic technique between animals.
2. For minor recovery procedures the instruments should be wiped clean of blood and tissues with sterile gauze, disinfected and rinsed in sterile saline or water.
3. One should use a new sterile pack for each cage of animals.
4. Segregation of instruments according to function helps insure aseptic technique (e.g. instruments used on skin should not be used within the abdominal cavity).
5. If using a cold sterilizer, follow manufacturers’ recommendations. Rinse them off with sterile saline before using them on the next animal.
6. Cold sterilants should be replaced when contaminated with body fluids or tissues.
7. The effectiveness of cold sterilization is directly dependent upon the contact time with the sterilants.

The surgeon should anticipate the number of surgical instruments required to guarantee uninterrupted conduct of the procedures while affording ample contact time.

8. The preferred method for sterilizing instruments between multiple animals involves wiping them clean with sterile saline solution, then inserting the tips of the instruments in a glass bead sterilizer. Follow the steps below for proper sterilization of instruments:



- Turn the power switch to “ON” and wait until the “STERILIZE” light illuminates. It will take approximately 30 minutes for the “STERILIZE” light to illuminate, thus indicating the beads have reached a minimum decontamination temperature of 450°F (233°C). The glass beads will continue to heat up and stabilize at approximately 500°F ± 15° with minor fluctuations from the on/off cycles of the heating element. These minor fluctuations will have no effect on the decontamination time.
- Sterilize clean and dry stainless instruments only.
 - Remove all debris from instruments prior to insertion into the glass beads. Any matter left on the instruments may get baked-on and will be difficult to remove. Instruments with visible debris will take longer to sterilize and could also cause the glass beads to adhere to the wet and contaminated portions of the instruments
- Gently insert the tip portion of the instrument into the sterilizer.
 - Only the portion of the instrument touching the glass beads will be decontaminated.
 - **NOTE:** The top ½ inch of glass beads will lose an excess amount of heat and will tend not to be within the recommended temperature for proper decontamination. Therefore, if you wish to decontaminate one inch of the instrument tip you must insert it at least 1½ inches into the glass beads.
 - Be careful not to force instruments into the glass beads to avoid damage to delicate tips.
 - It is also necessary to periodically stir the glass beads to prevent the growth of heat resistant microorganisms that could survive in the cooler top ¼ inch of the well from contaminating your instruments.
- Small instruments should remain in the glass beads for at least 15 seconds before they are removed. Larger instruments should remain in the glass beads for at least one minute.
 - Inserting more than two normal size micro dissecting instruments will drop the temperature of the glass beads below its operating temperature. If inserting more than one instrument into the glass beads, it is recommended that the decontamination time be doubled according to the instrument size.
 - Instruments can remain in the glass beads longer than their recommended time. **NOTE:** The longer instruments are left in the glass beads, the hotter the instrument will become.
 - The metal properties of some instruments could degrade if they are left in the glass beads for an extremely long period.

- When removing the instrument from the glass bead well make sure that none of the beads are attached to or stuck in the instrument. Failure to detect glass beads on your instruments could have an adverse effect on your research site. If necessary, tap the instrument lightly on the side of the glass bead well to remove beads. If beads remain lodged or attached, clean instrument thoroughly of visible contaminant and use a small sterilized probe to dislodge beads from the instrument.
- To avoid contamination of instruments during the surgical procedures:
 - Lay the sterilized instrument on a sterile field (i.e. sterile towel).
 - Always keep the sterilized tips pointed in the same direction.
- The instruments must be allowed to cool before applying them to the skin or other tissues.
- Between animals, the instruments may be covered with sterile gauze, hand towel, or drape.
- When finished with the unit for the day, turn the power switch off.
 - **NOTE:** If the glass bead sterilizer has been turned off for more than 30 minutes and the “STERILIZE” light comes on when the unit is turned back on, allow approximately 15 minutes for the temperature to stabilize before using again. This will ensure it is at the proper operating temperature.

Surgeon Preparations

Prior to scrubbing hands, the surgeon and any assistants working in the immediate surgical area should remove jewelry, don a surgical cap/bonnet, shoe covers, a facemask, and a clean laboratory coat or surgical scrubs. A sterile gown and facemask are preferred for major surgeries.

Hand Scrubbing:

1. Surgeons should wash and dry their hands before aseptically donning sterile-surgical gloves.
2. Scrubbing should be thorough beginning at the tip of the fingers all the way to the elbows using a surgical scrub containing a germicide (e.g. chlorhexidine, or iodophors e.g. Betadine®). Vigor and exposure times are critical, 3-15 minutes or 5-20 brush strokes per surface.
3. Rinse thoroughly from finger tips to elbows.
4. At the end of scrub, dry your hands with a sterile towel beginning at the tip of the fingers to the elbow. Rotate the towel and repeat the procedure on the other hand. When available put on a sterile gown, which may require assistance.

Scrubbing hands



Drying hands



Gloving Procedures:

1. Sterile surgical gloves are required by the “Guide”. Using sterile surgical gloves allows you to touch all areas of the sterile surgical field and surgical instruments with your gloved hands.

vs.

Tip only (post bead sterilization) technique restricts you to using only the sterile ends of the surgical instruments to manipulate the surgical field.

2. Remove outer cover; open the inner paper covering on the gloves as illustrated.
3. Make sure to not touch any non-sterile surfaces. If the gloves or gown touch non-sterile surfaces, discard them and proceed to regown and reglove.
4. Whenever performing multiple surgeries, a fresh pair of sterile gloves should be used for each cage of rodents.
5. Always maintain a zone of sterility (sterile zone) in front of you.
6. The sterile zone is defined by the area in front of the body between the shoulders and waist. Keep your hands above the table.
7. The gloved, but not sterile hand must never touch the working end of the instruments, the suture, suture needle, or any part of the surgical field. This technique is useful when working alone and manipulation of non-sterile objects (e.g. anes machines, microscope, stereotaxic etc.) is required.

Animal Preparation

1. Animals should be provided a period of stabilization, and acclimatized to the facility, to minimize risk of complications and reduce research variables. (Generally 3-5 days after arriving from the vendor; some instances may require this period be increased up to two weeks).
2. Proactive stress-reduction plan should be developed for all animals (including single housing, pre-operative monitoring, conditioning animal to proper handling, restraint, as well as to a procedure or administering analgesics preemptively).
3. Prior to surgery it is important that the animals are properly identified.
4. Note the weight (weigh for injectable anesthetics), age, sex, pregnancy status of each animal.
5. Fasting is generally not required in rodents, due to high metabolic rate, unless specifically mandated by the protocol.
6. In some cases, it may be preferable to initiate antibiotic or analgesic treatment prior to surgery.
7. Consider administering fluids pre-operatively.
8. Apply ophthalmic ointment to the eyes, following induction of anesthesia to protect the corneas from drying out. If the procedure is long reapply the ophthalmic ointment.

Skin Preparation:

1. The animal should be prepared for surgery at a location separate (bench or room) from where the surgical operation will be performed.
2. Enough hair should be removed from the area to ensure hair is not incorporated into the wound closure.
3. Hair removal should be done carefully using a # 40 blade to avoid skin abrasions and thermal injuries.
4. Remove clipped hair from the animal using a vacuum-system or tape.

Prep Surgical Site:

- A sterile gauze sponge or Q-tips can be used for prepping.
- Avoid wetting large areas of fur with prep solution due to potential to induce hypothermia.
- During the “prep” begin along the incision line and extend outward in a circular pattern. Never from outward (dirty) towards the center (clean). Do not go over the incision site twice with the same gauze / Q-tip.

General Path of Aseptic Skin Prep



----Incision ____ Path

Skin Disinfectants-Best Practices¹		
Using alternating disinfectants is more effective than using a single agent. An iodophor scrub can be alternated 3 times with 70% alcohol, followed by a final prep, with a disinfectant solution. “Alcohol by itself is <u>NOT</u> an adequate skin disinfectant” – NIH. The evaporation of alcohol can induce hypothermia in small animals.		
NAME	EXAMPLES²	COMMENTS
Iodophors	Betadine® Prepodyne® Wescodyne®	Inactivates a wide range of microbes but their activity is reduced in the presence of organic matter. Works best in pH 6-7.
Cholorhexidine	Hibiclens® Nolvasan®	Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses. Excellent for use on skin.
Alcohols	70% ethyl alcohol, 70-99% isopropyl alcohol	Not completely adequate for skin preparation. Contact time required is fifteen minutes. Not a high level disinfectant. Not a sterilant. Flammable. Effective against many viruses. Excellent for use on skin.
¹ AAALAC Council accepts alcohol as a skin disinfectant for rodent survival surgery noting, however, that it will not kill spores, certain viruses and protein rich materials.		
² The use of common brand names as examples does not indicate a product endorsement.		

Draping

The decision to drape depends on the procedures being done.

1. Minimal surgical intervention:

- Optional to drape. However tips of instruments must stay within the sterile field.
- Avoid contamination of instruments, surgical gloves, incision site and supplies.

2. Extensive procedure:

- Draping is necessary, using towels, stockinet, drapes, gauze, or plastic wraps.

3. While drapes play an important role in reducing contamination of the surgical site, faulty technique may increase contamination.

4. Drapes should cover the entire animal.

Heat Loss

1. Due to a large surface area to body weight ratio, rodents tend to lose heat rapidly and should always be kept warm.
2. Circulating hot water blankets, warmed fluid bags, warming blankets, and warming discs should be utilized.
3. Anesthesia alters thermoregulation and reduces metabolism.
4. Heat loss occurs from the tail, ears, feet, open body cavities and evaporation of body fluids.
5. Heat loss prolongs the duration of anesthesia and recovery which increases the risk of complications.

Fluid Loss *(Also see Body Condition Scoring Guide appendix)*

1. Fluid loss occurs as a result of evaporation from body cavities and blood loss.
2. Rodents are vulnerable to intra-operative fluid loss due to their small size and total body fluid content.
3. Reduce fluid loss by:
 - Irrigating the operative field with warm sterile saline (be careful not to wet drapes).
 - Normal maintenance volumes of Lactated Ringers Solution, or 0.9% saline, or glucose-saline can be injected in amounts of 1-2 ml/25 g mouse and about 5-10 ml/ 250 g rat per day. The subcutaneous administration of these volumes may begin prior to a study and continue once daily (or split in two doses a day) throughout the period of expected morbidity. Therapeutic fluids should be warmed prior to injection because fluids administered at room temperature will chill the animal. Fluids can be loaded into syringes and kept warm in rodent support areas. Analgesic treatments may be combined with daily fluid administrations (for hydration therapy). For convenience in treating multiple animals, you can figure the total fluid volume needed for the study and add the appropriate amount of analgesic to a concentration that will deliver the desired dose in each aliquot administered (**AALAS LEARNING LIBRARY** Post-Procedure Care of Mice and Rats in Research: Minimizing Pain and Distress, Lesson 9: Fluid and Electrolyte Balance).
 - If blood loss has occurred or if the animal is slow to recover provide additional fluids.
 - Control blood loss.

- Monitor water and food intake, body condition, and weight loss post surgically.

Principles of Operative Techniques

“Tip-only” Technique to maintain the sterility of the instruments

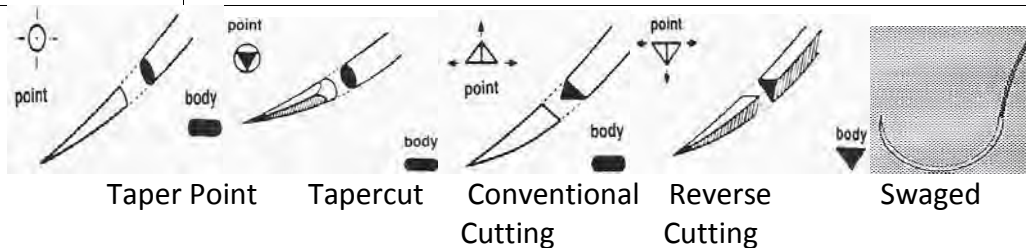
1. The animal must be maintained in a surgical plane of anesthesia throughout the procedure.
2. Begin surgery with sterile instruments and handle instruments aseptically.
3. When using “tip-only” technique, the sterility of the instrument tips must be maintained throughout the procedure.
4. Instruments and gloves maybe used for a series of similar surgeries provided they are maintained, clean, and disinfected between animals.
5. Monitor and maintain the animal’s vital signs and hydration.
6. Close surgical wounds with appropriate materials.

Sterile Field Technique

1. Follow aseptic surgical procedures.
2. Use procedures that limit microbial contamination so infection does not occur.
3. Utilize good surgical technique.
4. Tissue dissection should be done along natural fascial planes when possible.
5. Tissues should be handled gently with appropriate instruments.
6. Exposed tissue should be protected from drying.
7. When controlling hemostasis, only the vessel to be occluded should be incorporated in a ligature.
8. Antibiotics maybe used prophylactically, however, the use of antibiotics to compensate for a non-sterile surgical technique is unacceptable.
9. Vascular catheters should be tunneled to exit the interscapular area if possible.
10. Use appropriate size and type of needles and suture material.

Needles:

Taper Point	<u>Non-cutting</u> , <u>atraumatic</u> , and <u>round</u> . The body of this needle tapers down to a fine point, permitting minimum tissue damage. This needle has NO edges to cut through tissue. This needle is especially suitable for soft tissue, abdominal viscera, peritoneum, intestines, connective tissue, vessels, and other fragile tissues.
Tapercut	Taper body and cutting tip. Readily penetrates dense tissue but does not cut through it. For tough tissues. Like two needles in one
Conventional Cutting	Smooth penetration for passage through dense connective tissue (skin, tendons).
Reverse Cutting	For tough, difficult-to penetrate tissues, minimizes excessive cutting of transfixed tissue, such as skin.
Swaged	Joins the needle and the suture.
Auto clip	Stainless wound clips, staples for skin closure



Suture Material:

1. In general closure of the body wall or other wound closures should be completed using an absorbable suture material. Blood vessels should be ligated with slowly absorbable or nonabsorbable sutures. Nonabsorbable monofilament suture material should be used for skin closure.
2. The smallest appropriate suture material that will perform adequately should be used (e.g. Vicryl, sizes 3-0 and 4-0 in a rat; 4-0 and 5-0 in a mouse).
3. Non-absorbable suture material including sterile staples or wound clips should be removed 7-10 days after surgery, or when the wound has completely healed.
4. Sterile staples/wound clips used to close skin incisions are acceptable. Wound clips have a higher potential for post-operative infection, tissue tearing and other side effects. Attention should be given to placement and spacing (5mm apart) to prevent tearing of skin, or catching on caging.
5. Sterile surgical adhesive can be used for skin closure. It is important to align wound edges together using a probe; good for small incisions (1/2" or less). Area needs to be dry and free of blood. Apply drops of adhesive 3-5mm apart. Do not apply to fur.

Suture Types: (Also see *Surgical Technique appendix*)

SUTURE SELECTION	
Suture Characteristics and Frequent Uses	
Polyglycolic Acid Polyglactin 910 [®] , Vicryl [®] , Dexon [®]	Absorbable; 60-90 days. Ligate or suture tissue where an absorbable suture is desirable.
Polydioxanone (PDS [®]), or Polyglyconate Polypropylene (Maxon [®])	Absorbable; 6 months. Ligate or suture tissues especially where an absorbable suture and extended wound support is desirable.
Prolene [®] Nonabsorbable, Inert	
Nylon [®] (Ethilon [®]) Nonabsorbable, Inert, General Closure.	
Silk Nonabsorbable. (Caution: Tissue reactive and may wick microorganisms into the wound). Excellent handling. Preferred for cardiovascular procedures.	
Chromic Gut Absorbable. Versatile material.	
Stainless Steel Wound Clips, Staples Non-absorbable. Requires instrument for skin removal. (Mice 5mm) (Rat 9mm)	
Cyanoacrylate Surgical Adhesive (Vet Bond [®] , Nexaband [®] , Tissue Mend [®])	Skin glue Good for small incisions (½" or less), and non tension bearing wounds. Area needs to be dry and free of blood.

Suture patterns:

1. Simple interrupted suture pattern is preferred to continuous for skin closure.



Simple Interrupted

Continuous

Principles of Postoperative Care (Also see *Body Condition Scoring Guide, Post Procedural Pain Potential and Post-Operative Evaluation appendices*)

1. Monitor the animal regularly (at least every 15 minutes) during the procedure and until the animal is fully ambulatory. Written records on animals must be maintained for surgeries. The use of monitoring equipment to record clinical parameters is encouraged.
2. Body temperature needs to be maintained to minimize hypothermia. Place animal in a clean, dry recovery cage with absorbent toweling and provide warmth with a circulating water blanket, warm water bottle, blankets, blue diaper pads, heat discs, or other method (remove regular animal bedding from the recovery cage—absorbent toweling should be used).
3. Frequent checking of the animal is necessary to prevent hypostatic congestion. Provision must be made so that an awake animal can move away from the heat source.
4. Observe to make sure animal's head is not down in absorbent toweling or in a corner of the recovery cage which would compromise respiration.
5. Turn animal from one side to the other periodically (every 15 minutes) until they are able to maintain sternal recumbence.
6. House rodents individually until they are fully ambulatory, to prevent cannibalism or suffocation. Do not return them to the vivarium until they are stable and awake. Place them in a clean bedded cage once stabilized.
7. Monitor animals, observe breathing activity level and mucous membrane color (should remain pink).
8. Post-surgical animal should be seen at least daily for 7-14 days by a member of the Project Director's staff or other identified trained individual to ensure that there are no complications.
9. If the animal appears ill, or the surgical wound appears abnormal, contact the facility coordinator and research staff immediately.
10. Provide analgesics as appropriate and as described in approved protocol. (Refer to Central Michigan University IACUC Policy/Guideline/Principle of Care in Animal Pain and Distress Management CMU-P-010-00)

Potential signs associated with pain or distress in rodents: (Also see *Guidelines for Pain and Distress in Laboratory Animals: Responsibilities, Recognition and Alleviation appendix*)

- Decreased food and water consumption, weight loss
- Self-imposed isolation/hiding
- Rapid, open mouth breathing
- Biting, aggression, vocalization
- Increased/decreased movement
- Unkept appearance (rough, dull hair coat)
- Abnormal posture/positioning (hunched back, head-pressing)
- Dehydration, skin tenting, sunken eyes
 - Fluid therapy is indicated whenever surgery is performed, or if the rodent becomes dehydrated due to illness. Decreased water intake may occur due to pain, disease, or inability to access the water bottle. Hydration status can be checked by pinching up or "tenting" the skin over the nape of the neck. The skin should immediately relax into its normal position. If the skin remains tented longer than normal, the rodent is dehydrated. The eyes may also appear sunken.

% Dehydration

<5%

5%

6-8%

10-12 %

12-15%

Detectable Signs

Not detectable

Subtle loss of skin elasticity

Definite delay in return of skin to normal position

Tented skin stands in place, eyes are sunken

Signs of collapse and severe depression; on the verge of death

Rodents that are dehydrated often do not eat well, become depressed, and will not heal well following surgery. Fluid replacement therapy is quick and easy to perform, and can be lifesaving. Either lactated Ringer's solution or sterile saline for injection may be used as replacement fluids. These are both inexpensive and readily available.

- Change in body temperature
- Twitching, trembling

**Attached is an example of a score sheet used to evaluate these signs. Score sheets are used as an aid to determine the appropriate time to humanely euthanize an animal.

11. If appropriate consider giving fluids and/or nutritional support, determine cause of pain/distress, provide supplemental therapy and support, and contact veterinarian and facility manager.

Postoperative records (*Also see Body Condition Scoring Guide, Post Procedural Pain Potential and Post-Operative Evaluation appendices*)

Maintain a surgical record with important operative and post operative information. A postoperative card (i.e. Mark cage card with procedure and date, body weight on the day of surgery, analgesics administered, wound closure removal date, etc.) should be completed and affixed to the animal's cage card. Having the record in the room accomplishes several functions:

1. It explains the animal's condition to the animal care staff.
2. It assures animal care staff and inspectors that the animal is being cared for.
3. It informs animal care staff how recently the investigator has seen the animal; this knowledge helps them decide whether there is a need to contact the investigator to inform him or her of the present condition of the animal.

Maintain daily post operative score sheet monitoring 5-10 days out. Follow NIH body scoring system.

Although individual records are desirable, a composite post-operative record may be used for a group of rodents.

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Guidelines for Pain and Distress in Laboratory Animals: Responsibilities, Recognition and Alleviation

Introduction

Animals can experience pain and distress. It is the ethical and legal obligation of all personnel involved with the use of animals in research to reduce or eliminate pain and distress in research animals whenever such actions do not interfere with the research objectives. The Institutional Animal Care and Use Committee (IACUC) have the delegated responsibility and accountability for ensuring that all animals under their oversight are used humanely and in accordance with a number of Federal Regulations and policies. 1, 17, 23, 24, 25 Key to fulfilling the responsibilities for both the principal investigator (PI) and the IACUC are:

- Understanding the legal requirements.
- Being able to distinguish pain and distress in animals from their normal state.
- To relieve or minimize the pain and distress appropriately.
- Establishment of humane endpoints.

Regulatory Requirements

The IACUC must assure that all aspects of the animal study proposal (ASP) that may cause more than transient pain and/or distress are addressed; alternatives to painful or distressful procedures are considered; and that methods, anesthetics and analgesics to minimize or eliminate pain and distress are included when these methods do not interfere with the research objectives; and that humane end points have been established for all situations where more than transient pain and distress cannot be avoided or eliminated. Whenever possible, death or severe pain and distress should be avoided as end points. A written scientific justification is required to be included in the ASP for any more than transient painful or distressful procedure that cannot be relieved or minimized.

The obligation to reduce pain and distress **does not end** with the review of the ASP. It is the responsibility of the animal care staff, the research staff, veterinarians and the IACUC, to continue to monitor animals for pain, distress, illness, *morbidity* or mortality during the course of the research study.

If unexpected pain or distress occurs, and is more than an isolated incident, the PI must submit an amendment delineating the unexpected problem and stating their proposed resolution to the issue (i.e. administration of analgesics, lowering the dose of a drug that was administered, etc.).

Alternatively, the PI could justify the need for unrelieved pain or distress in the amendment, or in the case of regulated species as a Column E Justification.

If it is necessary to make changes in the ASP procedures, the PI must submit an amendment to the IACUC and receive approval prior to instituting the modification. For example, if unexpected pain or distress was noted following treatment with a specific dose of an experimental agent, the PI must submit an amendment delineating the unexpected problem and stating their proposed resolution to the issue (i.e. administration of analgesics, lowering the dose administered, etc.). Alternatively, the PI could justify the need for unrelieved pain or distress in the amendment, or in the case of regulated species as a Column E Justification.

Recognition of Pain and Distress

Animals should be monitored by trained individuals for pain and distress as appropriate for the species, condition and procedure. Critical to the assessment of the presence or absence of pain or distress is having the ability to distinguish between normal and abnormal animal behavior. This is especially true when dealing with species that often exhibit pain and distress with only subtle

changes in their behavior (see Table 1). Therefore, it is critical that the individuals assessing an animal be trained in the species specific signs of pain and distress, as well as be knowledgeable of the potential outcomes of the procedure, surgery or treatments administered to the animal. Pain and distress scoring is a method to convert subjective animal observations into objective scoring system which some have found to be helpful in assessing animal behavior.

Whenever more than transient pain or distress is anticipated preemptive measures should be taken to minimize or prevent the development of pain and/or distress. Following the implementation of preemptive or palliative measures, animals must be monitored to ensure the efficacy of the measures taken and determine if or when additional treatment will be necessary. The extent and frequency of monitoring will depend on the level of post-surgical/procedural pain and/or distress anticipated and the chosen intervention strategy(s). For example, animals undergoing a procedure known to produce no more than minimal/transient pain or distress may be adequately monitored by the daily observation of a trained animal caretaker. Whereas, the monitoring of an animal undergoing a procedure known to result in severe pain and/or distress may require more frequent monitoring by a team of trained individuals (e.g. trained animal care staff, technicians, veterinarians, investigators, etc.). Animals undergoing pilot studies or procedures new to the investigator or facility may also require a higher frequency of monitoring and a team approach. It is ultimately the responsibility of the PI and the personnel conducting the procedure to ensure the timely and adequate identification, monitoring and documentation of the animals undergoing potentially painful or distressful procedures. Investigators may request the assistance of institute and facility veterinary and technical personnel when monitoring their animals, but all individual(s) responsible for monitoring an animal must be identified prior to conducting the procedure and their accountability clearly delineated and accepted.

Animals should be observed a minimum of once daily or more often based on professional judgment and the research being conducted. The animals should be monitored for expected and unexpected signs of more than transient pain or distress and, if observed, appropriate intervention strategies implemented (e.g. non-pharmacological approaches, analgesics, anesthetics, euthanasia, etc.), unless the withholding of treatment is scientifically justified. Observations and actions taken to relieve pain or distress must be documented.

The documentation of monitoring of the animal is important and required. The nature and the frequency of the documentation are dependent on the species and the potential for pain and/or distress. For example, the identification of cages containing an animal which has undergone a potentially painful or distressful procedure with a "special observation" cage card has proven helpful in drawing special attention to the animal during the caretaker's daily health check. Cages containing animals requiring more intensive monitoring should also be appropriately identified and their monitoring and/or treatments documented either at the room, cage or animal level (e.g. room log, cage card, medical record, etc.), in addition to the investigator's notations in their laboratory notebook. Documentation must be available to all personnel monitoring the cage or animal (i.e. IACUC, veterinarians, animal care staff, etc.).

Intervention Strategies

Strategies for the management of pain and distress may include non pharmacological considerations (e.g. modified housing and husbandry practices, dietary modifications, desensitization and acclimation strategies, etc.), pharmacological interventions or euthanasia. The chosen strategy will vary with the species, the procedure(s) being performed, duration of action needed, route of administration preferred, degree and type of analgesia required and research being conducted (see Table 2). It is strongly suggested that PIs consult their IC veterinarian during the development of an animal study protocol, prior to its submission to the IACUC.

This approach has been demonstrated to expedite the protocol approval process.

Excellent resources and formularies are now available which provide extensive information on the recognition and alleviation of pain and distress in laboratory animals (See Plumb Veterinary Drug Handbook²⁶ and other references below). These resources, coupled with trained and skilled animal care personnel and the professional judgment of your IC and animal facility veterinarian provides each investigator and their IACUC with powerful tools for the recognition and alleviation of pain and distress in laboratory animals.

Preemptive measures should be taken to minimize or prevent the development of pain and/or distress. For example, a skilled surgeon can often minimize tissue trauma which in turn minimizes post-operative pain and distress. The use of ketamine or opioids preemptively, even in low doses has been demonstrated to prevent the development of some forms of pain.¹⁹ In addition, the use of a single dose of a non-steroidal anti-inflammatory agent (NSAID) or other analgesic agent can have a positive effect on the speed with which animals return to normal behavior.^{12, 13,29,30,31} It has been repeatedly demonstrated in humans that the provision of effective analgesia reduces the time taken for post-operative recovery.⁸

There are also many pharmacological intervention strategies for the management of pain and distress. Traditional analgesics include local or regional anesthetics, opioids and NSAIDs. Using two or more of classes of these analgesics together, or combining these analgesics with nontraditional analgesics such as NMDA antagonists,^{16,33} alpha₂-agonists,²⁸ tramadol,⁴ and even the antiepileptic drug gabapentin²¹ has been shown in both human and veterinary patients to enhance analgesia and allow a reduction in the use of more powerful analgesics. This approach, called multimodal analgesia also has the advantage of providing even analgesic dosing thus promoting the animal's well being.

For procedures in which the pain intensity is anticipated to be high, techniques such as constant rate infusions of local anesthetics and or opioids either systemically, locally at the surgical site or via an epidural catheter and transdermal preparations of drugs provide uninterrupted analgesia and are being used successfully in larger laboratory animals.⁶ The analgesic regimen chosen should always be made in consultation with your veterinarian.

Summary

The relief of pain and distress in research animals is ethically sound, humane, and promotes good science. The establishment of clear lines of responsibility coupled with appropriate endpoints, monitoring and intervention strategies are key to the prevention, minimization and/or alleviation of pain and distress in laboratory animals. Several excellent references and formularies are available to the researcher, veterinarian and husbandry personnel to facilitate their ability to recognize and modulate pain and distress in laboratory animals. Experience has demonstrated that a dynamic, interactive team approach to the recognition and alleviation of pain and distress in laboratory animals yields results that protect animal-welfare while promoting good science.

Approved by ARAC, 3/8/00

Revised - 7/14/04; 05/16/07; 7/14/10

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Sterilization

Key Concepts you should know about Steam Sterilization:

Sterilization destroys all microorganisms, including bacterial endospores.

Sterilization should be used for instruments, surgical gloves and other items that come in direct contact with the blood stream or normally sterile tissues. It can be achieved by high-pressure steam (autoclave). Because sterilization is process, not a single event, all components must be carried out correctly for sterilization to occur.

Effectiveness:

To be effective, sterilization requires time, contact, temperature and, with steam sterilization, high pressure. The effectiveness of the method of sterilization is also dependent upon four other factors:

1. **The type of microorganism present.** Some microorganisms are very difficult to kill. Others die easily.
2. **The number of microorganisms present.** It is much easier to kill one organism than many.
3. **The amount and type of organic material that protects the microorganisms.** Blood or tissue remaining on poorly cleaned instrument acts as a shield to microorganisms during the sterilization process.
4. **The number of cracks and crevices on an instrument that might harbor microorganisms.** Microorganisms collect in, and are protected by, scratches, cracks and crevices such as the serrated jaws of tissue forceps.
5. **Finally without thorough cleaning,** which removes any organic matter remaining on the instruments that could protect microorganisms during the sterilization process, sterilization cannot be assured, even with longer sterilization times.

****Remember:** When instruments and equipment are sterilized by high pressure steam (autoclaving), it is essential that steam reach all surfaces. For example, steam sterilizing closed containers will sterilize only the outside of the containers and don't wrap the packs too tight!

High-pressure, saturated steam using and autoclave is the most common and readily available method used for sterilization.

Steam sterilization requires four conditions: Adequate contact, sufficiently high temperature, correct time and sufficient moisture. Most common failures are lack of steam contact or failure to attain adequate temperature.

Standard Conditions for Steam Sterilization:

Steam sterilization (Gravity): Temperature should be 121⁰ C (250⁰ F).
Pressure should be 15lbs/in²; 20 minutes for unwrapped items; 30 minutes for wrapped items.

Or a high temperature of 132⁰ C (270⁰ F), pressure should be 30lbs/in²; 15 minutes for wrapped items.

Allow all items to dry before removing them from the sterilizer.

Follow manufacturers' recommendations.

Materials used for wrapping:

The material used for wrapping instruments and other items must allow adequate penetration of sterilant, provide a barrier to microorganisms and allow for sterile presentation. Packaging material (e.g. wrapped or container systems) allow penetration of the sterilization agent and maintain sterile processed item after sterilization. Materials for maintaining sterility of instruments during transport and storage include perforated instrument cassettes, peel pouches of plastic or paper, and sterilization wraps (woven or unwoven).

It is essential that the wrapping material be evaluated for properties such as acting as a barrier against dust particles, ability to repel water, as well as the capacity to provide an adequate seal on the contents. The wrapper should resist tears and punctures, and be free of holes and toxic ingredients. The sizes necessary to wrap the instruments and items to be processed must be available in sufficient quantities and be stored properly.

All wrappers must be lint free. Wrappers have to completely enclose the instrument or item being wrapped. The edges need to be properly folded so the tool can be aseptically presented during a procedure. While the edges and corners of the wrapper need to be tucked in, there should not be excessive wrapping material around the item as this interferes with the sterilant's penetration. Plus, if the wrapper is to be used as a sterile field, it should provide a field of at least six inches beyond the four sides of the table, mayo stand etc.

Pins, staples, paperclips and other sharp objects should never be used to secure a wrapped item. All sterile packages should be handled as little as possible.

There are several techniques available to wrap an item so it can be presented aseptically. Two of these methods are

1. **Square fold**, also known as the straight method.
2. **Envelope fold** sometimes called the diagonal method.

Figure 5. Sequential double-wrapping: Envelope fold.

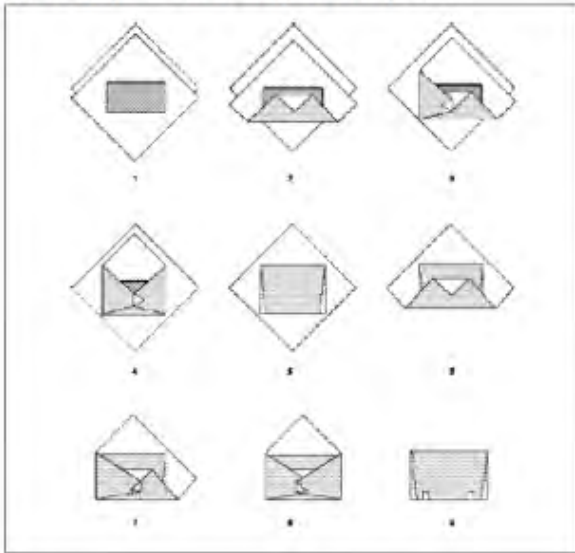
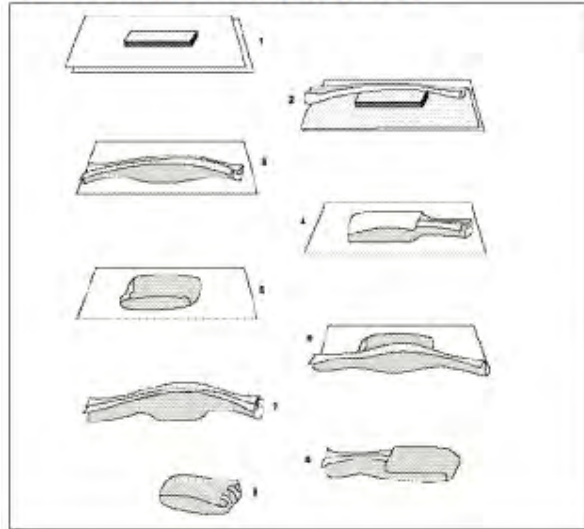


Figure 6. Sequential double-wrapping: Square fold.



Steps in wrapping:

- First make sure that the instrument being wrapped is clean.
- Make sure that the correct size wrapper for the instrument or item being wrapped is available
- Check the wrapper for holes, tears, lint, residue and defects.
- If linen is being used, the wrapper will need to be examined for holes using a light. Please remember that linen can only be processed a certain number of times and still hold its integrity, so be sure to check the corners. You may devise a marking system as to how many times the linen has been washed (no more than 50-60 times).
- Choose a wrapper that is compatible with the sterilization method. For example: non-paper materials should be used to package sharp instruments which can easily puncture paper packaging.
- To help prevent dulling of sharp points and cutting edges, wrap the sharp edges and needle points in gauze before sterilizing.
- A chemical indicator/integrator should be placed among the instruments, inside the package (sterilization monitoring).
- Place the item in the center of the wrapper. Wrap the item **sequentially** which requires using two separate wrappers. Or **simultaneously** which involves using two wrappers with their edges attached by the manufacturer, folding in a manner that will cover the entire contents.
- Secure the wrapped instruments with the appropriate autoclave tape.
- Label the package appropriately. Felt tipped indelible ink markers work nicely.
- Information noted on the label should include; contents, initials, date sterilizer, cycle, shelf life and destination of the item.
- Do not write on the wrapper; always write on the tape or a card that is taped to the item.
- Remember that all packages should be labeled before sterilization occurs.
- Put the pack through the sterilization process: Load, record, sterilize, cool, and place in sterile storage.

Monitoring Sterilization Procedures:

Steam sterilizers:

- Biological Indicators monitor for *Bacillus Stearothermophilus*
- Chemical indicators include indicator tape or labels, which monitor time, temperature and pressure. Location inside and outside of each pack.
- Mechanical Indicators provide a visible record of the time temperature and pressure for a sterilization cycle.

How should items be stored following sterilization?

Sterile items and disposable (single use) items should be stored in an enclosed storage area e.g. cabinet or drawer supplies and instruments should not be stored under sinks or in other locations where they might become wet.

Storage:

- Deep the storage area clean, dry, dust-free and lint-free
- Control temperature and humidity (24°C and relative humidity less than 70 %)
- Store items 10" off the floor, 20" from the ceiling and 8" away from the walls if not stored in enclosed cabinets or drawers.
- Do not use cardboard boxes for storage. They shed dust and debris and may harbor insects.
- Date and rotate the supplies (first in/first out).

Shelf Life:

The shelf life of an item (i.e. how long items can be considered sterile after sterilization is event-related. The item remains sterile until something causes the package or container to become contaminated-time elapsed is not the determining factor. An event can be a tear or worn area in the wrapping, the package becoming wet or anything else that will enable microorganisms to enter the package or container. A common length of time of item stays on a shelf before re-autoclaving is 6 months.

Shelf life depends on the following factors:

- Quality of the wrapper or container
- Number of times a package is handled before use
- Number of people who have handled the package
- Whether the package is stored on open or closed shelves
- Condition of storage are
- Use of plastic dust covers and method of sealing

Most packages are contaminated as a direct result of frequent or improper handling or storage. To make sure items remain sterile until you use them:

- Prevent events that can contaminate sterile packs
- Protect them by placing them in plastic covers

Before using any sterile item, look at the package make sure the wrapper is intact, the seal unbroken and is clean and dry as well as having no water stains, then you can be reasonably sure it is sterile regardless of when it was sterilized. If dropped on the floor, or damaged, it should not be used. The instruments should be recleaned, packaged in new wrap, and sterilized again.

Asepsis

Asepsis is defined as preventing exposure to microorganisms and prevention of infection. Three things that are very important in achieving asepsis are the reduction of time, tissue trauma and contamination.

- **Time** of surgical procedure is an important factor, as the longer a procedure takes the greater the risk of contamination and therefore infection.
- **Trauma** that is sustained by the tissue as a result of rough handling, drying out upon exposure to room air, implants, foreign bodies will contribute to infections.
- **Contamination** is initiated by bacteria or foreign matter in the surgical site.

Typically rodent surgical times are short, incisions are small and the amount of tissue trauma is minimal. These all minimize the risk of infection.

Surgical Technique

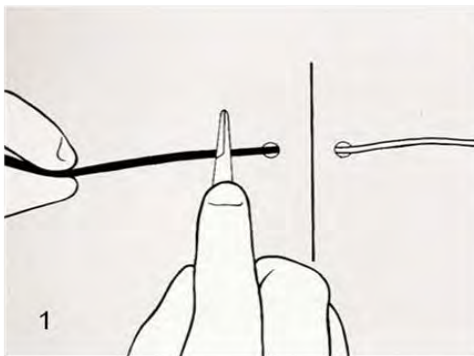
Surgeons themselves have been recognized to be one of the greatest influences on the incidence of post-procedural infection rates. Prolonged surgical times expose tissues to contaminants, dry out tissues and compromise the blood flow to tissues. Tissues damaged by crushing or drying, certain types of suture and other surgical implants serve as a nest for infection. There are a number of things that surgeons can do to prevent post-operative infections.

- Be aware of instrument and hand position at all times. If an instrument or glove touches something outside the sterile field the instrument or glove should be replaced immediately.
- Be gentle when handling tissues.
 - Do not use toothed or crushing instrument if it is not necessary.
 - When tying off vessels include only a minimum of surrounding tissues.
 - Use electrocautery or electroscapels sparingly. They cause significant tissue necrosis.
- Use appropriate suture techniques
 - Any suture that will be buried in tissues should be either absorbable or

monofilament (non-absorbable braided suture is irritating and can harbor bacteria).

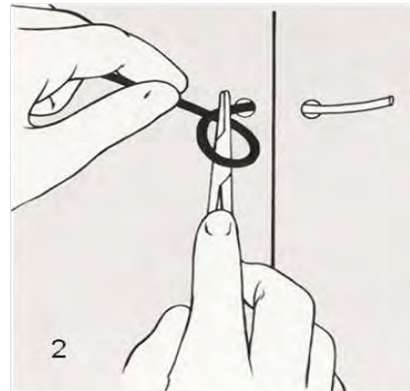
- Sutures should be placed evenly and as close to the tissue edge as possible to prevent obstruction of blood flow-typically no more than 0.2cm from the edge is necessary in small animals.

Sutures should only be tightened enough to appose the tissue edges. Any tighter will obstruct blood supply, retard wound healing and may result in dehiscence.

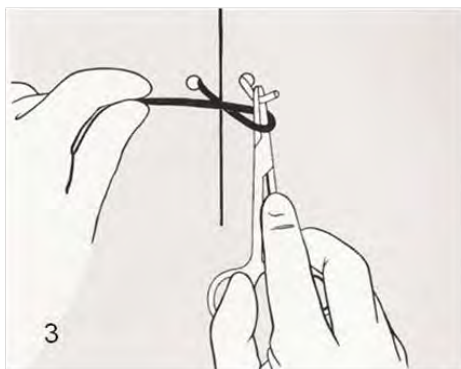


For the 1st throw the needle holder is in dominant hand. Place the needle holder perpendicular to and above the suture end held by the non-dominant hand

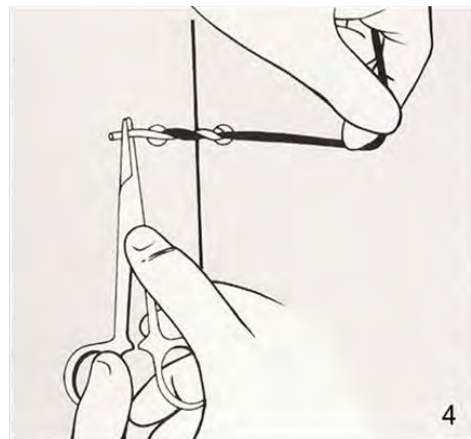
Square Knot Instrument Technique



Suture held by left hand is wrapped over and around the needle holder. (If the suture is wrapped twice around the holder, the first double wrap, throw of the surgeon's square knot will be formed.



Grasp the free end of the suture with the needle holder and withdraw it through the loop. Loop of the first throw will have a figure "8" shape.

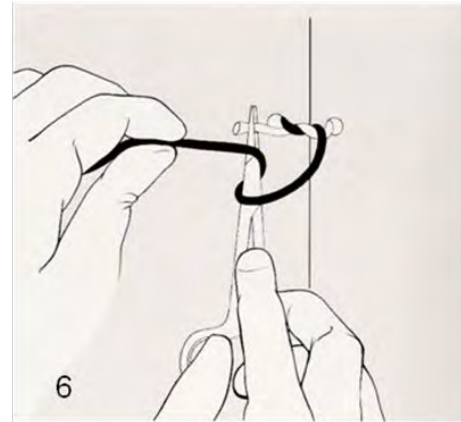


The figure "8" loop needs to be converted into a rectangular loop by crossing hands. Even tension is applied as the throw is tightened just enough to appose the skin edges. Over tightening can result in excessive swelling of the wound edge.

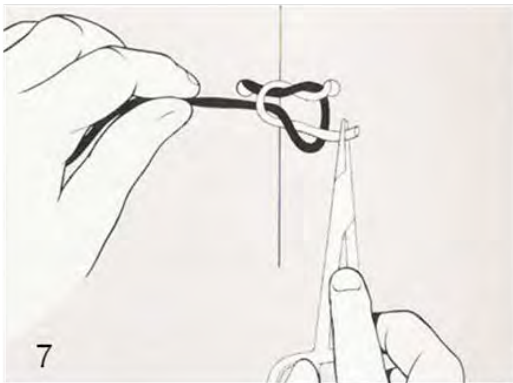
Problems: 1) a half hitch will result if hands are not reversed 2) avoid making the tail too long and do not grasp the suture at the base of the tail-prevents completion of the knot 3) care should be taken to keep the ends of the suture on the sterile surgical field to avoid contamination.



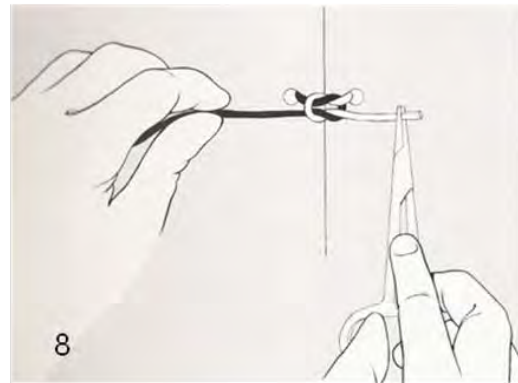
The second loop is initiated by passing the needle holder over the needled end of the suture, held by the left hand. A second loop is formed by wrapping the suture around the tip of the needle holder.



With a loop of suture wrapped around the needle holder, the needle holder is moved to grasp the free (white) end of the suture.



By withdrawing the free end (white) of the suture through the loop, a rectangular shaped second throw is formed.



This second throw is advanced against the first by applying even tension to the suture ends in lateral directions.

TABLE 1**POTENTIAL SIGNS ASSOCIATED WITH PAIN OR DISTRESS IN RATS and MICE**

	<u>Mice</u>	<u>Rats</u>	<u>Rabbits</u>
Decreased Food and Water Consumption	X	X	X
Weight loss	X	X	X
Self-imposed isolation/hiding	X	X	X
Self-mutilation, gnawing at limbs	X	X	X
Rapid Breathing	X	X	X
Opened-Mouth Breathing	X	X	X
Abdominal Breathing	X	X	X
Grinding Teeth		X	X
Biting/Growling/Aggression		X	X
Increased/Decreased Movement	X	X	X
Unkempt Appearance (Erected, Matted, or Dull Hair coat)	X	X	X
Abnormal Posture/Positioning (e.g., Head-pressing, Hunched Back)	X	X	X
Restless Sleep			X
Tearing (including Porphyria), Lack of Blinking Reflex		X	X
Dilated Pupils X			
Muscle Rigidity, Lack of Muscle Tone	X	X	X
Dehydration/Skin Tenting/Sunken Eyes	X	X	X
Twitching, trembling, tremor	X	X	X
Vocalization (Rare)	X	X	X
Redness or Swelling Around Surgical Site	X	X	X
Increased Salivation			X

Table 2: Post Procedural Pain Potential ^{a, b}

Minimal to Mild ^c	Mild to Moderate ^d	Moderate to Severe ^e
Catheter implantation	Minor laparotomy incisions	Major laparotomy/organ incision
Tail clipping	Thyroidectomy	Thoracotomy
Ear notching	Orchidectomy	Heterotopic organ transplantation
Subcutaneous transponder placement	C-section	Vertebral procedures
Superficial tumor implantation	Hypophysectomy	Burn procedures
Orbital sinus venotomy	Thymectomy	Trauma models
Rodent embryo transfer	Embryo transfer in non-rodents	Orthopedic procedures
Multiple injections	Bone marrow collection	
Non-corneal ocular procedures	Corneal procedures	
Intracerebral electrode implantation		
Vasectomy		
Vascular access port implantation		
Craniotomy (periosteal pain)		
Superficial lymphadenectomy		

^a Table adapted from “Guidelines for the Assessment and Management of Pain in Rodents and Rabbits”. 2006, American College of Laboratory Animal Medicine

^b The analgesia and monitoring required may vary due to a number of factors; such as the invasiveness of the procedure, degree of tissue trauma, surgical time, skill of the surgeon, and the tissues or organs involved.

^c Post procedural pain relief for minimal to mild pain may be adequately addressed with preemptive analgesia, tissue infiltration with a long acting local anesthetic, and a single dose of a long acting NSAID or mixed opioid agonist-antagonist, or other agent.

^d Post procedural pain relief for mild to moderate pain may be adequately addressed with tissue infiltration with a long acting local anesthetic combined with one or more doses of a long acting NSAID and/or an opioid or other agent in addition to pre-emptive analgesic administration.

^e Post procedural pain relief for moderate to severe pain should encompass multimodal analgesia (e.g. combining a pure opioid agonist with a NSAID, tissue infiltration with a long acting local anesthetic, etc.)

From: NIH – **Guidelines for Pain and Distress in Laboratory Animals: Responsibilities, Recognition and Alleviation. 2/11**

ANESTHETIC RECORD

NOTE: A copy of this record MUST be maintained in the animal's permanent record.

Animal ID: _____ Protocol # _____

Page: _____ of _____

Species: _____

Start Time: _____ am-pm

Weight: _____

Female Male

End Time: _____ am-pm

Operative Procedure: _____

Surgeon: _____

Anesthetist: _____

Pre Op Meds Given:

	Dose	Route	Time
<input type="checkbox"/> Atropine	_____	_____	_____
<input type="checkbox"/> Acepromazine	_____	_____	_____
<input type="checkbox"/> Ketamine	_____	_____	_____
<input type="checkbox"/> Meloxicam	_____	_____	_____
<input type="checkbox"/> Buprenorphine	_____	_____	_____
<input type="checkbox"/> Acetaminophen	_____	_____	_____
<input type="checkbox"/> Carprofen	_____	_____	_____
<input type="checkbox"/> Topical Anes	_____	_____	_____

IV Fluids Used:

Lactated Ringers Saline 5% dextrose

Volume Given: _____

Duration of Administration: _____

Anesthetic Gas Used:

Isoflurane Halothane N2O

Intubated: Yes No Time: _____

Use of respirator: Yes No

Date: ____ / ____ / ____

TIME	RESP	HEART RATE	MM COLOR	RETURNING REFLEXES

Recovery: (Circle One)

Rapid Prolonged Smooth Rough Exudates Time: _____ Returned to Cage Time: _____

Post Op Meds:

	Dose	Route	Time
<input type="checkbox"/> Meloxicam	_____	_____	_____
<input type="checkbox"/> Buprenorphine	_____	_____	_____
<input type="checkbox"/> Acetaminophen	_____	_____	_____
<input type="checkbox"/> Topical Anes	_____	_____	_____
<input type="checkbox"/> Others:	_____	_____	_____

POST OPERATIVE EVALUATION

Date of Operation _____ Species _____ Animal # _____

Pre-operative weight (g or kg) _____ Protocol # _____

Procedure _____

Date:										
Time:	Pre-Day 0	AM	PM	AM	PM	AM	PM	AM	PM	AM
Observations (Yes or No)										
Active?										
Rough Hair Coat?										
Eating?										
Drinking?										
Feces?										
Urine?										
Physical Examination										
Check extremities temperature in rodents. Rectal Temp										
*Rate & Type of Breathing										
**Dehydration										
Normal Gait/Posture										
Diarrhea?										
Urination?										
Vocalization?										
Suture/Staple Line										
Wound edges red?										
Swelling around incision?										
Exudates from incision?										
Other Medication Given? Agent Dosage										
Pain Score (1-4)										
Observer Initials										

PAIN ASSESSMENT SCORING

0 - Normal behavior and physiology

1 - Mild behavior and physiological changes (decreased food/water consumption, slightly depressed, minor guarding of incision site)

2 - Moderate pain (includes observation in assessment scoring 1 plus swelling/redness/discharge at surgical site, reluctance to move, guarding with vocalization or aggression)

3 - Severe pain/distress (includes all observation in assessment scoring 1 and 2 plus, immobility, dehiscence of incision, profound dehydration/weight loss)

4 - Moribund

Please contact a member of the veterinary staff for group 2 or higher animals or if you have questions about analgesia or post operative monitoring.

*N = normal, L = labored, R = rapid, S = shallow

** Gently pinch up a fold of skin. Skin of dehydrated animals will stay pinched up.

Body Condition Scoring Guide



BC 1

Mouse is emaciated.

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



BC 2

Mouse is underconditioned.

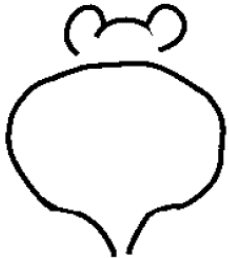
- *Segmentation of vertebral column evident.*
- *Dorsal pelvic bones are readily palpable.*



BC 3

Mouse is well-conditioned.

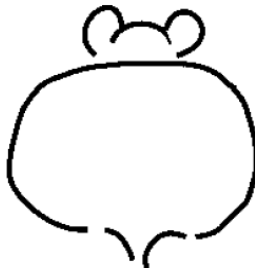
- *Vertebrae and dorsal pelvis not prominent; palpable with slight pressure.*



BC 4

Mouse is overconditioned.

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



BC 5

Mouse is obese.

- *Mouse is smooth and bulky.*
- *Bone structure disappears under flesh and subcutaneous fat.*

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