Aseptic Surgical Techniques Wetlab

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ASEPTIC SURGICAL TECHNIQUES WET LAB

Introduction: The purpose of this lab is to introduce you to the principles of aseptic surgical techniques which are required for *all* survival surgeries conducted on *any species* at NUS. Aseptic surgical techniques are mandated by legislation and are regulated by the AVA (Agri-food and Veterinary Authority of Singapore). Applicable documents include the Animals and Birds Act (Care and Use of Animals for Scientific Purposes Rules 2004), NACLAR (National Advisory Committee for Laboratory Animal Research) Guidelines and the *Guide for the Care and Use of Laboratory Animals (NRC).*

Aseptic techniques are designed to reduce bacterial contamination of the surgical wound to the lowest possible level, and must be addressed at four levels. These levels include: (1) cleaning and disinfection of the surgery room or dedicated rodent surgery area, (2) proper preparation of the incision site and draping of the animal, (3) aseptic preparation of the surgical team, and (4) sterilization of all instruments and materials to be used in the procedure. Techniques demonstrated today should always be a part of your preparation for any surgical procedure.

It is a common misconception that rodents cannot become infected during surgery or other invasive procedures such as the placement of indwelling catheters. This is totally false. In fact, the rat is a commonly used research model for investigating the pathophysiology of sepsis. If rodents were not susceptible to infection by bacteria, they could not be used for such research. You must remember that every surgical wound created, whether on humans or animals, becomes contaminated with bacteria during the procedure. Thus, it is the surgeon's responsibility to ensure bacterial wound contamination is kept to the lowest possible level. It is impossible to completely sterilize the skin; however, you can minimize bacterial numbers on the skin's surface. Also keep in mind that surgical wounds can have a significant number of bacteria present without the visible presence of pus. Included within your packet is a paper documenting the behavioral and physiologic effects of inapparent wound infections in rats.

Survival surgery may be conducted on mice and rats in an area of the laboratory dedicated solely for that activity. The guidelines covering rodent surgery have been included in your packet and should be read by all those who will be doing surgery on those species. Surgery on any species other than purpose-bred mice and rats (i.e. covered species) must be conducted in a dedicated operating suite. If you will be doing surgery on covered species, please contact Dr. Shannon Heo at ext 7870 or Mr James Low at ext 3062 prior to starting any surgical procedures.

Wet Lab Procedures:

1. *Always assess the animal's health before administering anesthesia.* Weigh the mouse or rat on a scale, calculate the anesthetic dose required, and check with your group helper to make sure the dosage is correct. Do not put the cage containing the animal on the seats or anywhere that they can be knocked off.

2. Inject the animal intraperitoneally (IP) with the anesthetic solution. If you are unfamiliar with this technique, or with proper restraint and handling of the animal, please ask for help. The intraperitoneal injection technique is also described and illustrated in this handout.

3. Observe the animal as it becomes anesthetized. It may go through an excitement phase, so be sure that the animal is in its cage with the top secured until it becomes quiet. If left on the table, or in the cage without the cage top, it may fall off the table or jump out of the cage and be injured. If the animal is properly anesthetized for surgery, it will be unresponsive to a toe or tail pinch, and should not blink when the inner corner of the eye is touched. When performing surgery, do not proceed until the animal is unresponsive to these tests. In addition, the muscles of the animal should be relaxed. *When you are actually performing surgery, there should be no response when the incision is made. If the animal flinches during surgery, it is feeling pain and the anesthetic plane is not deep enough. Do not proceed with the surgical procedure until the anesthetic plane has been adjusted.*

4. After the animal is anesthetized, place a small amount of bland ophthalmic ointment in each eye. *Use care when applying the ointment and avoid touching the eye with the end of the tube to maintain the ointment's sterility.* The ointment prevents the cornea from drying out and becoming inflamed, and must be used for all surgical procedures. Make sure the animal is maintained on a heat source after it is anesthetized, and never leave the animal unattended while it is unconscious.

5. If you are using an electric heating pad and not the warm recirculating water pad, be sure that there are layers of material between the animal and the pad. This will allow the heat to diffuse up and will keep the animal from being burned. *Careless* u*se of electric heating pads can result in severe burns to an unconscious animal that is lying unprotected on the pad.* Periodically monitor the animal's respiration, and make sure that the mucous membranes, ears, and footpads remain pink. Pink tissue color indicates the animal has adequate blood circulation and peripheral tissues are being properly oxygenated.

   After performing an actual surgery, keep the animal in a warm environment until it is sternal (in an upright position as opposed to lying on its side) to avoid hypothermia. Animals that are too cool may die or recover from the anesthetic extremely slowly. *It is always the surgeon's responsibility to monitor the animal until it recovers from anesthesia.*

6. Practice clipping a surgical field. Use the clippers with #40 blade to remove the hair from mice and rats. Shave as closely as possible without irritating or nicking the skin. If you will be using an animal such as a rabbit which has much thicker hair, you will need to prepare the field first with a #10 blade, and then finish shaving with the clippers. For mice and rats, the intended incision site must have an additional margin of hair removal of about 0.5-1 cm or 1-2 cm, respectively. Larger margins are needed for larger, long-haired species such as rabbits. *If a suitable size surgical field has been created, the long hairs at the edge are unable to contact the incision, and the hairs at the edge will not be included when the wound is sutured closed. It is inappropriate and unacceptable*

surgical technique to have long hairs from the margin included in the closed incision. Alternatively you can also use depilatory cream for hair removal but be sure to rinse the area thoroughly after using the cream as the cream can be an irritant to the skin. Hair removal with scissors is unacceptable as it does not remove the hair close enough to the skin, and carries a high risk of cutting the skin.

7. The surgical site is prepared by scrubbing the skin with a disinfectant followed by 70 % alcohol. This technique is performed a total of three times each with the disinfectant and three times each with the alcohol. The rodent surgery guidelines have an excellent description of suitable skin disinfectants. The skin disinfectant should have a soap base (e.g. Hibiscrub® or Betadine® scrub – do take note that this is different from the Betadine® solution which is non-soapy) to help remove small clipped hairs and accumulated skin debris and sebaceous secretions. *Proper skin preparation is essential to achieve surgical asepsis in animals, as the hair coat traps bacteria and debris, and the animals are not bathed on a regular basis.* The animal's skin is the primary source of bacterial wound contamination.

8. When preparing an animal for an actual surgery, change gloves between clipping the site and preparing the skin. Reserve one hand as your "clean" hand for dipping the gauze into the Hibiscrub®/Betadine®, then transfer the wet gauze to the other hand and use it to scrub the site. Start in the middle of the surgical site and circle outward towards the haired area. Do not retrace the motion or drag the gauze across the middle of the field if it has been through the haired margins. Follow the Hibiscrub®/Betadine® scrub with the same scrubbing motion using 70% alcohol. This will be repeated two additional times for each solution. Following the last scrub with Hibiscrub®/Betadine® scrub, use enough alcohol scrubs to thoroughly remove the soap from the surgical field. *When you are performing an actual surgery, wash your hands with soap and change into new sterile gloves before you start the surgical procedure.*

   Be careful not to wet the entire animal while scrubbing the surgical site. Rats and mice are small animals with a high surface area to mass ratio, and are prone to hypothermia very quickly when wet.

9. Following surgical site preparation, drape the animal so that only the surgical field is exposed, and the animal and its hair are under the drape. Take a piece of the draping material and cut an appropriate size area to place around the designated surgical field. *Drape material used for surgery should be sterile, and can be autoclaved as a part of your surgery pack.*

10. If you wish to practice suturing or using the wound clip applier and remover, we will euthanize the animal in order for you to practice. *Please ask the veterinarian how to make skin incisions and how to use the instruments to properly close the wound.* If you need additional help with surgical techniques, you can also make an appointment for one-on-one training at a later date with one of the LAC veterinary staff.

11. Other important aseptic surgery components are using sterile instruments and maintaining a sterile working surface on which your instruments, suture material, and other surgical supplies are placed. Always autoclave instruments prior to use and

handle them in a manner during surgery that maintains their sterility. A piece of sterile draping material or a sterile metal tray or pan will hold the instruments and other materials while you are working.

Always be aware of where your hands are, and if you touch anything that is not sterile change your gloves immediately to avoid contaminating the surgical wound. It is helpful to have an assistant nearby who can retrieve any supplies you might need during the surgical procedure.

Before autoclaving instruments, make sure they are clean and free from blood or tissue debris. Bacteria remain within any debris on the instruments, and they will not be sterile after autoclaving. If you are performing surgery on multiple rats or mice during a session, the instruments must be disinfected between animals. Please see the rodent wet-lab handout for more details. Use a hot bead sterilizer to re-sterilize the instrument tips between animals. Remove the blood and tissue debris before placing the instruments in the hot bead sterilizer. If you are using a hot bead sterilizer, dip the tips in sterile saline prior to touching the animal's tissues to ensure they are cool. If you do not have a hot bead sterilizer, disinfect the instruments thoroughly with a suitable disinfectant (see Table 3 on page 19) before commencing surgery on the next animal. If you are performing surgery on any other species besides rodents, you must use a new set of autoclaved instruments for each animal.

12. *It is mandatory for animals undergoing any major survival surgery at NUS to receive analgesics (medication to relieve pain) for 3 days post-surgery, or longer if the animal continues to experience pain. The only exceptions to this policy are those protocols where the IACUC has granted an exemption based on scientific justification.* The handouts you received today, and those provided at the rodent users training session, contain information on monitoring animals for signs of pain and distress. The veterinarians can assist you with choosing an analgesic.

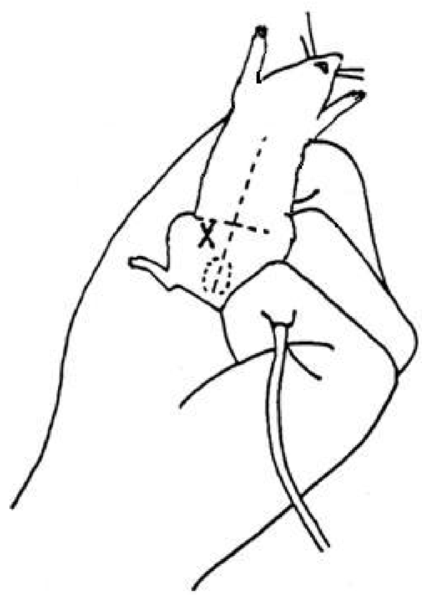
13. Monitor the incision site for 10 days following the surgical procedure or longer if there are problems. Observe the wound for dehiscence of sutures or wound clips (opening of the incision line), any discharge, swelling, redness, or pain. If you are unsure of the animal's condition, please call one of the veterinary staff for assistance and we will be happy to look at the animal with you. Remove sutures and wound clips 7 to 10 days after surgery.

    The handouts you have been given contain a great deal of information relevant to good surgical technique. Remember that good surgical technique will result in less pain and distress for the animal, and in better research data for your publications. Please take the time to carefully read the material provided. If you have additional questions concerning aseptic surgery in the future contact one of the LAC veterinarians.

Intraperitoneal Injection Technique

Intraperitoneal (IP) injections: Administer intraperitoneal injections in the lower right quadrant (the animal's right) of the abdomen to avoid vital organs. Tip the animal's head downward to allow organs to fall out of the way (not shown in the figure). Introduce only the needle's tip as penetration of the abdomen too deeply may result in intestinal puncture. Before any substance is injected into the animal's abdomen, it is good practice to aspirate gently by pulling back slightly on the plunger. If yellow or brown fluid is aspirated, your needle may have penetrated the bladder or the intestines. If this happens, you will need to withdraw the needle and repeat the procedure using a fresh needle and syringe. The smallest needle possible should be used, and will depend on the viscosity of the agent to be injected. Do not inject more than 5-10 ml into rats or 2-3 ml into mice using this route. This is the most common method of administering drugs and anesthetics to mice and rats.

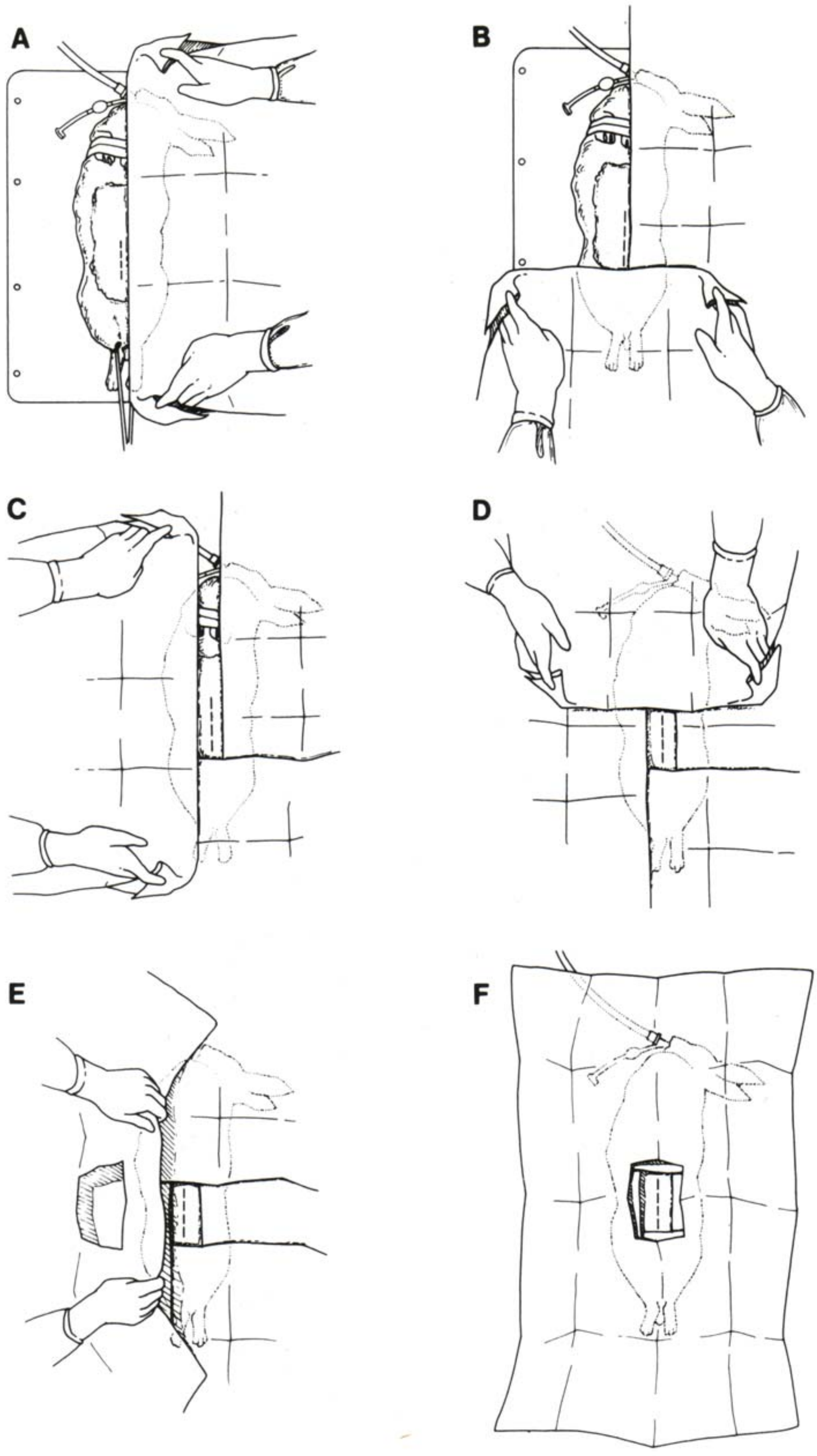
If you will be working with large rats, you may wish to have one person restrain the rat and another person administer the injection. Alternatively, you may use disposable rodent restraint cones to restrain the animal while giving an injection. The disadvantage to using the restraint cones is that you cannot hold the animal in a manner that allows the intestines to fall forward while giving the injection.



Restraint of the mouse using a one-hand grip for intraperitoneal injection. X is the injection site. Quadrants of abdomen and location of bladder are shown as *dotted lines*.

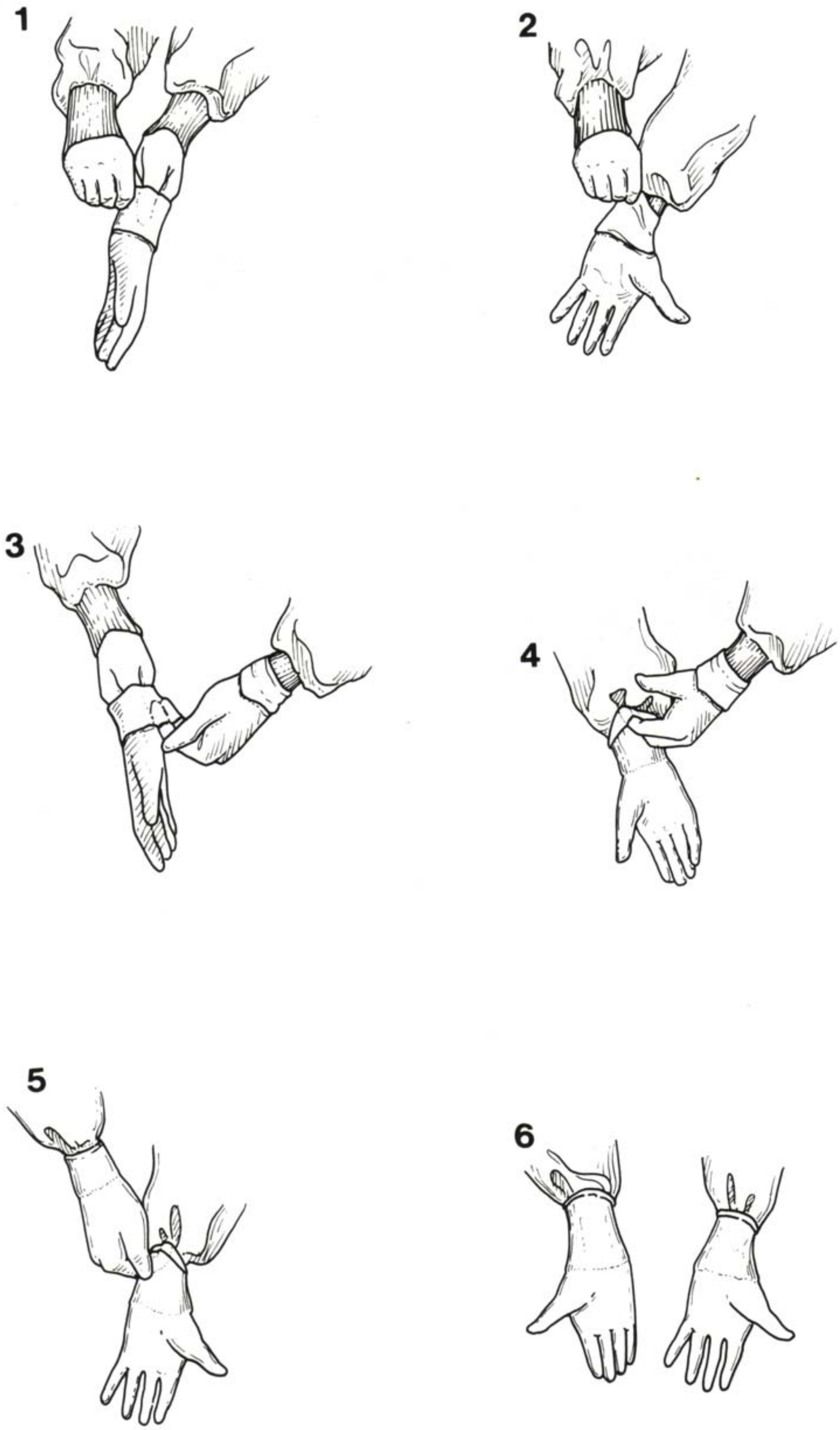
From: Clinical Laboratory Animal Medicine: An Introduction by Hrapkiewicz, Medina, and Holmes

Draping Technique using Multiple Drapes



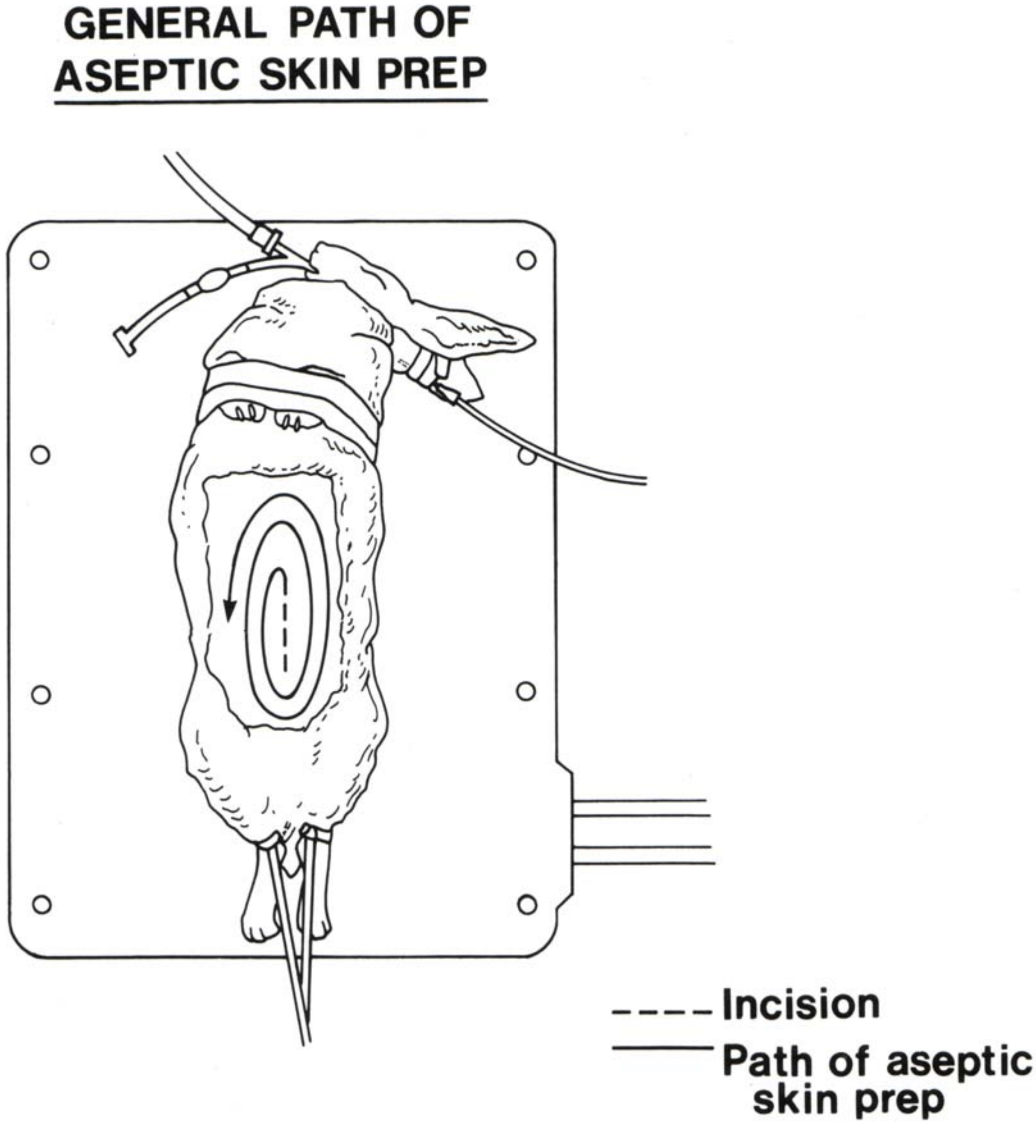
From: Essentials for Animal Research: A Primer for Research Personnel by USDA

Proper Donning of Sterile Surgical Gloves



From: Essentials for Animal Research: A Primer for Research Personnel by USDA

Aseptic Skin Preparation



From: Essentials for Animal Research: A Primer for Research Personnel by USDA

Principles of Surgical Asepsis

Bacterial Surgical Wound Contamination

The most common source of contaminating bacteria is the patient's own bacteria:

- -Preventing exposure is very important
- -Pre-operative hair clipping and skin scrubbing decreases the infection risk

We must also prevent exogenous bacterial sources

- -Surgical personnel are to wear a gown, cap, mask, sterile gloves, and use sterile instruments

Air in contact with the surgical wound can deliver microbes to the wound

- -Airborne bacteria are mostly from the animal and the non-scrubbed personnel in the room, so minimize the number of non-scrubbed personnel!

Sometimes pre-operative antibiotics are important

Keeping the surgical wound clean after surgery is essential

Infection Development

All clean surgical wounds are contaminated with many bacteria during surgery. Bacterial infection usually requires substantial bacterial growth in the wound. This growth usually occurs only when the surgical 'microenvironment' is favorable to the bacteria; for instance:

- -Necrotic tissue
- -Thrombosis
- -Dead space
- -Foreign bodies, including certain suture (e.g. silk, gut)
- -Blood clots or serum pockets

Preventing Surgical Infection

Proper surgical patient preparation (see later)

- -Can't completely sterilize the patient's skin
- -The aim of surgical prep is to kill the transient bacterial on the skin's surface
- -Can't kill resistant bacteria in hair follicles and sebaceous glands; during surgery these bacteria come to the surface and along with airborne bacteria, are sources of surgical wound contamination.
- -Patient draping decreases the exposure to the resident bacteria
  - -Cloth drapes allow bacterial penetration when wet, while waterproof paper drapes do not (therefore waterproof paper drapes are preferred over cloth)

Preparation of surgical personnel (see later)

- -Even you in the surgical suite should change out of your street clothes
- -Caps, masks, and shoe covers must be worn
- -Anyone approaching the surgical field must wear a sterile gown and gloves

Sterilization of surgical equipment and materials

Maintenance of the operating room

- -The area is to be isolated from all other hospital traffic, and used only for surgery
- -Surgical prep areas (for both surgeon and patient) should ideally be outside the operating room

-Before each surgical day, all surfaces (including floors, walls, tables, lights, counters) should be thoroughly cleaned with an appropriate disinfectant
-Between surgeries, all flat surfaces should be wiped with a disinfectant
Proper surgical operative technique
Signs of postoperative infection include pain, swelling, warmth, redness/other abnormal discoloration and/discharge from the incision
-Most common time of incision infection is 3 to 5 days after surgery
-However, incision infection can occur at any time

Preparation of a Surgeon's Skin
Anyone assisting in the surgery must scrub with Iodine (Betadine®) or Chlorhexidine scrub (Hibiscrub®) for 5 minutes before putting on surgical gloves and gown

Povidine-Iodine (Betadine®)
-Effective against Gram negative and Gram positive bacteria, fungi, and viruses
-Some activity against bacterial spores
-Organic material decreases efficiency
-Requires at least 2 minutes of contact time
Chlorhexidine
-More effective against Gram positive than Gram negative bacteria
-Effective against viruses
-Only somewhat effective against fungi
-Active for at least 6h after surgical scrub (longer than any other skin antiseptic)
-Activity is not affected by organic material

Preparation of the Surgical Patient

Hair Removal
-All methods cause some skin trauma; skin trauma allows bacteria to colonize the skin
-Remove hair just before surgery (incidence of post-surgical infection increases with time interval between hair removal and surgery)
-Shaving with a razor is not recommended, because it causes many small cuts that allow bacterial colonization
-Clipping causes less skin trauma than many other techniques
    -Be careful to cause the least skin trauma possible
    -Vacuum hair and debris after clipping
Patient Skin Disinfectants
-Quaternary ammonium compounds
    -e.g. Zephiran
    -Not recommended
    -Associated with *Pseudomonas* outbreaks
-Hexachlorphene (pHisoHex)
    -Activity is decreased by organic material and by alcohol
    -Not effective against Gram negative bacteria or spores
    -Not recommended
-Alcohol
    -Active against many bacteria, but not spores

- -Variable activity against fungi and viruses
- -Rapidly acting
- -Combine with Chlorhexidine or Betadine® to increase efficacy

-Betadine

- -See previous
- -May be inactivated by alcohol

-Chlorhexidine

- -See previous
- -Retains effectiveness when exposed to organic material, alcohol, and soaps

Prep Technique

-Use wet gauze sponges or a (sterile) gloved hand, not scrub brushes as scrub brushes increase skin trauma

-Never return the sponge to the central area once it's contacted a peripheral region

-The scrub detergent's activity is important for mechanical removal of debris and bacteria

-Avoid excessive vigor that can cause irritation and trauma

-After the scrub is completed, apply Betadine® or Chlorhexidine (Hibiscrub®) solution

Guidelines on Postoperative Care and Analgesia

BASIS:

NACLAR Guidelines, p.32 (on limiting pain): "Pain and distress cannot always be adequately evaluated in animals and investigators must therefore assume that animals experience pain in a manner similar to humans. Decisions regarding their welfare in experiments must be based on this assumption unless there is evidence to the contrary.

The investigator should anticipate any potentially adverse effects of a manipulation and take all possible steps to avoid or minimize pain and distress."

p. 38 (on postoperative care): "Consideration of and attention to pain relief is paramount in post-operative care. Investigators should ensure that adequate monitoring, treatment and care of post-operative animals is provided. They should ensure that they, or other experienced personnel, are fully informed of the animals' condition. The duties of all staff must be clearly defined and ways of dealing with emergencies established.

The comfort of the animals must be promoted throughout the post-operative period. Attention should be given to warmth, hygiene, fluid and food intake, and control of infection. The use of analgesics and tranquilisers may be needed to minimize post-operative pain and distress. Care should be taken that animals recovering from anaesthesia are housed to prevent injury and that conditions are such that they are not disturbed, attacked or killed by other animals in the same enclosure.

Regular observation of surgical wounds is essential to check the progress of healing. Any problems must be attended to promptly.

Any post-operative animal observed to be in a state of severe pain or distress which cannot be alleviated quickly must be killed humanely without delay and a veterinarian informed immediately."

Guide for the Care and Use of Laboratory Animals, p.63 & 64 (on postoperative care): "An investigator and veterinarian share responsibility for ensuring that postsurgical care is appropriate. An important component of postsurgical care is observation of the animal and intervention as required during recovery after anaesthesia and surgery…..

During the anaesthetic recovery period, the animal should be in a clean, dry area where it can be observed often by trained personnel. Particular attention should be given to thermoregulation, cardiovascular and respiratory function, and postoperative pain and discomfort during recovery from anaesthesia. Additional care might be warranted, including administration of parenteral fluids, analgesics etc.

After anaesthetic recovery, monitoring is often less intense but should include attention to basic biologic functions of intake and elimination and behavioural signs of postoperative pain, monitoring for postsurgical infections, monitoring of the surgical incision or bandaging, and timely removal of skin sutures clips or staples."

p. 64 (on limiting pain and distress): "An integral component of veterinary medical care is prevention or alleviation of pain associated with procedural and surgical protocols. . . Pain is a stress or and, if not relieved, can lead to unacceptable levels of stress and distress in animals. The proper use of anesthetics and analgesics in research animals is an ethical and scientific imperative. . . In general, unless the contrary is known or established, it should be assumed that procedures that cause pain in humans also cause pain in animals."

Guidelines:

- Analgesics must be provided to all animals following survival surgery unless scientific justification for withholding post-operative analgesics is provided by the investigator and approved by the IACUC, or if an LAC veterinarian examines the animal and determines that analgesic administration is no longer necessary. In cases where post-operative analgesics can /will not be administered for scientific reasons, the animals must be listed in Pain Category E (i.e., pain/distress cannot be relieved by use of anesthetics, analgesics, or tranquilizers as the use of these agents would interfere with the experimental design).
- The use of local pain-relieving drugs such as Xylocaine® (Lignocaine), in addition to systemic analgesia, may be indicated for some procedures resulting in significant disruption of the skin (e.g., Alzet® pump placement, catheter exteriorization), as these drugs may help to block the onset of the pain cascade due to disruption of the dermal nerve cells. Local analgesics are not intended for use in lieu of systemic analgesics, unless the withholding of systemic analgesia is scientifically justified.
- Major survival surgeries require at least 72 hours of post-operative analgesia, and then as needed if the animal still appears to be in pain. For major survival surgeries, consultation with the veterinarian should also include consideration of pre-operative analgesia. A major survival surgery is defined as any procedure that penetrates and exposes a body cavity, produces substantial impairment of physical or physiologic functions, or any procedure that requires the use of more than a single application of a short-term anesthetic.
- Minor procedures require at least 24 hours of post-operative analgesia, and then as needed if the animal still appears to be in pain.
- For guidance on analgesic selection and use, please contact a Laboratory Animals Centre (LAC) veterinarian.

Guidelines for Survival Surgery in Laboratory Animals

BASIS:

Recommendations for the performance of survival surgery on laboratory animals are based on the 1996 edition of the NIH *Guide for the Care and Use of Lithoratoy Animals* (the *Guide*, pp. 60-65, 78-79) and the NACLAR Guidelines (pp. 37-39).

DEFINITIONS:

Aseptic Technique

Aseptic technique reduces microbial contamination to the lowest possible practical level and includes preparation of the animal, such as hair removal and disinfection of the operative site; preparation of the surgeon, such as the provision of decontaminated surgical attire, surgical scrub, and sterile surgical gloves; sterilization of instruments, supplies, and implanted materials; and the use of operative techniques to reduce the likelihood of infection.

Survival Surgery

Any surgical procedure from which an animal regains consciousness for any period of time.

Major Survival Surgery

Any survival surgical procedure penetrating and exposing a body cavity or producing substantial impairment of physical or physiologic functions. Examples of major surgery include laparotomy, thoracotomy, craniotomy, joint replacement, spinal transection, and limb amputation.

Multiple Survival Surgeries

Multiple survival surgical procedures are not permitted on animals unless scientifically justified and approved by the IACUC.

GENERAL GUIDELINES:

Location of Surgery

A surgical area for rodents and birds can be a room or portion of a room that is easily sanitized and not used for any other purpose during the time of surgery. However, because dedicated surgical facilities provide advantages with regard to reduced investigator regulatory burden, consideration must be given for using LAC facilities (Room 16 at AHU, Front Laboratory Procedure Rooms at AHU, Operating Theatres at AHU, Procedure Rooms at sAHU etc.), whenever possible. Currently, there is no charge to use the facilities or the gas anesthetic machines (when used within the facilities). **An investigator's laboratory may be used as a survival surgery area provided the investigator provides scientific rationale for such use and the location is inspected and approved by the IACUC**. In selecting a surgical location, the investigator should bear in mind that "the number of personnel and their level of activity have been shown to be directly related to the level of bacterial contamination and the incidence of post-operative wound infection" (the *Guide*, p. 78-79). Thus, every attempt should be made to sufficiently physically separate the surgical area from other areas in the room to minimize unnecessary traffic and decrease the potential for contamination of the wound. Further, the location should be designed to include the following three areas.

(1) An area should be designated for *preparation of the animal*, including weighing, hair or feather removal, and initial skin disinfection. The prep area should be sufficiently separate from the surgery table to minimize the potential for contamination of the surgery area by aerosols generated during animal preparation. Again physical separation of the prep and surgical areas is expected.
(2) A separate area should be set aside for conducting surgical procedures (i.e., from skin incision to wound closure). The surgical table and immediate surrounding areas must be constructed of material that can be cleaned (washed with soap and water) *and* then disinfected using appropriate agents (see Table 1 below). The immediate surgical area should be disinfected prior to and between surgeries to decrease dust-borne contamination and may not be used for other purposes during the time of surgery.
(3) Finally, a separate recovery area should be established. This should be a quiet, undisturbed location where the animals can be observed.

Table 1. Recommended Hard Surface Disinfectants
(e.g., table tops, equipment) Always follow manufacturers' instructions.

Agents	**Examples**	**Comments**
Alcohols	70% alcohol	Minimum contact time required is 15 minutes. Contaminated surfaces take longer to disinfect. Remove gross contamination before using. Inexpensive. Flammable.
Chlorine	Sodium hypochlorite (Clorox® 10% solution) Chlorine dioxide (Clidox®, Alcide®)	Corrosive. Presence of organic matter reduces activity. Chlorine dioxide must be fresh (<14 days old). Kills vegetative organisms within 3 minutes of contact.
Aldehydes	Gluteraldehyde (Cidex®, Cide Wipes ®)	Rapidly disinfects surfaces. Toxic.

Surgical Instruments
Surgical instruments must be sterile. A list of acceptable methods for included in Table 2 below.

Table 2. Recommended Methods of Instrument Sterilization
Always follow manufacturers' instructions.

Agents	**Examples**	**Comments**
Physical: Steam sterilization (moist heat)	Autoclave	Effectiveness dependent upon temperature, pressure and time (e.g., 121.6°C for 15 mins vs. 13 1°C for 3 mins.

Chemical: Gas sterilization	Ethylene Oxide	Requires 30% or greater relative humidity for effectiveness against spores. Gas is irritating to tissue; all materials require safe airing time. Carcinogenic. Suitable for catheters and implants.
Chlorine Dioxide	Clidox®, Alcide®	A minimum of 6 hours required for sterilization. Corrosive. Presence of organic matter reduces activity. Must be freshly prepared (<14 days). Must be thoroughly rinsed from instruments using sterile distilled water before use.
Aldehydes	Gluteraldehyde	Many hours required for sterilization. Corrosive and irritating. Consult a Biosafety Officer on proper use. Must be thoroughly rinsed from instruments using sterile distilled water before use.

Surgical instruments may be used on more than one animal; however, **any item used on multiple animals must be carefully cleaned *and* disinfected between animals** (see Table 3 below). Hot bead sterilizers are the preferred method, although prolonged soaking in disinfectant is also acceptable. Because the effectiveness of disinfection is directly dependent upon the contact time with the disinfectant, the surgeon is expected to anticipate the number of surgical instruments required to guarantee uninterrupted conduct of the procedures while affording ample disinfectant contact time. Replace disinfectants when contaminated with body fluids or tissues.

Table 3. Recommended Instrument Disinfectants
Always follow manufacturers' instructions.

Agents	**Examples**	**Comments**
Dry heat	Hot bead sterilizer.	Fast. Instruments must be cooled before contacting tissue.
Alcohols	70% alcohol	Minimum contact time required is 15 minutes. Contaminated surfaces take longer to disinfect. Remove gross contamination before using. Inexpensive. Flammable.
Chlorine	Sodium hypochiorite (Clorox® 10% solution) Chlorine dioxide (Clidox®, Alcide®)	Corrosive. Presence of organic matter reduces activity. Chlorine dioxide must be fresh (<14 days old). Kills vegetative organisms within 3 mm. Must be thoroughly rinsed from instruments using sterile distilled water before use.
Peracetic Acid/ Hydrogen	Spor-Klenz®	Corrosive to instrument surfaces. Must be thoroughly rinsed from instruments using sterile distilled water before use.
Aldehydes	Gluteraldehyde	Minimum contact time required is 15 mins. Corrosive and irritating. Consult a Biosafety Officer on proper use. Must be thoroughly rinsed from instruments using sterile distilled water before use.

Pre-Surgical Evaluation & Treatment

Pre-existing health conditions may negatively affect the immediate or long-term success of the surgical procedures and the experiment. Performing pre-surgical evaluations helps ensure the animals are not overtly ill. This includes visual inspection of the animal and an assessment of their behavioral status. The animal should be alert and behaving normally, and should have a smooth coat and clear eyes. Bring animals with physical or behavioral abnormalities to the attention of the LAC veterinary staff.

Withholding food or water is generally *not* necessary in rodents or birds unless specifically mandated by the protocol or surgical procedure (e.g., gastrointestinal surgery). Withholding food or water for more than six hours should be discussed with an LAC veterinarian.

In some cases, it may be preferable to initiate antibiotic or analgesic treatment prior to surgery. All antibiotic or analgesic treatment regimens must be discussed with an LAC veterinarian.

Surgical Preparation

Animal preparation includes hair/feather removal from the surgical site with a generous border (at least 1 cm) to avoid contaminating the incision site. Perform the hair/feather removal in a location separate from the surgical area. When removing the hair/feather with a vacuum cleaner, it must be HEPA filtered. Scrub the surgical site with a germicidal scrub (e.g., Hibiscrub®, Betadine®), being careful to scrub from the center of the surgical site toward the periphery. Rinse the site with 70% alcohol. At least three alternating preparations of germicidal *scrub* and rinse are considered adequate, preferably followed by spraying the area with germicidal *solution*. Finally, drape the area with sterile drapes to prevent contaminants from entering the surgical field and provide a sterile area on which to lay sterile instruments during surgery.

The surgeon must thoroughly wash his or her hands with a bactericidal scrub. The use of *sterile* surgical gloves is required. Gloves dipped in bleach are <u>not</u> acceptable for this purpose. A surgical mask must be worn for major surgeries and implanted devices, but is also recommended for minor procedures. Wearing a *clean* lab coat is mandatory; however, a sterile gown is preferable, especially for major surgeries or surgeries where materials are implanted.

Anesthesia

The anesthetic regimen for any surgical procedure must be determined in consultation with an LAC veterinarian and must be described in the IACUC approved research protocol. Generally, injectable anaesthesia (e.g. ketamine-medetomidine, ketamine-xylazine etc.) or gas anaesthesia (e.g., isoflurane or halothane) is acceptable. In any case, the animal must be fully anesthetized prior to initiating the procedure and maintained in a consistent anesthesia plane throughout the surgery. Anesthetic depth may be monitored in a number of ways (e.g., respiration rate, corneal reflex, positive toe pinch) and may vary depending upon the species and the anesthetic agent used. For rodents and birds, it is generally not necessary or feasible to monitor the heart rate.

For guidance in selection and use of anesthetics, please contact an LAC veterinarian.

Surgical Procedures

All aspects of the surgical procedures must be conducted as described in the IACUC approved protocol. Animal evaluation during surgery is critical. In addition to monitoring anesthetic depth as described above, maintaining normal body temperature is of particular importance, as anesthetics can directly or indirectly induce hypothermia. Water-circulating heat pads are recommended for this purpose. Using electric heating pads may overheat or bum the animal; if these are used, the pad must be set on low, and a light cloth covering or bubble wrap should be placed between the animal and the pad, and the animal must be observed frequently for signs of hyperthermia. Even the use of rectal thermostat heating blankets can result in burned and overheated animals. Because heat lamps may cause severe hyperthermia or other thermal injury, their use is prohibited.

To prevent corneal desiccation, bland ophthalmic ointment must be placed in the eyes immediately following anesthetic induction. If the animals are undergoing survival stereotaxic surgery, blunt ear bars must be used to prevent tympanic membrane damage.

Paralytic agents must not be used without anesthesia. If a neuromuscular blocking agent is required for the surgical procedures, please refer to the Guidelines on Neuromuscular Blocking Agents.

Suture Selection

Use an absorbable suture material for body wall closure or other internal wound closures, and a nonabsorbable monofilament suture material for skin closure. Subcuticular suture placement, although more technically challenging, is acceptable for skin closure and may be performed using absorbable materials. The smallest gauge suture material should be used as practicable; typically 3-0 or 4-0 material is acceptable. A list of acceptable suture materials is included in Table 4 below.

Table 4. Acceptable Suture Materials

Suture	Characteristics and Frequent Uses
Vicryl®, Dexon®	Absorbable; 60-90.days. Suitable for internal wound closure.
PDS®, Maxon®	Absorbable; 6 months. Suitable for internal wound closure where extended wound support is desirable.
Prolene®	Nonabsorbable. Suitable for skin closure.
Nylon	Nonabsorbable. Suitable for skin closure.
Stainless Steel Wound Clips, Staples	Nonabsorbable. Suitable for skin closure. Requires instrument for removal from skin.

Because silk and chromic gut may cause tissue inflammation, these materials are not acceptable for wound closure.

Sutures, staples, or wound clips must be removed 7-14 days following surgery. Suture removal prior to euthanasia is not necessary for those animals euthanized within 14 days of surgery. Foreign substances (e.g.: suture material) remaining in the incision for an extended period serves as a nidus of irritation and infection. Please contact an LAC veterinarian to examine any incisions that do not appear to be healing.

For guidance in suture selection and use, please contact an LAC veterinarian.

Post-Operative Recovery

Observation during post-surgical recovery is imperative. The animal, whether recovering in or out of its 'home' cage, must be kept warm. Water-circulating heating pads are recommended for this purpose. Using electric heating pads may overheat or burn the animal; if these are used, the pad must be set on low, and a light cloth covering should be placed between the animal and the pad, and the animal must be observed frequently for signs of hyperthermia. Turn somnolent animals periodically to prevent bums or other thermal injury. Provisions must also be made for a conscious animal to escape the heat source when it becomes too warm. Because heat lamps may cause severe hyperthermia or other thermal injury, their use is prohibited.

A recovering animal must be watched continuously until in sternal recumbency *and* showing a degree of *purposeful* movement. **Unconscious animals must never be unattended**. To prevent undue risk, rodents must be housed individually following surgery until they are ambulatory.

Post-Operative Analgesic and Antibiotic Treatment

As described in the Guidelines on Post-Operative Care and Analgesia, analgesia must be provided to all animals following survival surgery, unless scientific justification for withholding such agents is approved by the LACUC as part of the investigator's research protocol, or if a veterinarian examines the animal and determines that analgesic administration is no longer necessary.

A list of commonly used analgesics is included in Table 5 below. At LAC, the analgesic drug of our choice is Rimadyl® (Carprofen).

Table 5. Frequently Used Analgesics

Drug	Species	Dose	Route	Frequency
Opiate drugs				
Buprenorphine	Mice	.05-.1 mg/kg	SQ	Every 12 hours
	Rats	.01-05 mg/kg	SQ	Every 12 hours
	Birds	.01-.05 mg/kg	TM	
Non-steroidal drugs (NSAIDs):				
Carprofen	Mice & Rats	5 mg/kg	SQ	Once daily
Ketoprofen	Mice & Rats	5 mg/kg	SQ	Once daily
Flunixin meglumine	Mice & Rats	1.1-2.5 mg/kg	SQ	Every 12 hours

The use of local pain-relieving drugs such as Xylocaine® (Lignocaine) or Marcaine® (Bupivacaine), in addition to systemic analgesia, may be indicated for some procedures resulting in significant disruption of the skin (e.g., Alzet® pump placement, catheter exteriorization), as these drugs may help to block the onset of the pain cascade due to disruption of the dermal nerve cells. Local analgesics are not intended for use in lieu of systemic analgesics, unless the withholding of systemic analgesia is scientifically justified. Post-operative antibiotic treatment should be discussed with an LAC veterinarian to determine whether routine antibiotic administration is necessary. In general, post-operative antibiotics should be provided if the animal will survive long enough to develop severe infection, but may also depend upon other factors such as the invasiveness of the procedure and the animal's immune status. Administering antibiotics prior to commencing a procedure can further minimize post-operative infections.

Long-Term Recovery and Monitoring
Post-surgical observations include a minimum daily observation, including weekends and holidays, of the animal's condition and the surgical site. Animals should be observed for continued recovery, which may include state of arousal; indices of pain or discomfort; condition of the surgical wound; appetite; hydration status; capillary refill time; mucous membrane color; or fecal and urine production.

Some surgical manipulations may require an extended period of post-operative monitoring. The LAC veterinary staff, in consultation with the investigator, can determine the appropriate duration and extent of monitoring. Some situations constituting prolonged monitoring periods include animals with chronic debilitating disease states (e.g., diabetes mellitus), animals undergoing organ transplantation or immunosuppressive 'therapy, and animals with chronically implanted instruments or catheters.

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Record-keeping Requirements
In accord with recommendations of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International, surgical records are required for rodents and birds. These records should include the administration of anesthetics, fluids, and any drugs; procedure details, including intra-operative monitoring; daily post-operative recovery observations and treatment, including administration of analgesics and antibiotics; monitoring of incision healing, including suture/staple removal if applicable; and the initials of the individual performing these tasks. Record all medications, including the name, dose, route, and time of administration. Additionally, any adverse outcomes should be noted.

To facilitate the veterinary staff's evaluation of post-operative healing and to ensure sutures are appropriately removed, **each cage card must be clearly marked with the date of surgery**. If animals need antibiotics in their drinking water, please communicate your needs to the laboratory officers at the respective animal facility and special treatment tags (orange *Animal under veterinary treatment/observation)* will be placed on the cages of animals that require treatment.

All records relating to surgical procedures and post-operative care may be subject to review during IACUC inspection or audit.

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Survival Surgery Guidelines

The following guidelines, which are based on the standards set forth in the Guide for the Care and Use of Laboratory Animals (Guide), and applies to survival surgeries performed on all laboratory animals. Investigators are responsible for ensuring all members of their laboratory adhere strictly to the IACUC-approved protocol and applicable IACUC policies and guidelines.

Pre-operative Management and Assessment

1. Preparatory steps should be taken to ensure the following:
   a. The surgical procedure(s) will be carried out efficiently, professionally, and with adequate record-keeping (including a record of all drugs administered and the dose, time, and route of administration).
   b. The animal's health has been appropriately evaluated prior to the surgery in order to minimize the risk of complications during and after surgery.
2. The surgeon is responsible for:
   a. Scheduling operating room time, determining the estimated procedure length, preparing necessary instruments and equipment, and arranging the anesthetic regimen to be used.
   b. Making appropriate logistical arrangements with the designated surgical assistant (within the investigator's own group) or surgical technician as outlined in # 3 below.
   c. Ensuring food is withheld from the animal for an appropriate period prior to the procedure. For assistance, please make these arrangements at the AHU (MD1) office at least two-business day prior to the expected surgical date.
   d. Assessing the overall status of the animal prior to the surgical procedure should include visual inspection of the animal and assessment of the animal's behavioral status. If there are previously undetected physical or behavioral abnormalities, these must be brought to the attention of the LAC veterinary staff.
3. The surgical assistant or technician is responsible for:
   a. Communicating with the surgeon to determine the nature of the procedure, the anticipated duration, the desired anesthetic technique, the necessary monitoring equipment and instruments, and the desired operating room configuration.
   b. Preparing all of the necessary records and informing the surgeon of any other records he/she must complete.
4. Consideration should be given to having pre-operative hematology and serum chemistry tests. The specific tests considered appropriate should be detailed in the protocol submitted to the IACUC; however, these may need to be included to address anticipated or historical veterinary care concerns.
   a. Minor surgical procedures usually involve some anesthetic risk, and multiple minor procedures may further complicate this risk. A hematologic assessment of packed-cell volume and total plasma protein may be appropriate in such cases.
   b. Major surgical procedures involve some anesthetic risk, and the nature of the procedure may further complicate this risk. It may be appropriate to carry out a complete blood cell count (red and white cell parameters, platelets, etc.)

plus an assessment of plasma or serum protein and kidney and liver enzymes. The need for these tests is heightened if there are induced or spontaneous medical or surgical abnormalities.

Intraoperative Procedures

1. Acute (terminal) and chronic (recovery) surgical procedures causing more than momentary or slight pain or distress require the appropriate use of anesthetics analgesics, and tranquilizers (AATs) as addressed in the NACLAR Guidelines and the Guide.
2. Most of the commonly used AATs can have both short-term and long-term effects on an animal's ability to maintain homeostasis. Monitoring of physiologic functions provides important indices of the animal's homeostatic state.

Intraoperative Monitoring

1. Unconscious and/or intubated animals must not be left unattended.
2. Monitoring is to be done by trained staff from LAC or the investigator's laboratory.
3. Cardiovascular
   a. Heart rate must be monitored continuously. This can be done via continuous ECG monitoring, blood pressure monitor, or pulse oximetry. The heart rate must be recorded every 15 minutes.
   b. Mucous membrane color and capillary rell time may be monitored periodically to help assess cardiac output and oxygen saturation.
   c. Blood pressure monitoring (by direct or indirect methods) should be considered for major surgical procedures lasting more than 2 hours.
   d. Hypotension may occur due to anesthetic agents, blood loss, and evaporative water loss of exposed tissues or expired gases. Blood pressure should be maintained through the appropriate use of intravenous fluid therapy, generally 5-15 ml/kg/hr IV of crystalloids (an average of 10ml/kg/hr IV is recommended).
4. Pulmonary
   a. If the animal is intubated, chest auscultation should be done to assess endotracheal tube placement. Airflow should be audible in all lung fields.
   b. Pulmonary function must be monitored. This can be done via continuous capnography and/or pulse oximetry, respiratory rate observation, or auscultation. The pulmonary function should be recorded at least every 15 minutes.
   c. Pulse oximetry and capnography should be considered for all major surgical procedures.
   d. Blood gas monitoring and recording should be considered for major surgical procedures lasting more than 3 hours.
   e. Mucous membrane color can be used to assess overall ability to oxygenate, but is not a very sensitive measure.
5. Temperature
   a. Body temperatures should be monitored by digital or conventional rectal thermometers or by electronic temperature (esophageal or rectal) probes. The temperature should be checked and recorded at least every 15 minutes.

b. Core body temperature should be maintained at normal levels with circulating water blankets, heated surgical tables, warmed intravenous fluid therapy, or other appropriate methods.

6. Anesthetic depth

   The animal's anesthetic depth should be monitored throughout the surgical procedure.

   Depending on the species, this can be done by observing responses to manipulation, jaw tone, palpebral reflexes and position of the eye.

Post-operative Care and Monitoring

1. Adequate post-operative care enhances the animal's recovery, minimizes pain and distress, and is a requirement of both professional and regulatory federal agencies. Adequate post-operative care includes monitoring and documenting the animal's recovery during the anesthetic recovery period, the acute post-operative period, and the long-term post-operative period.
2. The Principal Investigator must arrange for post-operative care. In some cases, surgical staff may perform part of the post-operative care. The Attending Veterinarian has appropriate authority to ensure provision of adequate veterinary care during all phases of the post-operative period. Communication between the Principal Investigator's staff and the LAC veterinary staff is crucial.

Anesthetic Recovery

1. Unconscious or intubated animals must not be left unattended.
2. Endotracheal tubes should not be removed until an animal has regained a swallow reflex. Animals must be monitored continuously until they are conscious and sternal.
3. All animals must be given analgesics unless scientific justification for withholding such agents is provided by the investigator and approved by the IACUC. Analgesics must be given such that these agents become effective before the animal emerges from anesthesia. Therefore, analgesia must be administered before or during surgery.
4. The animal's status must be recorded in the animal's post-operative chart every 5 minutes. The following parameters should be monitored and recorded at least every 15 minutes during the anesthetic recovery period:
   a. Respiratory rate, as assessed by observation, auscultation, capnography or other appropriate method. Any abnormal or deviant pattern or respiration should also be noted.
   b. Heart rate, as assessed by auscultation, palpation, ECG, pulse oximetry or other appropriate method.
   c. Temperature
5. It is the responsibility of the investigator to arrange for appropriate monitoring during the anesthetic recovery phase, either by a member of the investigator's laboratory or the surgery room staff.
6. During the anesthetic recovery period, the animal's core body temperature should be monitored and maintained in a normal range using circulating warm water blankets or warm water bottles. *The core body temperature of some animals is greater than a human being's (>37 °C).*
7. To avoid aspiration pneumonia, airway obstruction, pulmonary edema, tissue necrosis or edema at pressure points, a recumbent animal's position should be

adjusted every 15 minutes. In addition, capillary refill time, mucous membrane color, condition of the wound, and the animal's anesthetic plane should be monitored.

Acute Post-operative Period

1. The acute post-operative period is that period when the animal regains stable physiologic functions, usually over 24-48 hours. Monitoring during this time is usually done 2-3 times daily depending on the type of surgical procedure and the condition of the animal. The Principal Investigator's staff will monitor and record the post-operative condition of these animals at least once daily. An LAC veterinarian or veterinary technician will also check these animals at least once daily.
2. The acute post-operative period may require further treatments to stabilize the animal, such as fluid therapy, analgesics, antibiotics, and more intensive monitoring. Analgesia is required in all animals, excluding those in Pain Category E, as described in the *Guidelines on Post-operative Care and Analgesia.*
3. Depending on the species, monitoring parameters may include: respiratory rate and character; mucous membrane color; capillary refill time; hydration status; appetite; condition of the surgical wound; state of arousal; indices of pain or discomfort. If the animal is immobile, the heart rate and core body temperature should be monitored.
4. Post-operative treatments (analgesics, antibiotics, fluid therapy, etc.) must be administered to the animal according to the Principal Investigator's research protocol, or as determined by the LAC veterinary staff in consultation with the Principal Investigator. This may be done by the LAC veterinary staff or by the Principal Investigator's staff. All medications, including name, dose, route, and time of administration, must be recorded in the animal's post-operative record.

Long-term Post-operative Period

1. The long-term post-operative period is from the time of physiologic stabilization to normalization. This usually takes a minimum of 10 days for the animal to totally recover from most survival surgical procedures. Daily monitoring should continue during this time. Parameters that should be monitored/recorded during this time include: state of arousal; indices of pain or discomfort; condition of the surgical wound; appetite; hydration status; capillary refill time; mucous membrane color; fecal and urine production; and any medication administered.
2. If at the end of the 10-day period non-absorbable sutures remain, the animal's post-operative record must be continued at least until the non-absorbable sutures are removed.
3. Some surgical manipulations may require an extended period of post-operative monitoring. The LAC veterinary staff in consultation with the Principal Investigator can determine the appropriate duration and extent of monitoring. Some situations constituting prolonged monitoring periods are animals with chronic debilitating disease states (e.g., diabetes mellitus), animals having organ transplantation or immunosuppressive therapy, and animals with chronically implanted instruments or catheters.

Record Keeping

The following records are to be kept and maintained in the animal's record, as applicable, to assure compliance with the various regulatory agencies.

- Anesthesia Record (Required for survival and non-survival surgeries)
- Procedure Record (Required for survival and non-survival surgeries)
- Post-Procedure Record

Principal Investigators may keep copies of the record(s) or keep notes in their laboratory notebooks, but maintain the original records with the animal's record.

IACUC Policy on AVA Covered Species

All animals that are subject to the regulations covered in the AVA's Animal and Bird's Act should be house in LAC supervised facilities and all procedures performed on these animals should be carried out in procedure rooms and surgical suites maintained by LAC whenever possible. Exceptions will require scientific justification, including (but not limited to) advanced instrumentation that cannot be transported from the investigator's laboratory or the lack of adequate housing or procedure rooms within LAC supervised facilities. Requests for exceptions must include descriptions of the housing facilities, routine animal care and health monitoring activities, as well as any surgical procedures including pre and postoperative care plans and anesthesia and analgesia, in sufficient detail for LAC veterinary staff and the IACUC to assure adequate animal care.

Regulations contained in the Animal and Bird's Act (Rules on the Care and Use of Animals for Scientific Purposes 2004) pertain to all live vertebrate used, or intended for use for research, teaching, testing, experimentation, or exhibition purposes.

Multiple Survival Surgeries

Basis:

NACLAR Guidelines, p. 35 (Repeated use of animals in experiments): Individual animals should not be used in more than one experiment, either in the same or different Projects, without the express approval of the IACUC. However, it is noted that appropriate re-use of animals may reduce the total number of animals used in a Project, result in better design of experiments, and reduces stress or avoid pain to other animals.

Guide for the Care and Use of Laboratory Animals, p. 12: Multiple major survival surgical procedures on a single animal are discouraged but may be permitted if scientifically justified by the user and approved by the IACUC. For example, multiple major survival surgical procedures can be justified if they are related components of a research project, if they will conserve scarce animal resources, or if they are needed for clinical reasons. If multiple major survival surgery is approved, the IACUC should pay particular attention to animal well-being through continuing evaluation of outcomes. Cost savings is not an adequate reason for performing multiple major survival surgical procedures.

Guidelines:

Multiple survival surgeries on a single animal may be permitted only if scientifically justified by the investigator and approved by the IACUC. The number of survival surgeries performed must be limited to the minimum number necessary to achieve the research objectives and must be determined with due consideration for minimizing the pain and distress experienced by any one animal.

Whenever possible, all operative procedures should be done at one time to minimize post-operative discomfort and distress to the animal. Cost or convenience is not considered to be adequate justification for the conduct of multiple survival surgeries.

Emergency multiple surgeries may be done to correct complications due to previous surgical procedures or as part of the process of providing veterinary care; however, these must be done in consultation with an LAC veterinarian. Euthanasia should be considered as a humane alternative to surgical repair in these cases if the animal is in poor health or suffering pain or distress that cannot be alleviated.

Guidelines on Neuromuscular Blocking Agents

The use of neuromuscular blocking agents may be approved for research procedures where scientific justification is provided for paralysis of the animal. Few indications exist for the use of neuromuscular blocking agents in research involving animals, therefore, proposed use will be reviewed on a case by case basis. These drugs must not be used alone to provide restraint and immobilization, and may only be used in conjunction with drugs producing surgical anesthesia, and hence, unawareness of the paralytic state. Due to the inherent difficulties in assessing the level of surgical anesthesia in paralyzed animals, the use of these drugs will be approved only if it is clearly established that (1) neuromuscular blockers are essential for the proposed research, and (2) that the investigator is able to monitor the animals appropriately for signs of pain and distress.

The following guidelines apply to those protocols employing neuromuscular blocking agents:

A surgical plane of anesthesia must be induced and the animal intubated prior to administration of the neuromuscular blocking drug; furthermore, **a surgical plane of anesthesia must be maintained during the entire time the neuromuscular blocking drug is present and active *in vivo*.** Neuromuscular blockers must not be administered until after the initiation of the surgical procedure (i.e. the skin incision) to ensure that the depth of anesthesia is adequate and the animal does not feel pain. Use of neuromuscular blocking agents should be confined solely to that phase of the procedure for which they are indicated. Neuromuscular blocking agents must not be used as a matter of convenience or to substitute for poor control of anesthesia.

Nitrous oxide in most animal species is not an anesthetic and cannot provide a surgical plane of anesthesia; therefore, it must not be used alone with neuromuscular blocking agents during surgical procedures.

The use of a pre-operative analgesic is recommended in addition to the general anesthetic during surgical procedures where neuromuscular blockers are being used.

Controlled ventilation must be initiated prior to administration of the neuromuscular blocking drug.

During the period of paralysis, signs of reaction to pain and stress must be continuously monitored as appropriate to the species (e.g., heart rate, blood pressure). If these parameters increase by 20% or more without other explanation, pain/stress may be assumed to be present, and the anesthetic level should be deepened. Baseline measurements must be established at the initiation of anesthesia for comparison. If a surgical procedure is being performed, baseline measurements should be made at the initiation of the surgery (skin incision) to determine that the depth of anesthesia is adequate. Monitoring of electroencephalography (EEG) may also be helpful. However, the normal EEG appearance differs with different types of anesthetics, and confirmation of an anesthetized state may not always be possible based on the EEG.

Therefore, the investigator should be thoroughly familiar with the expected EEG pattern for the particular anesthetic used.

Core temperature, blood gases, and fluid and electrolyte balance must be maintained within normal levels during the period of paralysis. If animals will be paralyzed for long periods of time (e.g. greater than 4 hours) provision must be made for periodic voiding of the urinary bladder.

Prior to using neuromuscular blocking drugs in a procedure, investigators must be prepared to demonstrate the proposed procedure, at the request of the IACUC, in the absence of the paralyzing drug. This will assure that the anesthetic technique is sufficient to prevent pain and distress associated with the procedure, and to confirm that escape behavior does not occur in the absence of the neuromuscular blocking agent.

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Rodent Neonatal Anesthesia Guidelines

The scientific literature describes various neonatal rodent anesthetic methods, including injectable agents (e.g., ketamine-medetomidine, ketamine-xylazine, pentobarbital, fentanyl-droperidol), inhalant agents (e.g., methoxyflurane, halothane, isoflurane), and physical agents (hypothermia). Aside from anesthetic survival, parental cannibalism of the anesthetised neonate is the second biggest hurdle. Generally, low mortality exists with inhalants and hypothermia; injectable agents demonstrate a user-based bias. Hypothermia has significant ethical-based questions unanswered regarding its ability to truly anesthetize animals; furthermore, direct contact between the ice-water slurry and the neonatal rodents must be avoided. Although cannibalism concerns appear more closely aligned with hypothermia, all anesthetic-related procedures carry this risk. Maternal conditioning and procedure- related factors (e.g., removing blood, avoiding suture material) also impact cannibalism.

<u>Injectable Agents</u>
Several injectable agents have been described; however, a given agent's success appears subject to the individual accustomed to using a given agent. Commonly used agents sited include pentobarbital, ketamine-xylazine, and fetanyl-droperidol. In neonates a fine line apparently exists between successful outcome and the animal's death. Anesthetic agent dilution is a common point addressed in articles to titrate a given injectable agent to a desired anesthetic plane. Supplemental oxygen was not administered and its use may have yielded higher survival rates.

<u>Inhalant Agents</u>
Various inhalant agents have successfully provided general anesthesia to neonatal rodents in a manner consistent with general anesthesia in older rodents and larger species. Several methods are described including cleverly modified delivery devices for neonatal ophthalmic and other procedures lasting upwards to an hour. Inhalant anesthetics delivered via precision vaporizer utilize oxygen as a carrier gas, facilitating oxygenation and blunting hypoxia associated with injectable agents and hypothermia.

<u>Hypothermia</u>
Hypothermia is an older anesthetic method requiring minimal skill and technical equipment. Ethical concerns exist over its use in neonates because torpor could result, but it is unclear if a truly anesthetic state exists; hypothermia results in functional motor fiber loss prior to functional pain fiber loss. Furthermore, the immature state of the neonatal rodent's pain inhibitory pathways indicate they are more susceptible to noxious stimuli, resulting in an allodynia-like state. For these reasons, investigators are encouraged to consider other options, specifically inhalant anesthetic agents.

When a need for hypothermia is demonstrated, greater success has been associated with the following:

- As rapid cooling is painful, animals chilled in a latex sleeve (avoiding direct contact with the hypothermia media) should reduce this painful sensation.
- Avoid rapid warming because of the resulting tissue damage; an incubator maintained at 33°C is recommended. Once warmed to this level, water

recirculating heating pads may be used. The water recirculating heating pad is superior to heat lamps and uninsulated electric heating pads (even rectal-probe-regulated heating pads). Until animals are fully conscious they cannot 'escape' excessive heat generated by incandescent lamps and heating pads while in the peri-emergent sensory altered state associated with anesthesia.

- Supplemental oxygen may benefit the recovering animals, too.
- Proper wound management (See 'General' below).

General

The following items have resulted in the positive outcome in neonatal rodent surgeries:

- Handling and olfactory conditioning of pregnant females
- Proper animal oxygenation during anesthesia
- Visual monitoring during and after anesthesia
- Maintain body temperature during and after anesthesia
- Practice aseptic technique
- Use tissue adhesives (e.g., VetBond) for wound closure, if applicable
- Clean animals to ensure blood, etc. have been removed
- Allow appropriate anesthetic recovery prior to placing pups with the dam

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Guidelines on the Use of Pharmaceutical-Grade Compounds

LAC has established the following guidelines:

- Investigators are expected to use pharmaceutical-grade medications whenever they are available, even in acute (nonsurvival) procedures.

- Using non-pharmaceutical-grade chemical compounds in animals requires specific review and approval by the IACUC for reasons such as scientific necessity or non-availability of an acceptable veterinary or human pharmaceutical-grade product. Cost savings is not an adequate justification for using non-pharmaceutical-grade compounds in animals.

- All non-pharmaceutical-grade compounds must be filter-sterilized using a 0.22 j.tm filter prior to administration. Filtering will not removed endotoxin and other compounds that will interfere with research results.

Furthermore, LAC has taken the stance that questions the use of "antiquated anesthetics" (e.g.: urethane, avertin/TBE, chloral hydrate/equithesin) in contemporary scientific research indicating the use of such agents be justified and indicating, "...pilot studies may be in order to evaluate the need for such agents…"

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Expired Medical Materials

Expired Medical Materials

The use of expired medical materials such as drugs, fluids, or sutures on regulated animals is not considered to be acceptable veterinary practice and does not constitute adequate veterinary care as required by the NACLAR guidelines. All expired medical materials found in a licensed or registered facility are to be brought to the attention of the responsible official. The facility must either dispose of all such materials or segregate them in an appropriately labeled, physically separate location from non-expired medical materials. For acute terminal procedures, the use of expired medical materials, assuming if their use does not adversely affect the animal's well-being or compromise the validity of the scientific study, is permitted. Proper anesthesia, analgesia, and euthanasia are required for all such procedures. Drugs administered to relieve pain or distress and emergency drugs must not be used beyond their expiration date. Facilities allowing the use of expired medical materials in acute terminal procedures should have a policy covering the use of such materials and/or require investigators to describe in their animal activity proposals the intended use of expired materials. The attending veterinarian and the Institutional Animal Care and Use Committee (IACUC) are responsible for ensuring that proposed animal activities avoid or minimize discomfort, distress, and pain to the animal. These responsibilities cannot be met unless the veterinarian and the IACUC maintain control over the use of expired medical materials.

What does this mean?

FOR SURVIVAL PROCEDURES:
All drugs and medical materials (e.g., saline bags, suture materials, antibiotics) used in **survival procedures** must be disposed of after the expiration date.

> **Example**: Items stamped "12/03" may be used through December 31, 2003. It is outdated January 1, 2004. Dispose of the outdated items appropriately.

Procedures must exist for ensuring drugs and medical materials are within the expiration date. This includes labeling all drug preparations with the date of preparation and the earliest expiration date of the component drugs. All expired supplies must be labeled "Expired — Do Not Use" and stored separately from non-expired materials if immediate disposal is not possible.

FOR NON-SURVIVAL PROCEDURES:
With the exception of controlled substances and emergency, anesthetic, analgesic, or euthanasia drugs, expired medical materials may be used in **terminal procedures**, including non-survival surgeries, provided:

- Materials are marked as **"Expired — Use ONLY in TERMINAL Procedures."**
- Materials are stored in a different location (cabinet, drawer, etc.) than materials used for survival procedures.
- The use of expired medical materials does not adversely affect the animal's well-being or compromise the validity of the scientific study.
- Proper anesthesia, analgesia, and euthanasia are employed for all such procedures.

IDENTIFICATION AND REMOVAL OF EXPIRED MEDICAL MATERIALS:

The IACUC recommends that each laboratory establish a procedure to facilitate the identification and removal of expired drugs and other medical materials used for research involving animals. This may take the form of a drug log, signed by a member of the laboratory each month, indicating that they have checked for and discarded, or set aside for disposal, any expired drugs or other medical materials from their laboratory.

LAC has developed an internal system using a series of color dots to identify the expiration date (month and year) of medical materials. This system will be outlined in the next issue of the LAC Newsletter.