GUIDELINES FOR CONDUCTING SURVIVAL SURGICAL PROCEDURES

All major survival surgical procedures must be performed utilizing aseptic technique. The following definitions should be considered when determining if the procedures you are employing must meet these requirements.

Survival procedure - one in which the animal awakes from the anesthetic, even if for a short time.

Major procedure - a survival surgical procedure in which the surgical intervention penetrates a body cavity or has the potential for producing a permanent handicap in an animal that is expected to recover. Procedures such as **laparotomy, cannulations, ovariectomies, nerve sections** are all considered major survival surgery.

Although specialized surgical facilities are not required for surgeries in rodents, fish or birds, such facilities are required for lagomorphs (rabbits) and higher species. Please specify that procedures will be conducted utilizing aseptic techniques and provide the location of the laboratory where surgery will be performed when submitting a protocol that involves survival surgery. The Animal Facility has several procedure areas available, which can be utilized for conducting animal surgery. Contact the Animal Facility Manager for additional information.

The importance of utilizing aseptic techniques should not be underestimated. They are designed to reduce post-surgical complications (e.g., infections and wound dehiscence), improve animal survival rates, and hasten return to the basal physiological functions that were present in the animal prior to surgery. Specific regulations and guidelines can be found in the *Guide for the Care and Use of Laboratory Animals*.

The conduct of aseptic technique requires strict adherence to practices in a number of different areas. Consult Appendix I for highlights of the recommendations provided in this document.

Surgical Laboratory

As stated earlier, although dedicated surgical facilities are not required for rodents, fish or birds, it is still necessary to prepare the area in which surgery is to be performed. Although the investigator's laboratory is suitable, the site in which surgery is going to take place should be free of all ancillary equipment and should provide a **clean and clear work area** in which the investigator will perform the procedures.

Disinfecting surfaces - Prior to and after the completion of all surgical procedures, all organic debris should be removed and work surfaces cleaned. The surfaces should then be disinfected with an appropriate cleaning solution. The appendix contains a brief description and guidelines to follow when using these agents. We recommend covering the work surface with clean plastic backed absorbant paper, or an equivalent covering, for each procedure. Devices or equipment (i.e., animal restraining devices, monitoring equipment, stereotaxic devices, etc.) that will be required in the surgical field should be disinfected as described above. The above practices are performed in order to reduce or eliminate potentially infectious organisms and the substrates on which they grow.

Surgical Equipment and Instruments

Sterilization of surgical instruments - All surgical instruments, implantable devices, and equipment that will contact the surgical site or are implanted in the animal are to be sterilized, using any of the techniques described below. The methodology selected will depend on time considerations, specialized equipment available, and the composition of the material to be sterilized. Proper sterilization techniques must be followed for the particular method in order to obtain consistent results.

Steam sterilization - Conducted in an autoclave. An autoclave is available in the Animal Facility. Facility staff will autoclave your surgical packs. Please contact Sally Sockwell at 5512 for further information.

All surgical supplies and equipment must be cleaned prior to sterilization in order to remove any organic material that may interfere with the sterilization process. Surgical instruments may be cleaned in an ultrasonic cleaner or by hand, using a stiff bristle brush and a moderately alkaline, low sudsing detergent. Deionized or distilled water is preferred for cleaning.

Surgical supplies should be wrapped in cotton muslin or crepe paper. Materials should be placed in the autoclave in a manner that allows steam access to all surfaces. Supplies should be wrapped so that the autoclave packets can be opened easily without touching any of the sterilized equipment or instruments. Sterilization cycles are autoclave specific, but in general the following cycles can be utilized:

soft goods	30 min	250°F	15 psi
standard surgical pack	20 min	250°F	15 psi

flash sterilization (instruments only)	3 min	273°F	30 psi

All instruments and soft goods should be allowed to dry a minimum of 15 minutes and until cool following sterilization and before use.

Dry Heat Sterilization - Dry heat sterilizers containing glass beads can be used to sterilize surgical instruments (tips only). Clean instruments are inserted into preheated beads for 10 seconds to achieve sterilization. This method is extremely useful for sterilizing instrument tips between animals when multiple animals are surgically manipulated. Care should be taken when using this method with fragile instruments.

Chemical or cold sterilization - Refers to the process of soaking instruments in disinfectant or sterilant solutions. The agent utilized will determine the effectiveness of the sterilization process and the contact time necessary to achieve sterilization. Consult the product insert for details. The ideal disinfectant is one that will destroy all bacteria, bacterial spores, and viruses. The only agents that meet these criteria and are recommended for the cold sterilization process are the glutaraldehydes, available under the trade names Cidex and Sporicidin. As with all previously described sterilization procedures, instruments should be free of all organic debris prior to placement in solution. Instruments should be rinsed thoroughly with sterile water prior to use. Most chemical sterilants carry personal health and safety risks with their use.

In addition to the glutaraldehydes, chemical disinfectants such as Nolvasan^{R} can be utilized to sterilize instruments between surgical procedures on multiple animals. These methods should not be used for initial sterilization; however, when performing consecutive surgeries, placement of the instruments in these solutions for 20 minutes is permissible to eliminate potential bacterial contamination.

Surgeon Preparation

Although the requirements for preparation of the surgeon in rodent surgery are less rigorous than what is required for higher mammals, proper preparation is still necessary. Either a clean 3/4 length laboratory coat or a surgical scrub shirt must be worn by the surgeon and any assistant(s). In addition, a surgical mask (which serves to prevent contamination of the wound by droplets of saliva from the surgical team) must be worn. A number of steps should be taken prior to preparing the surgical site (see next paragraph). After preparing the surgical site, the surgeon should wash his/her hands with a disinfectant and then don the required **sterile surgical gloves** using a method that maintains the sterility of the gloves.

Surgical Site Preparation

Proper preparation of the surgical site (i.e., skin) involves a series of steps or processes. In rodents, one should define the site of incision and remove hair or fur from an area approximately 150% larger than the area of the incision, either by clipping or using a depilatory. All loose fur should be vacuumed or carefully dusted away in order to prevent translocation into the incision. An Oster surgical clipper with #40 blade or Oster Pro Trimmer, is ideal for clipping animal fur. In birds, feathers should be plucked from the surgical site. Once the site is free of fur or feathers, surgical preparation of the skin may commence. A number of agents are available for this purpose. We recommend the use of either povidone-iodine scrub (Betadine^{R} Scrub) or chlorhexidine scrub (Nolvasan^{R}). Both of these agents have good bactericidal activity and contain a detergent. Using 3x3 gauze squares, cotton-tipped applicators, or the equivalent, the area should be scrubbed beginning at the center of the incision site working out to the perimeter. After reaching the perimeter, a new gauze square should be selected and the process repeated. This should continue for approximately 3 minutes. After completing the above preparation, the area should be wiped with gauze 3x3's soaked in 70% isopropyl alcohol or 70% ethanol. The final step prior to making the incision is to paint or spray the surgical site with a 10% povidone-iodine (Betadine^{R} solution) or chlorhexidine solution (Nolvasan^{R}). The surgical site should be covered with a sterile drape. Sterilized surgical crepe paper should cover the entire animal with the exception of the head (when not performing surgery on that area) and the tip of the tail. The center of the drape overlying the site of the incision should be cut out in order to visualize the incision. Please note that the draping procedure should be performed utilizing sterile gloves.

Now all preparations have been made for surgery. The goal of aseptic technique is to prevent the surgeon, all instruments, implantable materials and equipment utilized, and the surgical site from contamination. Therefore, one should avoid touching or handling anything that has not been sterilized. The surgeon should restrict contact to the surgical site and previously sterilized equipment until the incision is closed.

Please note: Rodents - eyes must be lubricated with Puralube or an equivalent preparation while under anesthesia or they can suffer corneal damage.

Suture Materials and Wound Closure

Selection and use of appropriate suture materials is imperative for successful wound closure and healing. Sutures are either absorbable or nonabsorbable dependent upon the materials from which they are manufactured. Sutures are made from natural materials such as silk, cotton, or catgut; or synthetics such as nylon, polygalactin, or stainless steel.

Proper wound closure is essential to avoid wound dehiscence. Surgery in which a body cavity is entered, e.g., laparotomy or thoracotomy, requires a two-layer closure in which the body wall is closed separately from the skin. A two-layer closure reduces the potential of evisceration or pneumothorax following a laparotomy or thoracotomy, respectively. For routine surgical procedures in animals, commercial suture materials with swaged (attached) needles in sterile packets are ideal.

Materials should be selected that are the correct size, have appropriate absorption (if absorbable) and handling characteristics for the intended procedure and animal species. Absorbable suture should be used for closure of the muscle. The ends of the suture in the muscle layer should be clipped short so they do not extend through the incision in the skin. This prevents wicking bacteria into the incision and/or the animal manipulating the suture.

Silk suture should be avoided. Silk is braided and bacteria (skin microflora) penetrating the interstices of the braid may lead to infections. It is imperative that suture materials are sterile, since they are a foreign material and provide a substrate where bacteria may proliferate. Stainless steel wound clips may be used for skin closure in animals. The clips should be sterilized before each surgical session. Wound clips, like nonabsorbable sutures, must be removed 10 -14 days after placement.

Multiple Surgeries in a Single Session

Groups of animals are frequently surgically manipulated during a single session. Care must be taken to avoid contaminating one animal from another. New sterile gloves must be donned and preferably, separate sterile surgical instruments should be available for each animal subject. Always begin a surgical session with instruments that have been sterilized using an approved method. The following procedures are recommended when a limited number of surgical instruments are available and separate sterile surgical packs are not feasible:

1. Use two sets of instruments. One set is used for incising and manipulating the skin, which is considered a potentially contaminated site because of microbial flora. Once the initial incision and skin manipulation is complete, these instruments are set aside. A second set of instruments is used to manipulate deeper tissues that are sterile. Using this system care must be exercised to maintain the sterility of the surgical gloves by avoiding contacting tissues with fingers. The second set of instruments that are shared should be rinsed with sterile saline or water between animals.

2. Utilize a glass bead sterilizer to sterilize instrument tips between animals. Recognize that only the instrument tips are sterilized. Care must be taken to avoid touching tissues with instrument handles or other non-sterile instrument parts. Because non-sterile instrument handles are held with gloved fingers, contact of tissues with the fingers must be avoided.

3. Have two or more sets of sterile instruments available so that the contaminated set can soak in cold sterilant solution for at least 10 minutes to kill vegetative bacteria prior to reuse. Instruments must be rinsed thoroughly with sterile saline or water prior to reuse.

Post-Operative Care

It is the responsibility of the investigator, in consultation with the Attending Veterinarian and the Animal Facility staff, to promote the optimal recovery of the animal from surgery and anesthesia by providing appropriate postoperative care. A number of factors (e.g., type of surgery performed, type and amount of anesthetic used) will modify the nature, duration, and intensity of postoperative care required by the animal patient.

Postoperative care programs should be considered and designed before commencing any experimental procedures. The following minimal essential components should be routinely incorporated into postoperative management of animals:

1. The animal should be kept warm by the use of recirculating hot water pads, warming packs, hot water bottles, blankets or lamps, and, if animal size permits, body temperature should be monitored and recorded until it returns to normal. If electrical heating pads are used, they should be monitored for the development of hot spots which can cause burns.

2. Animals recovering from anesthesia should be rotated from side to side every 15 minutes until they are able to maintain sternal recumbency. They should not to be left unattended until they have recovered consciousness.

3. Animals recovering from anesthesia should not be placed directly onto contact bedding as they may inhale or ingest bedding particles. Placing the animals onto a paper towel or equivalent will prevent this from occurring.

4. Hydration should be assessed on a daily basis and fluid replacement administered at a volume of 60-80 ml/day/kg body weight for animals which are not eating and drinking postoperatively. In small laboratory animals, fluids may be given parenterally, either subcutaneously or intraperitoneally. Lactated ringers solution or an equivalent should be utilized. Fluids should be warmed prior to administration to animals.

5. Adequate nutrition is necessary in the healing animal patient. Caloric replacement should be instituted for animals that have not resumed eating by the second postoperative day. Caloric replacement may require supplemental feedings using specialized dietary formulations and feeding methods. Contact Veterinary Technicians in the Animal Facility (Sonia Acevedo or Sally Sockwell) for assistance.

6. The incision must be examined daily for evidence of wound dehiscence or infection until it is completely healed. Nonabsorbable sutures or wound clips should be **removed 10 -14 days** postoperatively. If not removed, the remaining suture or clips acts as a foreign body frequently leading to bacterial infections of the skin.

7. Analgesics should be utilized in animals which demonstrate pain related behavior, e.g. guarding of the incision, reluctance to move, anorexia, absence of normal behavior patterns, etc. Please consult Veterinary Technologists in the Animal Facility for additional information.

Appropriate records must be kept of your postoperative care evaluation and treatments. Contact Sonia Acevedo for sample postoperative care forms.

Appendix I

Animal Surgery
Chemical (Cold) Disinfectant/Sterilant Agents Summary

Work Surface Sanitation

Work surfaces are wiped down with the following agents and allowed to air dry.

Sodium Hypochlorite	(1:20 dilution of Bleach in water)
Chlorine Dioxide	(Clidox)
Roccal D	(1:200 dilution in water)
Chlorhexidine	(Nolvasan solution 1:40 dilution in water)
Quaternary Ammonia	(other than Roccal; use according to label instructions)

Chemical (Cold) Instrument/Implant Sterilization

Instruments must be rinsed thoroughly with sterile saline or water to remove sterilant before use.

Prior to surgery (to kill spores and vegetative cells)
Note: Alcohol is not a sterilant and is not acceptable for this purpose.

Agent	Required Contact Time
Glutaraldehydes (Cidex, Sporicidin)	10 hours

Between animals (to kill vegetative cells)

Glutaraldehydes (Cidex, Sporicidin)	10 minutes
Chlorhexidine (Nolvasan Solution)	20 minutes
Alcohol (70% isopropyl)	20 minutes
Chlorine Dioxide (Alcide, Clidox)	3 minutes (caution: corrosive to metals)

Preoperative Skin Preparation

Note: Alcohol alone is not acceptable for this purpose.

Two Recommended Procedures:

1.) **Povidone-Iodine scrub** (betadine) 3 minutes scrub/contact time.
Alcohol (70% isopropyl) wipe/rinse to remove iodine scrub.
Povidone-Iodine Solution - Provides residual activity.

2.) **Chlorhexidine scrub** (Nolvasan Scrub, Hibiclens) 3 minute scrub/contact time.
Alcohol (70% isopropyl) wipe/rinse to remove Nolvasan scrub.
Chlorhexidine Solution - Provides residual activity.

Surgical Attire

Sterile Surgical Gloves
Laboratory Coat or Scrub Top
Surgical Mask

DISINFECTANTS AND STERILANTS

Definition

Disinfectant is a germicidal, chemical substance that kills microorganisms on inanimate objects, such as instruments and other equipment that cannot be exposed to heat. Disinfectants differ in their spectrum of activity. They do not kill the tubercle bacillus or spores.
Antiseptic is a chemical agent that either kills pathogenic microorganisms or inhibits their growth as long as there is contact between agent and microbe. By custom, the term "antiseptic" is reserved for agents applied to the body.

Sterilization is the process of killing all microorganisms including all bacteria, fungi, viruses, and spores with the use of either chemical or physical agents.

Agents

The following agents are commonly used as disinfectants, antiseptics, and sterilants.

Glutaraldehyde: 2% glutaraldehyde solutions are used for cold sterilization. They are commercially available as **Cidex** (Surgikos-Johnson & Johnson) and **Sporicidin** (Ash-Dentsply). These agents are diluted with an activator prior to use and have limited shelf lives after dilution (14 days for Cidex; 30 days for Sporicidin). They can be used for disinfection or sterilization depending upon the time allowed for instrument contact. Cidex and Sporicidin require 10 hours of contact time for sterilization. Instruments may be soaked for 10 minutes in Cidex solution, to remove vegetative bacteria, when performing surgical procedures on multiple animals as long as they are sterilized thoroughly before the first procedure in the sequence. Glutaraldehyde is toxic to skin and mucous membranes. Therefore, it must be thoroughly rinsed from instruments and other items with sterile water before use. It is the "cold" sterilant of choice for cleansed instruments.

Chlorine Compounds: Household **bleach** (5% sodium hypochlorite) is effective against all classes of microorganisms but is inactivated by organic debris. It can be used as a disinfectant on previously cleaned surfaces at a dilution of 1:20. Full strength or a 1:5 dilution is recommended against hepatitis B virus, HIV or on surfaces soiled by potentially contaminated fluids. Some authors claim that the tubercle bacillus and other similar organisms are resistant to hypochlorite. It must be made fresh; solutions that are allowed to sit may deteriorate. Bleach will damage fabric and is an irritant to mucous membranes. **Chlorine dioxide** (Clidox), is available as a binary system, consisting of a base and an activator, which require mixing. Once prepared the useable life of the solution is 14 days. Chlorine dioxide is effective against all classes of microorganisms including bacterial spores. Three minutes of contact time is necessary for efficacy.

Alcohols: Alcohols destroy bacteria via the coagulation of protein. They have poor activity against bacterial and fungal spores, evaporate rapidly if kept in open containers, form flammable mixtures with air, are inactivated by organic matter, and dissolve lens cement mountings. In spite of these shortcomings, they are rapidly bacteriocidal and are useful antiseptics. Isopropyl alcohol is typically used as a 70% solution; ethyl alcohol is used between 75 and 90%. Ethyl alcohol has abuse potential and must be kept in locked cabinets. When used as antiseptics, they are applied to the skin after Nolvasan or Betadine Scrub. They are only effective as disinfectants as long as they remain in solution. As such they may be used for emergency disinfection of instruments by immersion for 20 minutes

Povidone-Iodine: Free iodine is complexed to the polymer povidone to produce a non-toxic antiseptic. Povidone-iodine is effective against all classes of microorganisms. It is most commonly used as a surgical scrub **(Betadine Scrub**, Purdue-Frederick) at 7.5%. It is also available as a 10% solution **(Betadine Solution);** the solution is used undiluted to paint the skin after an appropriate surgical scrub.

Chlorhexidine acetate: Chlorhexidine acetate is commercially available as both a 2% solution and 2% surgical scrub (**Nolvasan Solution; Nolvasan Surgical Scrub**, Aveco/Fort Dodge). The scrub is used undiluted as an antiseptic. The solution is used as a disinfectant by diluting 3 ounces to a gallon of water. Nolvasan solution is not effective aginst gram + cocci or *Pseudomonas aeruginosa* on inanimate objects.

Quaternary ammonium compounds (Benzalkonium chloride): Roccal D and other compounds in this class are used to decontaminate surfaces (i.e it is not used on instruments). A 1:200 dilution with water is recommended by the manufacturer. It is effective against a variety of viruses, gram + and gram - bacteria. Zephiran has been largely supplanted as an antiseptic by Betadine. These agents have no effect against the tubercle bacillus and are inactivated by soaps.