Training in Survival Rodent Surgery

Introduction:
This CD was developed to assist you in becoming proficient in performing aseptic rodent surgery. To get the most from this program we suggest you review the Definitions and the NIH Guidelines for Survival Rodent Surgery available in the References. You may also find it helpful to print the script and use it in following each module.

Module 1 - General Training in Rodent Survival Surgery:
We all have an ethical responsibility to animals in terms of minimizing pain and distress. This can be accomplished, in part, by using proper surgical technique. There is a scientific responsibility in terms of performing and reporting good science, but there is also a legal responsibility.

The Public Health Service Policy on Humane Care and Use of Laboratory Animals requires that research institutions base their animal care and use on the Guide for the Care and Use of Laboratory Animals (referred to as “the Guide”).

The following principles described in the Guide apply to rodent surgery:

- Appropriate pre-operative and post-operative care of animals in accordance with established veterinary medical and nursing practices is required.
- All survival surgery will be performed by using aseptic procedures, including sterile gloves, masks, sterile instruments, and aseptic techniques.
- A dedicated surgical facility is not required for rodents but surgery must be performed using aseptic techniques.
- Research personnel will be appropriately qualified and trained in all procedures.

The Guide states that it is important for personnel to have appropriate training to ensure that good surgical technique is practiced.

Good technique includes:

- asepsis,
- gentle tissue handling,
- minimal dissection of tissue,
- appropriate use of instruments,
- effective hemostasis, and
- correct use of suture materials and patterns.

The NIH has established specific recommendations for how rodent surgery should be conducted. The full text of the NIH Intramural Research Program Guidelines for Survival Rodent Surgery can be found in the Reference section.

The PHS Policy, the Guide, and NIH guidelines all specify the use of aseptic technique when performing surgery. What is aseptic surgery?

NIH guidelines define aseptic technique as surgery performed using procedures that limit microbial contamination so that significant infection or suppuration does not occur.

This includes preparation of the patient, preparation of the surgeon, sterilization of instruments, supplies, and implanted materials, and the use of operative techniques to reduce the likelihood of infection.
How do you achieve aseptic technique? Pre-surgical planning begins during the protocol development phase in consultation with your veterinarian. This includes:

- identification of personnel, their roles and training needs
- equipment and supplies required for the procedures planned
- the location and nature of the facilities in which the procedures will be conducted, and
- pre- and post-operative care.

In planning the location to perform your surgery you must select the best possible surgical area. Characteristics of a good surgical area include an uncluttered area that is easily organized and disinfected, and free of debris and equipment not related to surgery. The area should be dedicated for the duration of the procedure, but can be used for other purposes when not being used for surgery.

Avoid locations that are beneath supply ducts to minimize contamination from dust.

Avoid high traffic areas such as those near doorways to prevent unnecessary interruptions and creation of air turbulence.

Select the anesthetic depending on the type of surgical procedure, the length of the surgical procedure, the equipment available and the expertise of those who will be responsible for administering the anesthetic. Each anesthetic used must be approved in your protocol and administered in consultation with your veterinarian.

Most inhalant anesthetics are administered using precision calibrated vaporizers. When using gas anesthetics you must also account for scavenging of waste gases. One acceptable method of scavenging is the use of a downdraft table. It is important not to cover the surface of the downdraft table. This will cause a loss in its ability to effectively scavenge gases.

Downdraft tables are usually only effective up to a height of 6-8 inches from the surface. Do not use induction chambers taller than this for induction of anesthesia.

Chemical fume hoods can also be used to scavenge waste anesthetic gases. Type IIB Biosafety cabinets that are vented to the outside are another method used to scavenge waste anesthetic gases.

Charcoal canisters can also be used for scavenging. Note that charcoal canisters must be weighed before, and after, each use. Most must be replaced after an increase in the recommended weight. Depending on the size of the canister and the manufacturer’s recommendations, the canister should also be weighed during especially long procedures to assure its continued effectiveness.

Injectable anesthetics are widely used in rodent surgeries. The NIH Division of Veterinary Resources Pharmacy maintains a stock of commonly used rodent anesthetics available for purchase by NIH staff. Check their web site (http://dvrnet.ors.od.nih.gov/internal/pharmacy.asp) for more information. Approval by your Controlled Substances Custodian will be necessary to purchase controlled drugs. Controlled substances require additional record keeping.

If using injectable anesthetics, weigh each animal and dose each according to its body weight.

If making your own anesthetic, such as tribromoethanol, you should be cognizant of the proper methods for preparation and storage.
Some anesthetics, such as ketamine, abolish the blink reflex. Anesthetized animals should have their corneas protected with an ophthalmic lubricant. To avoid contamination of the lubricant, do not touch the tip of the tube to the skin or eye surface.

Anesthetized animals must be monitored during the procedure to assure that they stay in the proper anesthetic plane. That is, they must not be allowed to get too light or too deep.

Once the anesthetic has been given time to take effect, the anesthetic plane can be assessed by pinching the toes, tail or ear of the animal. Any reaction from the animal indicates that the animal is too light and that additional anesthesia should be given.

It is important to visually inspect the animals and not rely solely on monitoring instruments. The color of the mucous membranes and exposed tissues is easy to monitor. This will give an indication of tissue perfusion and oxygenation. The color should be a bright pink to red and not dusky gray or blue.

Respiratory pattern and frequency is also easily monitored and will give an indication of anesthetic depth and other potential complications. Core body temperature can also be monitored in rodents, including mice.

Pulse oximetry can be used in larger rodents to monitor pulse and oxygenation. Instruments must be properly calibrated as inaccurate information may be confusing, distracting, and misleading. Electrocardiograms can also be used in larger rodents. In protracted anesthesia procedures such as during MRI when the animal is inaccessible, capnography may be employed.

The most frequent complication of small animal anesthesia is hypothermia resulting in prolonged recovery or death of the animal. Animals should be provided with a heat source during the pre-operative, intra-operative, and post-operative periods. Because of the high air flow, the risk of hypothermia is heightened when using chemical fume hoods or biosafety cabinets.

Improper use of warming devices can lead to injury. Heat lamps can be very dangerous. Temperature at the level of the animal should be held between 85 to 95°F. Use a thermometer to measure the temperature adjacent to the animal.

Electric heating pads can also be very dangerous. They have varying temperatures across the surface. It is recommended that these not be used for rodents.

Slide warmers can be used as a heat source during recovery. Animals must be watched very closely and be placed in the recovery cage as soon as they begin to stir. The safest devices for providing heat are circulating hot water blankets or instant heat devices such as Safe-n-Warms® or hand warmers. These devices must be covered with a paper towel or other insulation so that the animal does not come in direct contact with the hot pad.

Planning the surgical procedure also requires consideration of the instruments required for the procedure and what type of pack will be utilized. A simple peel pack can contain small numbers of small to medium sized instruments. A complex pack consists of overlapping cloth or paper drapes folded together and sealed with autoclave tape. It can contain a large collection of instruments of various sizes. Tip protectors should be added to delicate instruments or those with sharp points.

When performing multiple rodent surgeries it is a good idea to have staging areas for the different steps of the procedure. Whenever possible, animals waiting for surgery should be kept at a visual and olfactory distance from those animals undergoing surgery.
Surgical preparation of the animal should occur in a location different than that used for performing the surgeries. This will help to prevent hair and dander from getting on the sterile packs.

If space constraints or requirements for use of the down draft table necessitates a single location for prepping and surgery, then the bench towel used to prep the animal should be replaced before performing the surgery. The surgical pack, if already open, must be covered to prevent contamination with hair.

Sanitize the area you have selected for performing your surgery with an appropriate disinfectant such as Clidox®, Alcide®, or Nolvasan®. Remember a dedicated surgical site is not required for rodent surgery.

Once everything is pre-selected and organized, you can begin by anesthetizing the animal and doing the surgical prep. Hair must be removed from the surgical site. The most common method is to use an electric clipper. You may want to use a piece of adhesive tape or moistened gauze dabbed over the clipped area to pick up loose hair that could otherwise migrate into the incision.

An easy alternative to clipping the fur is to remove it by plucking. Hair follicles in mice are usually in telogen or resting phase, and hair can be removed without injury. It is a fast and easy method that does not leave stubble.

The standard surgical prep consists of alternating scrubs of an iodophor such as Betadine® and 70% alcohol. Alternative scrubs such as chlorhexidine (e.g. Novalsan® Hibiclens®) may also be used.

Using a gauze sponge or cotton-tipped applicator, cleansing should be done in a circular motion beginning at the center of the shaved area and working toward the periphery. Never go back to the center with the same sponge. The scrubs should be alternated between an iodophor scrub and alcohol. This should be repeated at least 3 times, ending with the iodophor. Be careful not to excessively wet the animal as this can exacerbate hypothermia. For small incision sites cotton-tipped applicators work best.

Once the animal is prepared you must open the surgical pack. This is to be done before donning surgical gloves. Regardless of whether your surgical pack is simple or complex, you must make sure the sterilization indicator has turned the appropriate color before using.

Simple-peel packs are opened in a manner that preserves the sterility of the inside surface. Do not touch the inside surface as it can be used as a sterile surgical field on which to keep the instruments.

Complex surgical packs are opened in such a way as to keep the inside surface of the wrapping sterile so that it can be used as a sterile field.

If using cold sterilant solutions, make sure instruments are exposed for the proper length of time and that expiration dates of solutions are observed. Instruments must be removed from the solution and rinsed with sterile water, saline or alcohol. This is very important, as the sterilization solution is very irritating to tissues. Rinsed instruments must be placed on a sterile field.

Hot bead sterilizers may also be used. This method sterilizes only the tips of the instruments. The beads must be pre-heated to the recommended temperature and the instruments exposed for the recommended time. Gross debris must be removed from the instrument before sterilizing. Instruments must be allowed to cool before touching tissues. This method is best reserved for sterilizing instruments between surgeries.
“Flash” dry heat sterilizers are another alternative for sterilizing instruments. Remember to allow the instruments to cool before touching them to tissues.

Delicate instruments, materials for implantation such as catheters or items that otherwise may melt or become damaged when heated can be sterilized using ethylene oxide. The packs must be sufficiently aerated to prevent toxic effects from residual gas. This may require 24-72 hours.

Remember, alcohol provides disinfection not sterilization and should not be used to sterilize instruments.

Once instrument packs are opened, make sure all other sterile equipment, such as scalpels and suture material, are opened and placed on the sterile field. These items must be opened in such a way as to prevent contamination of the item and the surgical pack.

Proper surgical attire consists of cap, mask, and clean lab coat. Hands should be washed with an antibacterial soap and sterile gloves donned. Exam gloves used for handling animals and working in the laboratory are not the same as sterile surgical gloves!

It is important to don the gloves in such a way that prevents contamination of the outer surface of the glove.

- One of the gloves is lifted from the opened glove package by its turned-down cuff.
- The glove is pulled on the hand with a rotating motion. Do not touch the outside surface of the glove.
- Place the gloved fingers beneath the cuff of the other glove.
- With the gloved fingers under the cuff, the glove is placed on the ungloved hand. The folded cuff protects the gloved hand from contamination.
- It is pulled over the cuff of the lab coat following insertion of the hand.
- The fingers are then slipped under the cuff of the first glove to pull it over the lab coat cuff.

After the surgical gloves are donned, the prepared surgical site must be draped. The most common drape is a paper drape. It may be precut or one in which you must cut your own hole. The disadvantage of paper drapes is that they usually cover the entire animal, making patient monitoring difficult. Plastic drapes, usually with an adhesive, offer the advantage of more visibility and better patient monitoring. Sterile gauze sponges can also be used for drapes.

Organize the instruments in your surgical pack. Point all the tips in one direction. It is helpful to place them in the order used. Between surgeries or during breaks in surgeries cover the tips of the instruments with sterile gauze.

You are now ready to perform your surgery. Be aware of the space that is not sterile between your pack and the draped animal. Do not lay instruments in this space. They will become contaminated.

While performing surgery, be careful not to get paper or cloth instrument drapes wet. Wet material acts as a wick to pull bacteria through from the non-sterile surface below. When this occurs the instruments should be considered contaminated and re-sterilized before further use.

The selection of the type and size of suture material should be done in advance in consultation with your veterinarian and based on the type of surgery and species of animal. If you are inexperienced with common suturing methods used in rodents, please review Module 2.
For small animals, a 3 aught suture thickness or smaller is best. Cutting and reverse cutting needles have sharp edges and are best used for skin suturing. Non-cutting, taper or round needles are used for suturing easily torn tissues such as peritoneum, muscle or intestine.

If ligation of vessels or suturing of tissues other than skin is necessary during surgery, an absorbable material such as Vicryl®, Dexon®, PDS®, Maxon®, or Chromic Gut should be used.

For skin closure, non-absorbable suture such as Prolene® or nylon, stainless steel wound clips or staples may be used. Most rodents will gnaw at any externalized sutures, so a buried suture line or wound clips are recommended. Cyanoacrylate surgical adhesives, such as Vetbond® or Nexaband®, may be used to close incisions or to close the area between sutures. If you are unfamiliar with these products, you should ask your veterinarian for advice on their proper usage.

Silk is a non-absorbable suture material that can cause tissue reactions and may wick microorganisms into the wound. It is best used for cardiovascular procedures only and not for closure.

The Guide states that the application of prophylactic antibiotics is not a substitute for the practice of proper aseptic surgery. If prophylactic antibiotics must be used, for example in gastrointestinal surgery or an accidental break in aseptic technique, then you must choose an appropriate antibiotic that is given for the recommended length of time at the correct dose. In guinea pigs and hamsters the use of inappropriate antibiotics can cause fatalities. Your veterinarian should be consulted for the appropriate drugs and dosages for the species involved.

Remember to keep the recovering patient warm. Do not lay recovering animals directly on the bedding. They may aspirate and asphyxiate.

Part of your surgical planning should have given consideration to intra- and post-operative analgesia. Again, your veterinarian should be consulted during protocol planning for advice. Remember to keep records of controlled substances.

Recovery from anesthesia can also be aided by the administration of warmed fluids given subcutaneously or intraperitoneally. Your veterinarian should be consulted for appropriate volumes and routes of administration.

In neonates, or animals recovering from prolonged surgical procedures, hypoglycemia can be a problem leading to post-surgical complications. These animals may benefit from the administration of oral glucose. Glucose solutions should never be given subcutaneously (SQ) or intraperitoneally (IP).

Animals may be returned to their holding area once they are awake and appear to be making a normal recovery. Be sure to mark the cage card with the surgical procedure performed and the date.

Post-operative care does not end with the return of the animal to its home environment. Animals must be monitored for several days after the surgical procedure. Daily weighing of the animal during the post-operative period is a sensitive method of monitoring the animal. While subtle changes in the animal’s activity or appetite may not be clinically observed, changes in weight will be quickly detected allowing appropriate clinical intervention to be instituted. It is important to remember that some analgesics may depress the appetite causing secondary weight loss. This weight loss must be differentiated from that which occurs in an animal that is not feeling well.

Animals must be monitored for the continued need for analgesics. This assessment should be made at least twice daily in the first few post-operative days. Food intake may be difficult to monitor in
rodents, especially if they are group housed. However, if post-operative animals are singly housed and food rations are supplied in measured amounts this can be a useful monitoring tool.

Supplying a softer, more palatable, easily accessible diet may encourage the animal to eat.

The animal’s hydration can be monitored by “tenting” the skin along the back of the animal. In a well-hydrated animal, the skin should quickly fall back into place when released. If an animal is dehydrated, the skin will be slow to return to its original place. When this occurs, your veterinarian should be consulted for the appropriate use of subcutaneous or intraperitoneal fluids.

Wound closures must be removed at 10 to 14 days post-operatively. Suture scissors or staple removers must be used.

You have now completed the first module. If you have any questions about what has been presented, please consult with your veterinarian.

**Module 2 – Simple Suture Patterns for Rodent Surgery**
If you are inexperienced with suturing techniques, review this module with some practice suture and instruments in hand. Practice models work well to simulate the look and feel of suturing.

**Instrument Square Knot**
For most incisions, an instrument square knot can be used. To begin suturing, the needle is passed through the far edge of the tissue first and then the near edge. The needle holder is placed between the far or tail end of the suture, shown here as blue, and the near or needle end, shown here as white.

A loop is formed around the needle holder in the near strand. Note that the needle holder remains parallel to the incision while the throw is placed. The far or tail end is grasped in the needle holder. Avoid making the tail too long. Do not grasp at the base of the tail.

The left hand shown wearing a blue glove and the right hand shown wearing a cream glove are reversed in position so that the tail is now on the opposite side. Even tension is applied as the throw is tightened.

A half-hitch will result if hands are not reversed. This knot can slip easily and result in wound failure.

The knot is tightened just enough to oppose the skin edges. Over-tightening can result in excessive swelling of the wound edge.

To begin the second throw a loop is formed with the needle end of the suture. Grasp the tail end of the suture with the needle holder. Grabbing the needle-end rather than the tail end will not allow completion of the knot. The strands are tightened under even tension as they are held close to the incision. A correct square knot will have a small space between the first and second throws to allow for tissue swelling.

Placement of the needle holder between the two strands for one throw and exterior to them for the second throw will result in a granny knot.

Failure to take the tail end to the opposite side for each throw will result in a double half-hitch that can easily slip.
Begin the third throw by placing a loop from the needle end around the needle holder and grasping the tail to take it to the opposite side. To complete the knot the needle holder crosses the incision to the opposite side. Remember to apply even tension while tightening the knot and keep the needle holder parallel to the incision while the throw is being placed.

Your knot should now look like this. A fourth throw is made in the same manner as for previous throws. This will complete the double square knot.

**Instrument Surgeon’s Knot**

For incisions under tension, an instrument surgeon’s knot can be used. The instrument surgeon’s knot is tied in much the same fashion as the instrument square knot, but a double loop is placed around the needle holder for the first throw.

The tail end is grasped in the needle holder. The hands are crossed and even tension is applied.

This knot, when tightened, will not slip as readily as the square knot.

The second loop is made in the same manner as the square knot. A single loop is made around the needle holder, which is always placed between the two strands. The completed surgeon’s knot appears this way.

The knot is completed by placing one to two more single loop throws. The completed surgeon’s knot appears this way.

**Mistakes to Avoid**

A half-hitch will result if hands are not reversed. This knot can slip easily and result in wound failure.

Failure to take the tail end to the opposite side for each throw will result in a double half-hitch that can easily slip.

Placement of the needle holder between the two strands for one throw and exterior to them for the second throw will result in a granny knot.

Now that you have reviewed this module, to become proficient at suturing techniques, try practicing it with suture and instruments in hand.

**Module 3 – Special Considerations for Aseptic Surgery in a Transgenic Mouse Facility**

This presentation is meant as an example of how the existing NIH Guidelines for Survival Rodent Surgery can be practically used in one of the most common rodent surgery procedures performed at NIH. This is not the only way to perform transgenic surgery aseptically. However, when considering alternative practices, the following points should be followed:

- The animal must be maintained in a surgical plane of anesthesia throughout the procedure.
- Begin surgery with sterile instruments and handle them aseptically.
- Instruments and gloves may be used for a series of similar surgeries provided they are maintained clean and disinfected between animals.
- Provide analgesics as appropriate.

The most common anesthetic used for transgenic surgeries is tribromoethanol, usually referred to by its European brand name Avertin®. (Please see the addendum at the end of this section for additional anesthetic considerations concerning tribromoethanol.)
Frequently, transgenic surgeries require multiple stations and multiple animals anesthetized at any given time. An advantage of using tribromoethanol versus inhalants anesthetics is that the use of scavenging devices can be avoided.

Other anesthetics, such as a Ketamine-Xylazine mixture, may also be acceptable. Your veterinarian should be consulted for advice on selection and administration of anesthesia. Anesthetic agents selected must be approved on your Animal Study Proposal.

Since Avertin® is not commercially available in the U.S., it is made in the laboratory. Therefore, the solution should be filtered through a 0.2μ filter. This will remove debris, most bacteria, and some viruses.

The anesthetic solution should be stored at 4° Centigrade in an amber or aluminum foil-wrapped bottle. When diluting the stock solution of Avertin® to the working solution, it is important to use a buffered diluent.

Decomposition of the anesthetic can result from improper storage. The pH should be greater than 5. When the pH falls below 5, decomposition to toxic substances can occur. If pH falls, or if crystallization or any other discoloration is noted, the anesthetic should be discarded as it can cause death within 24 hours of injection.

The Avertin® is given as an IP injection. The most common dose is 0.24 – 0.4 milligrams per gram body weight. Remember to protect the animal’s corneas with an ophthalmic lubricant. To avoid contamination of the lubricant, do not touch the tip of the tube to the skin or eye surface.

While waiting for the mouse to become anesthetized, prepare your sterile surgical instruments. Cold sterilization with an agent such as Cidex® is commonly used in transgenic surgery. Follow required exposure times to ensure sterilization of instruments. When using cold sterilant, the instruments must be rinsed with sterile water, saline, or 70 % alcohol as they are removed from the sterilant solution. This step is critical, as cold sterilant is very irritating to tissues.

Glass bead sterilizers can also be used to sterilize the tips of the surgical instruments. If using a glass bead sterilizer you should remember the following:

- Only the tips of the instruments are sterilized.
- The tips of delicate instruments may become damaged during immersion in the glass beads.
- The tips of the instruments become extremely hot.
- The instruments must be allowed to cool before applying them to the skin or other tissues.

Place the tips of the sterile instruments on a sterile field. To avoid contamination of the tips of the instruments during the surgical procedure, always keep the tips on the sterile field and pointed in the same direction.

Once the mouse is anesthetized, the fur is removed from the surgical site by shaving. This should be done in a location different than that used for performing the surgeries. Hair can also be removed from the surgical site by gently plucking the fur.

The surgical site is prepped with alternating scrubs of an iodophor such as Betadine® and 70 % alcohol. Cotton-tipped swabs or 2x2 gauze sponges may be used. It is important to avoid excessively wetting the mouse, as this will lead to hypothermia and anesthetic complications.
Place the prepped mouse on several 4x4 gauze sponges or a small heating pad to provide warmth. After donning a clean pair of gloves, an incision through the skin and abdominal musculature is made.

Sterile surgical gloves do not have to be worn if the following criteria are strictly adhered to:

- the gloved hand never touches the prepped surgical field, and
- only the sterile tips of the instruments are used to handle the animal’s tissues.

The mouse is placed on the pre-warmed microscope surface. A surgical drape is placed and the ovary is exteriorized. Note how the tips of the instruments have been placed on a sterile gauze sponge to prevent contamination.

The embryos, 1-cell, 2-cell, or blastocysts, which have been collected and maintained in sterile conditions, are loaded into the pipette. Note how the tip of the pipette is resting in such a way as to prevent the tip from becoming contaminated. Also notice that there is a break between the pipette and the suction tube to prevent accidental aspiration. A filter is placed in the apparatus to protect the eggs from contamination due to the operator’s mouth.

A serrafin clip is placed on the ovarian fat pad in preparation for embryo implantation. The embryos are implanted. One-cell or two-cell embryos are implanted into the infundibulum. Blastocysts are implanted into the proximal uterine horn.

In a larger transgenic facility it is preferred that one person prep the mouse, one person makes the skin incision and closes it, and a third person does the implanting. This keeps the risk of contamination to a minimum.

Sterile ophthalmic surgical spears work well to blot blood or fluid from the surgical site. Sterile gauze sponges or cotton-tipped applicators may also be used.

The abdominal musculature is closed with an absorbable or monofilament non-absorbable suture. Note how only instruments are used to handle the tissue. After the abdominal wall has been closed, one to two drops of a long acting local anesthetic such as bupivacaine or ropivacaine should be applied to the surgical wound. This will supply analgesia to the surgical site.

The skin is closed with a non-absorbable suture or stapling device. Wound closures must be removed in 10-14 days. Surgical glue is an alternative product used to close the skin.

The mouse is placed on the warming tray for recovery from anesthesia. Once the mice begin to awaken from the anesthesia, they are placed in the pre-warmed recovery cage. Throughout the recovery and post-surgical convalescent period, the mice must be closely monitored for the need for additional analgesia.

The cage card or a special observation card should be marked with the surgery date, so that you know when to remove the wound closures and when to expect the birth of pups.

Since multiple surgeries are performed each day, the instruments should be rinsed with 70% alcohol between mice. Alcohol will disinfect, not sterilize. Alternatively, the glass bead sterilizer can be used to sterilize the tips of the instruments. Remember to allow instruments to cool before touching tissues!

Gloves should be rinsed with 70% alcohol between surgeries. If you have had to handle another mouse to anesthetize and prep it, you should change gloves before performing the next surgery.
Regardless, remember to never touch the tissue ends of the surgical instruments and always keep the tips of the instruments on a sterile field.

By following these guidelines, your mice will recover quicker without complications.

**Addendum - Anesthetic Considerations for the Transgenic Mouse Facility**

There is abundant literature available regarding the use of Tribromoethanol (TBE, Avertin®) in the production of genetically engineered mice (GEM). Many reports describe the safe and effective use of this drug given strict adherence to the precautions necessary for preparation and storage. However, there are also a growing number of reports describing complications associated with its use. Given this, the investigator should give thorough consideration to other anesthetic regimens that might be equally effective in their laboratory. The most common examples include; isoflurane inhalant, ketamine/xylazine injectable or ketamine and other injectable drug combinations. Your veterinarian should be consulted for advice on the optimal anesthesia regime based on your survival surgery area, the skills of the anesthetist and the equipment available.

**References**


This module was developed by the Transgenic Surgery Subcommittee of the NIH Animal Research Advisory Committee. Committee members included:

- Shelley Hoogstraten-Miller, NHGRI, Chair
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- Michael Bloom, NHLBI
- Judith Hewitt, NIAID
- David Bodine, NHGRI

Special thanks to the NHGRI Transgenic Core for their assistance in providing subjects for the photographic images throughout this presentation.
Definitions:

**Analgesia:** the relief of pain without loss of consciousness

**Asepsis:** The prevention of contact with microorganisms.

**Capnography:** The measurement of exhaled carbon dioxide values, providing both a graphical and numerical display.

**Cold Sterilants:** Chemical agents that destroy microorganisms. See table below

<table>
<thead>
<tr>
<th>Agent</th>
<th>Examples</th>
<th>Comments</th>
</tr>
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<tbody>
<tr>
<td>Chlorine</td>
<td>Chlorine Dioxide</td>
<td>Corrosive to instruments. Instruments must be rinsed with sterile saline or sterile water before use.</td>
</tr>
<tr>
<td>Glutaraldehydes</td>
<td>Cidex®, Cetylcide®, Metricide®</td>
<td>Several hours required for sterilization. Corrosive and irritating. Instruments must be rinsed with sterile saline or sterile water before use.</td>
</tr>
<tr>
<td>Hydrogen peroxide-acetic acid</td>
<td>Actril®, Spor-Klenz®</td>
<td>Several hours required for sterilization. Corrosive and irritating. Instruments must be rinsed with sterile saline or sterile water before use.</td>
</tr>
</tbody>
</table>

**Disinfection:** The chemical or physical process that involves the destruction of pathogenic organisms. All disinfectants are effective against vegetative forms of organisms, but not necessarily spores.

**Hemostasis:** To stop bleeding.

**Hypoglycemia:** An abnormally low concentration of glucose in the blood.

**Hypothermia:** A low body temperature.

**Instrument square knot:** A double knot formed using instruments in which the free ends of the second knot are in the same plane as the ends of the first knot.

**Major Surgery:** Any surgical intervention that penetrates and exposes a body cavity; any procedure that has the potential for producing permanent physical or physiological impairment; and/or any procedure associated with orthopedics or extensive tissue dissection or transection.

**Minor Surgery:** Any surgical intervention that neither penetrates and exposes a body cavity, nor produces permanent impairment of physical or physiologic function. Examples are superficial vascular cutdown, and percutaneous biopsy.

**Needles:**

**Taper needle:** (synonyms: non-cutting, atraumatic, round): the body of this needle tapers down to a fine point, permitting minimum tissue damage. This needle is especially suitable for soft tissue.
**Cutting needle:** used for suturing tough tissue (skin, tendon). Not as optimal as a reverse-cutting needle because tension of suture is at point of angle.

**Reverse cutting needle:** used for suturing tough tissue. Tension of suture is dispersed along edge of angle.

**Sterilization:** The process whereby all viable microorganism are eliminated or destroyed. The criterion for adequate sterilization is the failure of organisms to grow if a growth-supporting medium is supplied.

**Surgeon’s knot:** a knot in which the thread is passed twice through the first loop to prevent slippage.

**Suppuration:** The formation of pus.

**Suture material:** see table below:

<table>
<thead>
<tr>
<th>Wound Closure Material *</th>
<th>Characteristics and Frequent Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyglactin 910 (Vicryl®), Polyglycolic acid (Dexon®)</td>
<td>Absorbable; 60-90 days. Ligate or suture tissues where an absorbable suture is desirable.</td>
</tr>
<tr>
<td>Polydioxanone (PDS®) or, Polyglyconate (Maxon®)</td>
<td>Absorbable; 6 months. Ligate or suture tissues especially where an absorbable suture and extended wound support is desirable.</td>
</tr>
<tr>
<td>Polypropylene (Prolene®)</td>
<td>Nonabsorbable. Inert.</td>
</tr>
<tr>
<td>Silk</td>
<td>Nonabsorbable. (Caution: Tissue reactive and may wick microorganisms into the wound). Excellent handling. Preferred for cardiovascular procedures.</td>
</tr>
<tr>
<td>Chromic Gut</td>
<td>Absorbable. Versatile material</td>
</tr>
<tr>
<td>Stainless Steel Wound Clips, Staples</td>
<td>Nonabsorbable. Requires instrument for removal.</td>
</tr>
<tr>
<td>Cyanoacrylate (Vetbond®, Nexaband®)</td>
<td>Skin glue. For non-tension bearing wounds.</td>
</tr>
</tbody>
</table>

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

**References:**
1. Guide for the Care and Use of Laboratory Animals Ch. 3, Veterinary Medical Care pp 56-70, Institute of Laboratory Animal Resources, National Research Council, National Academy Press, Washington, DC, 1996. (http://www.nap.edu/readingroom/books/labrats/)

**Credits:**
This CD was developed by a subcommittee of the NIH Animal Research Advisory Committee to assist in the development of proper surgical skills when performing surgery on rodents. It illustrates the most common practices used in the NIH intramural research program and the rodent surgery standards established by the NIH “Guidelines for Survival Rodent Surgery.” Committee members included:
Disclaimer: Illustrations of products and materials in this program are not meant as endorsements by the NIH, but as examples of items commonly used in performing rodent surgery. Other similar products may work as well.