RODENT SURGERY GUIDE
STANDARD OPERATING PROCEDURE
EKU Institutional Animal Care and Use Committee (IACUC)
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Recommendations for the performance of rodent surgery are based on the eighth edition of the NIH *Guide for the Care and Use of Laboratory Animals* (2011; hereafter referred to as the '*Guide*') and 9 CFR, the Animal Welfare Act (AWA). Part 2 of the AWA states that ***major surgical procedures on rodents "must be performed using aseptic procedures."*** This would include the use of sterile instruments, sterile surgical gloves, and aseptic preparation of the surgical site in order to prevent postoperative infections. A separate facility for rodent surgery is not necessary. A rodent surgical area can be a room or portion of a room that is easily sanitized and not used for any other purpose during the time of the surgery. However, EKU does have a dedicated surgical space for rodents at the time of production of this document.

Anesthesia of rodents will not be discussed in detail here. A summary of inhalant and injectable anesthetics recommended by the Eastern Kentucky University (EKU) Institutional Animal Care and Use Committee (IACUC) is presented in Appendix 1 of this document.

Rodents include hamsters, gerbils and guinea pigs, as well as rats and mice. Guinea pigs, gerbils, and hamsters are U.S. Department of Agriculture (USDA) covered species, meaning that they are not exempt from USDA regulations and the provisions of the AWA.

Rodent Surgery can be Classified as Minor or Major in Nature.

PART I MINOR SURGERY

"Minor survival surgery does not expose a body cavity and causes little or no physical impairment" (the "*Guide*," p 117) and includes injections, vena-puncture, and subcutaneous implants. When conducted with proper care, these techniques present few difficulties. "Minor procedures are often performed under less stringent conditions than major procedures but still require aseptic technique and instruments and appropriate anesthesia." (the "*Guide*,"
p 118) For example, although heat sterilization, steam (autoclave) is required for the initial preparation of instruments, dry heat (glass bead sterilizer) or cold disinfection can be used for instruments in between animals during minor surgery. Agents such as chlorine dioxide or gluteraldehyde can be used for cold sterilization. ***Adequate contact time with the chemical disinfectant is required to achieve sterilization of the instruments, and manufacturers' recommended contact times should be followed***. Chlorine dioxide is not documented as being toxic to animal tissue but will corrode stainless steel instruments, whereas gluteraldehyde is known to be caustic to animal tissues. Chemical sterilants must be thoroughly rinsed off of instruments with sterile saline or water before use. Deionized or distilled water are not sterile from the tap. It is mandated by AAALAC (American Association of Laboratory Animal Care) and the USDA that expiration dates on the solutions be observed.

It is strongly recommended that surgeries be performed in a HEPA-filtered laminar flow hood to minimize the amount of contamination during surgery and protect the animals from unwanted infections, such as mouse hepatitis virus, rat coronavirus or mouse parvovirus. Be aware that much rodent research is performed within human medical centers and that implants or instruments can also contaminate rodents with human pathogens if improper technique is used.

The implanting of a chronic intravenous catheter is intermediate in nature, but is the technique that presents the most severe post-surgical infections, based on previous experiences of the author of this document. Because one is opening a direct venous access, surgical technique needs to be

meticulous, as for major surgery. Post-surgically, use sterile technique when accessing the catheter (s). The most critical requirement is to inject only sterile solutions into the catheter. ***Remember to observe expiration dates of solutions and supplies.***

Solutions should be freshly prepared or stored under refrigeration if prepared in advance. The top of the vial or mouth of the container containing solutions for injection must be kept clean and wiped with alcohol, or flamed, before drawing up the solution. Inoculation of even a few microorganisms into an intravenous catheter may result in death of the animal due to sepsis. ***Expiration dates for laboratory produced solutions and surgical supplies should be determined, or at least, solutions and supplies labeled with a production date.***

PART II MAJOR SURGERY

Major surgery includes invasion of the cranial, abdominal, or thoracic cavities. Any procedure that might leave the rodent with a permanent handicap, whether physical or physiological, would also be considered major surgery. ***The use of aseptic technique is mandatory in these surgeries to minimize the possibility of post-surgical infection***. Consultation with the attending veterinarian or EKU clinical laboratory animal veterinarian is recommended if you have questions regarding techniques appropriate for these situations.

Facility and Instrument Preparation

The area used for major rodent surgery should be located in a portion of the laboratory that is not heavily traveled. The surgical "table" must be constructed of a material that can be disinfected using appropriate agents (see Table 1) or that can be heat sterilized. The area immediately surrounding the surgery should be disinfected prior to surgery to decrease dust borne contamination. The majority of disinfectants are less effective in the presence of gross debris. Use soap and water for the initial cleaning of gross debris from the surface, then follow with the disinfectant. ***It is important to remember that chemical disinfectants require a minimum amount of contact time with the surface that they are applied to in order to achieve their maximum effectiveness. Follow manufacturers recommended contact times and allow to air dry before using the area for surgery.***

Surgical instruments must be sterile. Steam heat sterilization (autoclave) is required for the initial preparation of instruments, dry heat (glass bead sterilizer) or cold disinfection can be used for instruments in between animals during minor surgery. Catheters, implants and delicate instruments such as drills and burrs can be sterilized using ethylene oxide or ionizing radiation (see Table 2).

Preparation and Monitoring of the Animal

A pre-surgical evaluation should be performed to insure that your prospective patients are not overtly ill; for example, is the animal alert with a smooth coat and clear eyes?) The withholding of food is not necessary in rodents unless specifically required by the protocol or surgical procedure. Water should **NOT** be withheld unless stipulated in the protocol. Withholding of food for more than six hours should be discussed with a veterinarian.

Anesthetized animals are unable to blink, and as a result, the cornea is very susceptible to drying. ***As soon as the animal is anesthetized, apply a sterile lubricating ophthalmic ointment*** (such as Artificial Tears® or Lacrilube®) in the anesethetized animal's eyes to prevent drying of the cornea. To avoid contamination of the lubricant, or possible corneal trauma, the tip of the lubricant should not touch the eye surface, or any other surface.

Hypothermia is the most commonly overlooked complication in rodent surgery and can result in a prolonged recovery period and death. A supplemental heat source should be provided in the pre-operative, intra-operative, and post-operative periods. ***Proper use and selection of supplemental heat sources must***

be considered to prevent injury to the anesthetized animal. Heat lamps can cause hyperthermia and severe burns of the skin and should not be used. The temperature of the area surrounding the animal should be held between 30-35º C. Electric heating pads are not recommended for use with rodents due to the possibility of "hot spots" across the pad surface that can cause thermal burns of the animal's skin. Circulating warm water blankets are the safest devices for providing supplemental heat. Instant heat devices or hand warmers available from outdoor supply stores or first aid supply companies are safe as well. Always insulate the animal from the heat source with paper towels, cloth towels or other insulation to prevent thermal burns to the animal's skin, keeping in mind that if there is too much padding between the animal and the heat source, the heat source may not be able to penetrate the padding enough to provide benefit to the animal.

Evaluation of the animal during surgery is critical. Monitoring of anesthetic depth is vital to insure that the animal remains in the proper plane of anesthesia. There are several methods that can be used to assess anesthetic depth in order to ensure that the animal is not too lightly anesthetized, a condition which can result in the animal experiencing pain or regaining consciousness; or so deeply anesthetized that vital functions are compromised and death results. One method of assessing the anesthetic plane is to pinch the toes, tail, or ear of the animal. Any reaction from the animal indicates that it is too lightly anesthetized. Mucous membrane color and the color of exposed tissues can also be assessed, and is an indicator of tissue perfusion and oxygenation. Mucous membrane color should be bright pink to red. A dusky gray or blue coloration is an indication of inadequate oxygenation and tissue perfusion. Respiratory pattern and frequency will give an indication of anesthetic depth as well. A decreasing respiratory rate is an indicator of increasing depth of anesthesia.

Animals waiting for surgery should be kept at a visual and olfactory distance from those animals undergoing surgery when possible to minimize pre-operative stress. Surgical preparation of the animal should occur in a location different than that used for performing the surgeries. Pre-emptive analgesics should be administered during this time based on consultation with the attending veterinarian or EKU clinical laboratory animal veterinarian.

While under anesthesia, preparation of the animal for surgery should include clipping the surgical site with enough border (at least 1cm on all sides) to keep hair from contaminating the incision. Hair can be removed by clipping with a small electric clipper/trimmer, e.g., Oster® clippers with a #40 blade, by plucking, or by using a depilatory cream. Loose hair must then be removed from the animal and the environment. This can be done with a vacuum, a piece of adhesive tape, or moistened gauze dabbed over the clipped area. The surgical site should be scrubbed with a germicidal scrub (see Table 3). Carefully scrub the area with a new clean surgical sponge or sterile cotton swab (for small incision sites in mice). Scrub in a gradually enlarging circular pattern from the center of the proposed incision to the periphery. The sponge or swab should not be brought back from the contaminated periphery to the clean central area. Repeat with a 70% alcohol (or sterile water) soaked sponge or sterile cotton swab. The ***surgical scrubbing should be alternated between the germicide and alcohol and repeated at least three times, ending with the germicide***. Avoid using excessive amounts of liquid on the animal, particularly outside of the surgical site, as it is an important contributor to hypothermia in rodents. Move the sedated animal to the surgical area taking care not to contaminate the scrubbed skin. Place the animal on a clean absorbent surface and maintain body temperature using one of the aforementioned heat sources.

Preparation of the Surgeon

The surgeon must thoroughly scrub his or her hands with a bactericidal scrub (see attached Table 3). A cap, mask, and clean lab coat comprise proper surgical attire. A sterile gown is preferable for major surgeries**.** ***The use of sterile surgical gloves is required for all recovery surgeries.*** Exam gloves used for handling animals and working in the laboratory are not the same as sterile surgical gloves, and should not be substituted. Gloves should be donned so that contamination of the outer surface is prevented.

Draping and Instrumentation

The surgical area should be draped with sterile drapes; this helps prevent stray hair from entering the surgical field and provides a sterile area on which to lay sterile instruments during surgery. Clear, adhesive draping material is available (e.g., 3M™ Steri-Drape™ 2 Incise Drapes) and recommended because it greatly enhances the ability to monitor the anesthetic depth of the animal during the surgical procedure, adheres to the wound edges (reducing bacterial migration), and reduces the risk of surgical site contamination.

Surgical instruments should be placed on sterile surfaces only. While performing surgery, care should be taken to not get paper or cloth drapes wet. Wet material can pull bacteria through the drape from the non-sterile surface. Instruments in contact with a wet surface are contaminated and should be re-sterilized before being used again. Surgical instruments, gloves and other paraphernalia may be used on more than one animal. Surgical instruments must be autoclaved prior to the first surgery of the session. ***Any item used on multiple animals must be carefully cleaned and disinfected between animals*** (see Table 4). Chemical sterilants should not be used due to the time required to achieve sterilization, and the potential for tissue damage if they are not rinsed properly. Hot bead sterilizers are easier to use, but gross debris must be removed from the instruments before sterilizing. It is important to allow the instruments to cool before touching tissues to avoid tissue injury. Alternating two or more sets of instruments is one way to allow time for cooling. Surgical gloves must be kept sterile in between animals; if anything that is not sterile is touched between animals, a fresh, sterile pair of gloves must be donned.

Techniques which are important and often difficult to perfect are the following:

- Touch only "prepped" areas with instruments and hands.
- Keep operating fields draped.
- Do not let catheters or implants become contaminated.
- Use sterile solutions.
- Disinfect the tops of containers of solutions.
- Use sterile technique to access implanted catheters.
- DO NOT USE OUTDATED ANESTHETICS OR DRUGS

Incision Closure (sutures or staples)

The type and size of suture material should be chosen in advance (see Table 5), in consultation with the attending veterinarian or EKU clinical laboratory animal veterinarian, with special consideration given to the type of surgery and the species of animal. In rodents, a 3-0 or smaller suture thickness is recommended. Cutting needles have sharp edges and are best used for skin suturing. Needles for suturing tissues that are easily torn (i.e., peritoneum, muscle, and intestine) are taper or round needles. Vessel ligation and soft tissue suturing (other than skin) are generally performed with an absorbable material such as polyglactic 910 (Vicryl®), polydioxanone (PDS®), polyglycolic acid (Dexon®), polyglyconate (Maxon®), or chromic gut. Skin closure should be performed with a non-absorbable suture such as polypropylene (Prolene®, Surgilene®) or nylon. Stainless steel wound clips or staples may also be used to close the skin. It is preferable to perform skin closure with an absorbable suture in a subcuticular pattern (buried suture line) to decrease the likelihood that the animal will be able to chew on the suture material, and to increase the potential for the animal to be group housed with other animals without them chewing on each others sutures. Cyanoacrylate surgical adhesives may also be used to close short (1 cm) incisions or to close the area between sutures. Consultation with the attending veterinarian or EKU clinical laboratory animal veterinarian on the proper usage of surgical adhesives is recommended to avoid complications. Silk is a non-absorbable suture material that can wick bacteria into the wound and can cause tissue reactions. ***Silk as a suture material is not approved for use at Eastern Kentucky University for survival surgeries.***

Postoperative Care

After surgery, warmed sterile fluids (saline or lactated Ringers solution) should be provided. ***Rodents, because of their small size and smaller total body fluid contents, are particularly vulnerable to intra-operative fluid loss***. Volumes of 0.5 – 1.0 ml for mice and 3-5 ml for rats (300-500g body weight) should be given subcutaneously after surgery and prior to recovery from anesthesia. Use caution in warming

the fluids, as fluids that are too hot can cause thermal injury/burns to the animal. Fluids should be warmed to approximately the normal body temperature for most rodents, 37º C (98º F). Any tissues exposed for very long during surgery should be kept moist with these same warmed solutions during the surgery. Neonates and animals recovering from prolonged anesthesia can become hypoglycemic, and may benefit from the administration of an oral glucose solution once they are awake enough to swallow and not aspirate the solution. Glucose solutions should not be given subcutaneously or intraperitoneally.

Observation of animals during post-surgical recovery is important. The animal, in or out of its cage, must be kept warm. Warm water pads, blankets, or blue "diaper" pads work well. Electric heat pads or heat lamps may overheat the animal; their use is discouraged. If electric heat pads or heat lamps must be used; provision must be made to make frequent observations of, and turning of, a somnolent animal so that the animal will not overheat. Provision must also be made so that an awake animal can escape the heat source when it becomes too warm (such as placing the heating pad under only half of the cage). A recovering animal should be watched very closely until securely in sternal recumbency, be able to move around without plugging its nostrils or mouth with bedding, and should appear to be making normal behavioral adjustments. Some rodents left overnight on pads or paper bedding will eat that bedding, so they should not be left unattended on this type of bedding. Recycled newspaper pelleted bedding is available for post-operative recovery to prevent the plugging of airways with bedding as well as preventing bedding from sticking to surgical wounds. An animal should not be placed in a group cage after surgery until it is capable of protecting itself from cage mates.

Post-surgical observations include a minimum daily observation of the condition of the animal and the surgical site. Sutures (see Table 5 for data on suture types and uses) and/or staples need to be removed by 10 days following surgery, if the rodent has not already done so. Any foreign substance left in the incision for long period of time serves as a nidus of irritation and infection. Incisions that do not appear to be healing should be examined by the attending veterinarian or EKU clinical laboratory animal veterinarian. The use of prophylactic antibiotics is not a substitute for the practice of proper aseptic surgical technique. There are some instances where antibiotics may be appropriate, such as in gastrointestinal surgery, bone surgery, or when an accidental break in aseptic technique occurs. In these instances, the attending veterinarian or EKU clinical laboratory animal veterinarian should be consulted concerning the appropriate drugs and dosages for the species involved. Antibiotics should be administered for the recommended length of time to help prevent the emergence of antibacterial resistant bacteria. Guinea pigs and hamsters are particularly sensitive to the development of diarrhea that can be caused by certain antibiotics, and death can result if an antibiotic inappropriate for the species is administered.

Not only are the preceding surgical recommendations more humane to our animal charges, but following these recommendations should improve one's research by providing a less stressed animal and thereby decreasing the number of variables in a research protocol. For example, the rat has always been considered "hardy" and not subject to post-surgical infections. Published research has documented that ***post-surgical infections in rats are subtle***. The rat appears to eat and act normally, but will not respond appropriately to research stimuli. As with all new and improved techniques, patience and practice are required to harvest full benefits from the use of aseptic surgical techniques in rodents.

Another misconception regarding surgery on rodents is that the animals do not feel, or exhibit pain. ***Rodents are a prey species and have adapted by not always showing obvious external signs of pain and distress***. Daily weighing of the animal in the immediate post-operative period is a sensitive method of monitoring the animal. Animals must be monitored for the continued need for analgesics, and observations should be made at least twice daily in the first few days post-operatively. See Appendix 2 for a sample post-operative monitoring checklist for rodents.

Recovery of rodents from surgery is enhanced by providing nursing care. Supplying a softer, more palatable, easily accessible diet may encourage the animal to eat. A soft "dough diet" should be considered. Hydration can be monitored by "tenting" the skin along the back of the animal. The skin should quickly fall back into

place when released. If the animal is dehydrated, then skin will be slow to return to its original position. ***If dehydration is suspected, the attending veterinarian or EKU clinical laboratory animal veterinarian should be consulted regarding the appropriate use of subcutaneous or intraperitoneal fluids***.

There is ample literature available supporting the recommendations presented in this document. The attending veterinarian or EKU clinical laboratory animal veterinarian is available for assistance or to provide referrals to other researchers with applicable knowledge or skills. One reference that was instrumental in developing these guidelines was "Principles of Aseptic Rodent Survival Surgery: General Training in Rodent Survival Surgery - Part I and Part II", P.A. Brown and S. Hoogstraten-Miller *in Laboratory Animal Medicine and Management*, Reuter J.D. and Suckow M.A. (Eds.).

Table 1. RECOMMENDED HARD SURFACE DISINFECTANTS (e.g., table tops, equipment). Always follow manufacturer's instructions.

NAME	EXAMPLES *	COMMENTS
Alcohols	70% ethyl alcohol 70% - 99% isopropyl alcohol	Contact time required is 15 minutes. Contaminated surfaces take longer to disinfect. Remove gross contamination before using. Inexpensive. Flammable.
Quaternary Ammonium	Roccal®, Cetylcide®	Rapidly inactivated by organic matter. Compounds may support growth of gram negative bacteria.
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	Glutaraldehyde (Cidex®, Cide Wipes®)	Rapidly disinfects surfaces. Toxic. Exposure limits have been set by OSHA.
Phenolics	Lysol®, TBQ®	Less affected by organic material than other disinfectants.
Chlorhexidine	Nolvasan®, Hibiclens®	Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses.

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

Table 2. RECOMMENDED INSTRUMENT STERILANTS. Always follow manufacturer's instructions.

AGENTS	EXAMPLES *	COMMENTS
Physical: Steam sterilization (moist heat)	Autoclave	Effectiveness dependent upon temperature, pressure and time (e.g., 121°C for 15 min. vs 131°C for 3 min).
Dry Heat	Hot Bead Sterilizer Dry Chamber	Fast. Instruments must be cooled before contacting tissue.
Ionizing radiation	Gamma Radiation	Requires special equipment.
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 (23-72 hours). Carcinogenic. Excellent for items that are unable to withstand the heat of an autoclave such as catheters or plastics,
Hydrogen Peroxide	(Sterrad®)	Gentler for delicate instruments than steam.

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

[1] Instruments must be rinsed thoroughly with sterile water or saline to remove chemical sterilants before being used. Distilled and deionized water are not sterile from the tap.

Table 3. SKIN DISINFECTANTS . Alternating disinfectants is more effective than using a single agent. For instance, an iodophore scrub can be alternated 3 times with an alcohol, followed by a final soaking with a disinfectant solution. Alcohol, by itself, is not an adequate skin disinfectant. The evaporation of alcohol or alcohol based products can induce hypothermia in small animals.

NAME	EXAMPLES *	COMMENTS
Alcohols To be used between applications of scrub solutions listed below	70% ethyl alcohol 70-99% isopropyl alcohol	**NOT ADEQUATE FOR SKIN PREPARATION! Contact time required is 15 minutes.** Not a high level disinfectant. Not a sterilant. Flammable.
Iodophors	Betadine®, Prepodyne®, Wescodyne®	Reduced activity in presence of organic matter. Wide range of microbe killing action. Works best in pH 6-7.
Chlorhexidine	Nolvasan®, Hibiclens®	Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses. Excellent for use on skin.

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

Table 4. RECOMMENDED INSTRUMENT DISINFECTANTS Always follow manufacturer's instructions. These agents should only be used to disinfect instruments between animals during MINOR surgical procedures.

AGENT	EXAMPLES *	COMMENTS
Alcohols ***PRIMARY USE is as a disinfectant soak between animals when starting with sterilized instruments.***	70% ethyl alcohol 70 % - 99% isopropyl alcohol	Contact time required is 15 minutes. Contaminated surfaces take longer to disinfect. Remove gross contamination before using. Inexpensive. Flammable. Low level disinfectant.
Chlorine[1]	Sodium hypochlorite (Clorox ® 10% solution) Chlorine dioxide (Clidox®, Alcide®)	Corrosive. Presence of organic matter reduces activity. Chlorine dioxide must be fresh (< 7 days old); kills vegetative organisms within 3 min.
Peracetic Acid / Hydrogen Peroxide	Spor - Klenz®	Corrosive to instrument surfaces. Must be thoroughly rinsed from instruments before use.
Chlorhexidine	Nolvasan® , Hibiclens®	Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses.

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

Table 5. INCISION CLOSURE (SUTURES OR STAPLES)

SUTURE *	CHARACTERISTICS AND FREQUENT USES
Vicryl®, Dexon®	Absorbable; 60-90 days. Ligate vessels or suture tissues where an absorbable suture is desirable.
PDS® or Maxon®	Absorbable; 6 months. Ligate vessels or suture tissues especially where an absorbable suture and extended wound support is desirable
Prolene®	Nonabsorbable. Inert.
Nylon	Nonabsorbable. Inert. General closure.

Silk	Nonabsorbable. (Caution: Tissue reactive and may wick microorganisms into the wound). Silk is very easy to use and knot. **Silk is not currently used at EKU for survival surgeries.**
Chromic Gut	Absorbable. Versatile material. Causes mild inflammation, but is absorbed more rapidly than synthetics. **Chromic gut is not acceptable for suturing skin.**
Stainless Steel Wound Clips, Staples	Nonabsorbable. Requires instrument for removal from skin.

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

Suture gauge selection: Use the smallest gauge suture material that will perform adequately.

Cutting and reverse cutting needles: Provide edges that will cut through dense, difficult to penetrate tissue, such as skin.

Non-cutting, taper point or round needles: Have no edges to cut through tissue; used primarily for suturing easily torn tissues such as peritoneum or intestine.

Appendix 1. Recommended Rodent Anesthetic Agents

Recommended Mouse Anesthetic Agents

1. Isoflurane: Administered at 1 to 4% in air or oxygen via mask or intubation
2. Ketamine/Xylazine: 90-150 mg/kg BW IP and 5-10 mg/kg BW IP
3. Ketamine-Dexmedetomidine (Dexdomitor) Cocktails

Mouse Cocktail #1: Recommended for Shorter Procedures and/or Restraint

	Drug Stock Concentration (mg/ml)	Volume Used for Cocktail	Volume of Cocktail Administered to Mouse	Dose Administered to Mouse
Ketamine	100 mg/ml	1.25 ml	-	75 mg/kg
Dexmedetomidine	0.5 mg/ml	2.5 ml	-	1 mg/kg
Saline	Sterile, isotonic	1.25 ml	-	
Combination	Total	5 ml	0.1 ml / 25 gm IP	

Mouse Cocktail #2: Recommended for Longer Surgical Procedures

	Drug Stock Concentration	Volume Used for Cocktail	Volume of Cocktail Administered to Mouse	Dose Administered to Mouse
Ketamine	100 mg/ml	1.25 ml	-	75 mg/kg
Dexmedetomidine	0.5 mg/ml	2.5 ml	-	1 mg/kg
Ace promazine	10 mg/ml	0.12 ml	-	1 mg/kg
Saline	Sterile, isotonic	1.13 ml	-	
Combination	Total	5 ml	0.1 ml / 25 gm IP	

Atipamezole (= Antisedan) Reversal Agent

This anesthetic reversal agent is an alpha-2 antagonist. It is highly recommended to use with Mouse Cocktail #2. It is used to reverse the effects of xylazine or dexmedetomidine.

It is recommended to make a 0.25 mg/ml stock solution (from 5 mg/ml solution in purchased bottle).

To make 0.25 mg/ml stock solution, make a 1:20 dilution: Add 0.5 ml Atipamezole to 9.5 ml sterile water or sterile saline.

Mouse Dosage: ~1-2.5 mg/kg
Give 0.3 ml SC or IP of 0.25 mg/ml stock solution to 30 gm mouse.
Give 0.2 ml SC or IP of 0.25 mg/ml stock solution to 20 gm mouse.

Note: SC or IP fluid support following procedure is recommended with use of these cocktails. Less bladder tone is evident and rats urinate frequently while anesthetized.

Recommended Rat Anesthetic Agents

1. Isoflurane: Administered at 1 to 4% in air or oxygen via mask or intubation
2. Ketamine/Xylazine: 40 - 90 mg/kg BW IP and 5 - 7 mg/kg BW IP
3. Ketamine-Dexmedetomidine (Dexdomitor) Cocktails

Rat Cocktail #1: Recommended for Shorter Procedures and/or Restraint

	Drug Stock Concentration (mg/ml)	Volume Used for Cocktail	Volume of Cocktail Administered to Rat	**Dose Administered to Rat**
Ketamine	100 mg/ml	0.8 ml	-	**50 mg/kg**
Dexmedetomidine	0.5 mg/ml	0.8 ml	-	**0.25 mg/kg**
Saline	Sterile, isotonic	2.4 ml	-	
Combination	Total	4 ml	**0.25 ml / 100 gm IP**	

Rat Cocktail #2: Recommended for Longer Surgical Procedures

	Drug Stock Concentration	Volume Used for Cocktail	Volume of Cocktail Administered to Rat	**Dose Administered to Rat**
Ketamine	100 mg/ml	1.2 ml	-	**75 mg/kg**
Dexmedetomidine	0.5 mg/ml	0.8 ml	-	**0.25 mg/kg**
Saline	Sterile, isotonic	2 ml	-	
Combination	Total	4 ml	**0.25 ml / 100 gm IP**	

Atipamezole (= Antisedan) Reversal Agent

This anesthetic reversal agent is an alpha-2 antagonist. It is highly recommended to use with **Both Rat Cocktails # 1 & 2**. Rats may sleep for 4-5 hours if reversal agent is not administered.
It is used to reverse the effects of xylazine or dexmedetomidine.
It is available as a 5 mg/ml injectable solution.

It is recommended to make a 0.25 mg/ml stock solution (from 5 mg/ml solution in purchased bottle).

To make 0.25 mg/ml stock solution, make a 1:20 dilution: Add 0.5 ml Atipamezole to 9.5 ml sterile water or sterile saline.

Rat Dosage: 1 mg/kg

Give 0.6 ml SC or IP of 0.25 mg/ml stock solution per 150 gm rat.

Note: SC or IP fluid support following procedure is recommended with use of these cocktails. Less bladder tone is evident and rats urinate frequently while anesthetized.

Appendix 2. Recommended POST- OPERATIVE EVALUATION FORM

Animal # _____Species_____Date of Operation_____

Pre-operative weight _____(g or kg) Procedure _____

Date					
Day post-procedure					
Time					
Active					
Inquisitive					
Rough hair coat					
Crusty red eyes					
Feces					
Urine					
*Rate & type of breathing					
Normal gait/paralysis					
Fecal/urine soiling of coat					
Diarrhea					
**Dehydration					
Bony/thin appearance					
Vocalization					
Body weight					
% change from pre-op weight					
Wound edges red					
Swelling around/under incision					
Sutures/staples missing					
Discharge from incision					
Sutures/staples removed **(date)**					
ANALGESICS GIVEN (drug, dose in mg/kg, route)					
OTHER TREATMENT					
OBSERVER INITIALS					

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

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