Institutional Animal Care and Use Committee Guidance 304

TITLE:  
Guidelines for Survival and Non-Survival Rodent Surgery

PURPOSE:  
To standardize approved methods for survival and non-survival rodent surgeries.

REVIEW/REVISIONS:  
The IACUC will review and revise this guidance as needed.

DATE OF LAST REVIEW:  
12/05/2016

This document outlines the regulatory requirements for conducting survival or non-survival surgery in rodents, only. While many requirements are similar, it should be noted that not all requirements can be applied to non-rodent species. Whenever possible, the same conditions should be used for non-survival surgery as for survival surgery.

This document also serves as a reference when writing a protocol or amendment involving surgery. A specific lab SOP should also be written for each surgery, combining the pertinent information in this Guidance along with the IACUC-approved surgical procedures.

A good surgical outcome requires:
- Sufficient pre-operative preparation and surgical support
- Asepsis, or sterile technique, which is used to reduce the chance of infection
  - Includes surgery set-up, instrument/tool sterilization, and surgeon preparation
- Appropriate anesthesia
  - Includes selection of drugs and anesthetic monitoring
- Good surgical technique
  - Includes gentle tissue handling, correct use of surgical instruments and adequate knowledge of anatomy and procedure being performed
- Adequate post-operative care and pain management
- Consistent recordkeeping
  - Identifies trends that that can be used for surgical refinements
  - Necessary for documentation of scientific data

IACUC expectations:
- All surgical procedures must be reviewed and approved by the IACUC.
  - Any modifications to the procedures, drugs, and time lines must be reviewed and approved by the IACUC before they are implemented.
  - If you have to ask whether a change needs to be reviewed and approved, then you need to submit an amendment.
  - **If you are not sure what is approved, read the protocol.**
- Anyone participating in pre-op, surgery, or post-op care of animals must:
  - Be appropriately trained [includes CITI; GRST; UAC veterinarian; experienced protocol participant].
  - Be familiar with the approved procedures.
  - Perform every aspect of the procedure as approved by the IACUC.
- **Adverse outcomes** such as anesthetic failure, death under anesthesia, failure to recover, infection, or poor outcome must be reported to the IACUC Office immediately [http://rgw.arizona.edu/compliance/IACUC/report-adverse-or-unanticipated-event].
  - Reporting to a UAC staff member is not reporting to the IACUC Office.
Surgery location and set-up:

- The ideal location is a dedicated room or alcove.
  - Avoid placing the surgical location near a doorway, due to increased foot traffic and potential disturbances.
  - If an entire room/alcove is not available, the back of the lab, away from foot traffic, is recommended.
  - If a permanent space is not possible, reserve the area for the day of the surgery, remove unnecessary equipment and materials including anything hanging over the surgical area and disinfect the area the day before.
- Separate the 1) pre-op, 2) surgery and 3) post-op areas for survival surgery.
  - Make sure the surgical area has adequate lighting.
  - Separation of areas is not required for non-survival surgery.
- Ensure all instruments are sterilized, that sufficient sterile drapes, suture material, and gloves are available and that all equipment, reagents, and drugs are prepared and are in the surgical location.
  - Ensure the sterilized instrument pouches, sutures, gloves, drugs, and fluids are within their use-by-date.
    - In-house sterilized instruments are considered sterile for one year as long as the pack is not damaged and remains dry.
    - Make sure all injectable drugs are sterile.
- Turn on the glass bead sterilizer and pre-warm heat pad[s], if appropriate.
- If using isoflurane, check the vaporizer set up for leaks, add isoflurane, and position it in the surgical area.
  - Medical grade O2 gas must be used with the vaporizer.
    - Make sure there is sufficient O2 gas in the cylinder for the duration of the surgery.
- Disinfect the surgical area with 10% commercial bleach solution, Versaclean, Clidox, MB-10, etc...
  - 70% alcohol alone is not sufficient for disinfection for survival surgery.
- Disinfect devices such as a stereotaxic device, microscope knobs, etc. to be used in the surgery, as described for the surgical area.
  - Cover any knobs that will be operated by the surgeon with a sterile drape, gauze or glove.
- A source of heat must be provided to prevent hypothermia during surgery.
  - The safest methods for providing thermal support are circulating water blankets or instant heat devices.
    - Electric heating blankets and heat lamps are discouraged because of the increased potential to cause burns.
  - Animals must never be placed directly on a heat source as this could cause thermal burns. Use of a clean drape [surgical set up] and a sterile drape [pre-op] over the heat source will prevent this issue.
  - Heat sources are not required for non-survival surgery.
- Cover the surgical area, including the heat source, with a clean drape or plastic pad.
- The sterile surgical area is encompassed by the area between the lab bench surface and ~mid chest height of the surgeon and the width of the draped area, which includes space for sterile instruments.
- A sterile surgical field is not required for non-survival surgery.

Instrument/tool sterilization:

- Use of sterile disposable instruments and drapes are encouraged, when possible.
- Make sure that blades and scissor are sharp, or use disposable sterile scalpels.
- Place instruments in a “peel open” pouch, wrap them in brown paper or place them in an instrument tray wrapped with a cloth drape or brown paper.
  - Instruments can be singly wrapped, or complete sets of instruments can be wrapped.
  - If autoclaving or using gas sterilization, the wrapping material must be permeable. Steam or gas cannot permeate a tightly sealed metal container or something wrapped in aluminum foil.
• Sterilize an extra drape or instrument tray so that there is a sterile surface on which to place opened instruments.

• Acceptable methods of sterilization are as follows:
  o Autoclaving:
    ▪ Some materials such as PE-10 tubing can be autoclaved by placing in a small container of water.
    ▪ Dry heat sterilization at 340°F/170°C for 1 hour, 320°F/160°C for 2 hours or 300°F/150°C for 3 hours:
      ▪ Instruments must be dry and must be held at the correct temperature for the entire period.
    ▪ Sterilization with ethylene oxide gas [ETO; performed by UAC for a fee].
    ▪ Vaporized hydrogen peroxide [VHP; performed by UAC for a fee].
    ▪ Ionizing radiation [usually only performed by a commercial service].

• Appropriate monitoring of sterilizer effectiveness is required. Only adequately sterilized instruments can be used.

• Some devices, such as those with electronic components cannot be sterilized by these methods. There are two options:
  o Use of a cold sterilant solution:
    ▪ Cold sterilants are not recommended for general use as there is no reliable way to determine sterilization efficacy.
    ▪ Use only the cold sterilant that IACUC approved in the IACUC protocol.
    ▪ Acceptable cold sterilants include Cidex OPA, SporexII and Sterilox.
    ▪ Follow the manufacturer’s recommendations about dilution and duration.
    ▪ Make sure to adequately rinse the device in sterile water or saline before use.
  o Disinfection by soaking in 70% alcohol for at least 15 minutes, followed by rinsing in sterile water.
    ▪ This method should only be used when all other methods will damage the device.

• After surgery, soak instruments in an appropriate detergent and rinse thoroughly in clean water.
  o Use of an ultrasonic cleaner provides superior cleaning, but is not required.

• Sterilization of instruments in not required for non-survival surgery.

Surgeon preparation:
• Surgeon preparation is required for survival surgery, or for a non-survival surgery that opens a body cavity and lasts for more than 3 hours.
  o For other non-survival surgery, surgeon preparation is highly recommended, but it not required.

• Remove jewelry from the hands and wrists, tie back long hair and roll up long sleeves.
• Don a clean lab coat or scrub top, or a sterile gown, a hair bonnet, and a mask.
  o When wearing a sterile gown, remember that gowns are sterile from the mid-chest to the waist only.
• Wash and scrub hands with disinfectant soap or a surgical scrub brush. Dry hands with a clean towel.
• Don sterile gloves and if you have an assistant, tuck lab coat/gown sleeves under the top of the gloves.
  o Individually wrapped and autoclaved unused nitrile gloves may be used, although sterile surgical gloves are preferred.
  o Gloves are sterile from the finger tips to the top of the gloves.
  o Gloves lose their sterility if they touch anything that is not sterile.

Selection of anesthetic drugs and use of anesthetic gas vaporizers:
• Selection of the appropriate anesthetic regimen depends of the species, the type and duration of the procedure, and is made in consultation with a UAC veterinarian.
  o Only the anesthetic regimen approved in the IACUC protocol must be used.
• An inhalant anesthetic, such as isoflurane, should be the first choice as animals are anesthetized quickly and recover within several minutes after the gas is discontinued.
Use of an inhalant anesthetic requires a vaporizer and nose cone to administer the drug and a method to capture the waste anesthetic gas.

- No more than two lines must actively deliver gas from a single precision vaporizer (also known as “splitting” the vaporizer line), as the amount of gas going to each anesthetized animal cannot be controlled separately with this modification. With more than two lines active, animals will not receive the appropriate amount of anesthetic gas.
- F/AIR charcoal canisters used for waste anesthetic gas scavenging must be weighed, and weights recorded before each surgical session. Canisters must be discarded when they increase in weight by 50g.

- Injectable anesthetics require no specialized equipment; however, the commonly used agents may be DEA controlled substances or non-pharmaceutical grade.
  - The effects of injectable anesthetics are more variable, and can even be strain dependent.
  - For long procedures, booster doses will be required, and recovery from anesthesia will not occur until sufficient of the drug has been metabolized.
  - If a reversal agent is used, this must be included as part of the anesthetic regimen in the IACUC protocol.
  - The use of non-pharmaceutical anesthetic agents must be scientifically justified.

Anesthetic monitoring:

- Anesthesia must be monitored a minimum of every 15 minutes throughout the surgical procedures, to assure the appropriate depth of anesthesia is maintained.
  - Whenever possible, anesthesia should be monitored and supplemented, if necessary, by the surgical assistant.
- If there is insufficient anesthetic depth, anesthesia can be supplemented by adjusting the isoflurane or providing a booster injection, as approved in the IACUC protocol.
- There is a possibility that too much anesthesia can suppress respiration to an extent that death occurs.
  - If using isoflurane, the amount can be reduced and the depth of anesthesia can be reassessed.
  - If using an injectable anesthetic, the animal should be monitored and a UAC veterinarian consulted, if possible.
  - If death under anesthesia occurs, this must be reported to the IACUC as an adverse event.
- Response to a toe pinch is a commonly used method to assess anesthetic depth. Using two fingers, the animal’s foot is squeezed hard enough to cause slight blanching of your fingernail. If there is no withdrawal reaction, the anesthesia is judged deep enough to commence surgery.
  - A sterile gauze pad or glove should be placed over the animal’s foot to prevent contamination of the surgeon’s glove.
- There are also several visual methods of anesthesia monitoring that can be performed easily and frequently:
  - Respiratory rate and pattern: Anesthesia slows the respiratory rate, which can be seen as the chest rising and falling. The respiratory rate increases when there is insufficient anesthetic depth.
  - Movement: If anesthesia is not deep enough, movement of the whiskers may be observed. If the animal moves in response to surgical procedures, then there is insufficient anesthetic depth.
  - Mucous membranes: Mucous membranes are evaluated by the color of gums, the pinna [ears] and the toes, which should be pink. A bluish color indicates insufficient oxygen due to a decreased respiratory rate from too much anesthesia. A darker pink to red color indicates that the animal is overheating, most likely from the thermoregulatory support.
  - Instrumentation: Animals may be attached to a device which provides a read out of respiration rate.

Pre-operative preparation and surgical support:

- Food and water should not be withheld from rodents prior to surgery.
  - If food or water must be withheld for any reason, the duration must be specified in the approved IACUC
protocol.
- Whenever possible, a surgical assistant should be responsible for preparing animals for surgery [pre-op], as this is a mostly non-sterile process.
- Start a new surgical record and document pre-op, intra-op, and immediate post-op information.
- Evaluate the animal to ensure it is apparently healthy.
- Induce anesthesia as described in the approved IACUC protocol and assure adequate depth of anesthesia by using the toe pinch method.
  - For injectable anesthetics, if adequate depth of anesthesia is not achieved, one or more booster injections may be administered as described in the approved IACUC protocol.
  - If the animal fails to become adequately anesthetized, stop the procedure and consult a UAC veterinarian.
- Apply sterile, bland ophthalmic ointment to the eyes to prevent drying, if anesthesia will last for more than 20 minutes.
  - The ointment must be labeled for ophthalmic use.
- Administer pre-op fluids, e.g., warm 0.9% sterile saline, as described in your protocol.
  - Fluids help the animal stay hydrated for longer surgeries.
- Administer analgesia, as described in your protocol.
  - Consider whether analgesia should be administered immediately prior to surgery, or should be administered 1-2 hours before surgery, if the surgery is of a short duration. Remember to request a protocol modification if a change is made.
- Administer any additional pre-op drugs as approved, e.g., antibiotics, topical anesthetic, etc...
- Remove hair at least 2-3 cm around the surgical site using well maintained clippers and/or depilatory cream.
  - When using depilatory cream, apply and immediately remove to prevent chemical burns.
- Using clean gloves, scrub the shaved skin with gauze soaked in either a povidone iodine/betadine solution or a chlorohexidine surgical scrub preparation. Start with the gauze in the center of the shaved area where the surgical incision will be and work in concentric circles towards the outer edge of the shaved area. Next scrub the area with gauze soaked with 70% [isopropyl] alcohol. Using a new pad each time, repeat these alternating scrubs 2 additional times. Finish by spraying area with betadine spray.
- The animal is moved to the prepared surgical area and is positioned in a stereotaxic device or is positioned with tape, taking care to maintain limb circulation, if applicable.
- Cover the animal with a sterile drape, leaving an opening over the surgical site.

**Surgical technique:**
- Perform the surgery **exactly** as described in the approved IACUC protocol. This includes use of only the approved drugs and techniques.
  - If surgery is planned/in progress and cannot be completed exactly as described, contact the IACUC Office or a UAC veterinarian to determine whether a modification is possible.
- If performing more than one surgery, or if sterility is broken:
  - Use a new sterile pack between animals or if sterility is broken [preferred].
  - “Sterilize” instruments between animals or if sterility is broken by using a glass bead sterilizer [see below].
    - Instruments must be replaced with a sterilized pack after no more than 7 uses of the glass bead sterilizer, or up to 8 animals total.
- Sterile gloves must be changed:
  - If they become contaminated during the surgery by touching anything non-sterile.
  - If they move out of the sterile area.
  - Between animals.
Aseptic

- The specific techniques used will depend on the type of surgery. However, there are a number of common practices that should be incorporated in any surgery.
  - Minimization of the size of incisions to only that necessary for the procedure.
  - Gentle tissue handling to reduce tissue damage around the surgical site.
  - Effective hemostasis to minimize blood loss.
  - Effective wound closure technique to ensure good healing and minimal pain.
  - Appropriate type of wound closure, such as suture type and pattern.
- Consider whether surgical refinements can be made, and if so, amend the protocol.
- Aseptic technique is not required for non-survival surgery lasting less than 6 hours.

Aseptic tip technique:
- Also known as “tips-only” technique, this is a modified set of procedures used to achieve asepsis in rodent surgeries where the surgeon only utilizes the sterile working ends of the surgical instruments [or “tips”] to manipulate the surgical field.
  - This technique is well suited for less invasive surgeries, such as subcutaneous mini-pump implantation, tumor implants, and intracranial cannula/head cap placement.
  - The technique is best suited to situations where an assistant is available to perform pre-op and other non-sterile activities.
- Use of this technique must be specifically approved in the IACUC protocol for each surgery in which it will be implemented.
- Instruments must be first sterilized as for non-“tips” surgery and sterile gloves must be worn for the first surgery. Therefore, the first surgery follows classical aseptic principles.
- Prior to the subsequent surgeries, the tips of instruments are hot bead sterilized and the instruments are placed on the existing sterile field so the tips remain sterile for the next surgery.
- As the surgeon’s hands and instrument handles will become contaminated, only the tips are to be used to manipulate any tissues in the surgical field to maintain asepsis.
  - If the surgeon performs pre-op or other non-sterile activities, new sterile gloves must be worn for the next surgery.
- When suture is used for incision closures while utilizing this technique, additional caution needs to be taken to ensure the suture remains in the narrow sterile field and is not moved through areas contaminated by previous surgeries.
- Instruments must be replaced with a sterilized pack after no more than 7 uses of the glass bead sterilizer, or up to 8 animals total. Fresh, sterile gloves must be worn every time a new instrument pack is opened.

Post-operative care and pain management:
- Post-operative recovery must occur in a separate location to pre-op and surgery.
- Animals must be recovered in a clean cage without bedding to prevent accidental aspiration of bedding; a plastic pad or paper towel can be placed on the bottom of the cage.
  - The recovery area should provide thermal support. The ideal method is to have the recovery cage half on, half off a heat source, as this allows the animal to move away from the heat as they recover. A heating lamp can be used, as long as it is set on the lowest setting and placed at a sufficient distance to prevent overheating. Similarly, an incubator may be used.
- For prolonged or invasive surgeries, fluid replacement should be administered prior to waking from anesthesia.
  - Warm 0.9% saline can be administered IP or SC, if approved in the IACUC protocol. The usual amounts are 0.5-1.0 ml for mice or 3.0-5.0ml for rats. Larger rodents may have an indwelling IV catheter placed, if necessary, and approved in the protocol.
  - Use of oral hydration gels [Napa nectar] or water-softened food do not require IACUC approval and can
assist in recovery from particularly invasive surgeries or surgeries with long anesthetic periods.

- Monitor the color of the pinna and/or footpad and watch for the respiration rate and movements to increase as the animal recovers from anesthesia.
  - Once the animal has righted itself and is ambulating normally, it is considered recovered from anesthesia. At this point, the animals can be transferred to their home cage.
  - It is recommended that the animals be monitored for an additional 30 minutes before being returned to the animal housing room.
- Administer analgesics or other drugs as stipulated in protocol, or approved by a UAC veterinarian.
  - Repeat as necessary.
  - Maintain a record of administration of all drugs, including animal information, dosage, and time.
- Monitor daily: appetite, wound healing, energy/normal movement. Repeat analgesics as necessary.
- Consult a UAC veterinarian if complications arise.
- Remove skin closure materials 10-14 days post-surgery [record removal in lab records].

**Recordkeeping:**

- Records must be kept for non-surgical anesthesia, survival, or non-survival surgery.
- Records must contain:
  - The protocol number and procedure
  - The species and animal or cage identifier
  - Pre-surgical assessment
  - Route and dose of all drugs, including anesthesia, ophthalmic ointment, fluids, topical anesthetic, analgesics, antibiotics, etc...
- Records must be initialed and dated.
- Records must be kept in a central location and available to all protocol participants and IACUC inspectors.
  - Copies should be placed in individual lab books.
  - Records must be maintained for the duration of the protocol approval period and for any additional time required for research records, as applicable.
DEFINITIONS:

Aseptic technique: A combination of techniques used to create and maintain a controlled environment free of microbial contaminants [bacteria, viruses, and fungi].

Aseptic tip technique [aka “tips-only” technique]: A modified combination of techniques wherein the surgeon only uses the sterile working ends of the surgical instruments [or “tips”] to manipulate the surgical field in order to achieve asepsis. The gloved, but non-sterile, hand never contacts the instrument tips, suture, suture needle, or any other part of the surgical field during use.

Disinfection: Also known as sanitization. Disinfection removes the majority of microorganisms. Some bacteria and viruses are resistant to use of alcohol as a disinfectant. Most bacterial spores and protozoan parasites are resistant to many disinfectants.

Drape: A sterile drape is a porous [cloth, paper] or non-porous [plastic] sheet that is used to provide a temporary sterile barrier. A dedicated roll of Saran Wrap or Glad wrap, especially the Press n’ Seal variety, can be used as a see-through, non-porous drape. Due to the way the plastic is formed at extremely high heat, the inner surface of the roll is sterile, as long as it is untouched.

Glass bead sterilizer: Glass bead "sterilization" uses ~1.5mm glass beads and high temperature [217-232°C] for brief exposure times to inactivate microorganisms. The instruments must be placed about halfway into the beads for at least 45 seconds. Only the portion of the instrument placed in contact with the beads will be sterilized. You may need to rinse the instrument covered in blood or tissue with sterile saline or distilled water prior to using the bead sterilizer. Allow the instrument to cool before touching the animal again.

Non-survival surgery: An animal is subjected to surgical anesthesia and surgical penetration of the body occurs or invasive measurements are performed over a long period and the animal is euthanized without waking from anesthesia. Aseptic technique may or may not be required.

Rodent: Rodents are characterized by a pair of unremittingly growing incisors in the upper and lower jaws, and include mice, rats, guinea pigs, hamsters, gerbils, and squirrels.

Sterile: Free of all forms of life and biological agents, such as bacteria, viruses, and fungi.

Survival surgery: Surgery in which the animal is revived from anesthesia. Aseptic technique must be used for all survival surgeries.