



Animal Care and Use Program

Institutional Animal Care and Use Committee

IACUC Guidelines

Title: Surgery Guidelines

Policy: Index:

| | Pages |
|--|-------|
| A. Definitions | 1 |
| B. Survival Surgical Procedures | |
| 1. Rodents (Rats, Mice, Gerbils, etc.) | 1-5 |
| 2. Large Animals (Rabbits, Swine, Sheep, Dogs, etc.) | 5-9 |
| 3. Non-mammalian Species (Fish, Amphibia, Reptiles, Avian) | 9-14 |
| C. Terminal Surgical Procedures | 14-16 |
| D. Record Keeping Procedures | |
| 16-17 | |
| E. Who to Contact for Help | 17 |
| F. Sample Laboratory Animal Anesthesia Record | 18 |
| G. Sample Post-Operative Recovery Record | 19 |

A. Definitions

1. Aseptic technique: Encompasses a number of practices and procedures to reduce microbial contamination to the lowest practical level.

2. Survival surgery: A surgical procedure from which an animal is expected to recover from anesthesia.

3. Non-survival (Terminal) surgery: A surgical procedure from which an animal is euthanized before recovery from anesthesia.

4. Major survival surgery: A surgical procedure that penetrates and exposes a body cavity or produces substantial impairment of physical or physiologic functions. Examples include: laparotomy, thoracotomy, craniotomy, orthopedic procedures, limb amputation and enucleation.

5. Minor survival surgery: A surgical procedure that does not expose a body cavity and causes little or no physical impairment. Examples include wound suturing, peripheral vessel cannulation, and placement of subcutaneous implants.

B. Survival Surgical Procedures

1. Survival Surgical Procedures for Rodents (Rats, Mice, Gerbils, etc.)

Pre-Surgical Planning

Appropriate planning is important for all successful surgical outcomes. Pre-surgical planning requires input from all members of the surgical team and includes the investigator, surgeon, anesthetist, technicians, veterinarians, and animal care staff. Investigators are encouraged to contact ULAR veterinarians for assistance in planning surgeries and post-surgical care.

A surgical plan should be developed for each procedure and identify the following:

- All necessary personnel, as well as their roles, experience and training needs.
- Equipment and supplies required for the planned procedures

- Location and nature of the surgical facilities (shared facilities may require reservation)
- Preoperative animal health assessment criteria and a description of presurgical medications if needed
- Expected postoperative care and identification of all needed supplies

Personnel Training

Personnel involved with surgery in a research setting often have a wide range of educational backgrounds and may require various levels of training before performing surgery on animals. Personnel trained to perform surgery in humans may require additional training for interspecies variations in anatomy, physiology, and response to anesthetics and analgesics.

Regardless of an individual's responsibility or educational background, all personnel performing surgery must have thorough knowledge and understanding of the approved IACUC protocol procedures and must possess knowledge and familiarity with the relevant anatomy of the species and the surgical site.

At a minimum, training of surgical personnel must include:

- A thorough knowledge of aseptic technique
- Administration and assessment of anesthesia
- Appropriate tissue handling (tissue trauma contributes to postoperative infections)
- Appropriate use of instruments
- Effective methods of hemostasis
- Correct use of sutures and/or skin staples
- Post-surgical care and monitoring, including the ability to recognize and alleviate pain and distress

Surgical Facilities

A dedicated surgical suite is not required for the performance of surgical procedures on rodents. However, the space must be designated for surgery at the time of the procedure and appropriately managed to minimize contamination from other activities in the room during surgery.

- Rodent surgical space should be designed and managed to achieve the following:
- Separation of the animal preparation area from the surgery area
- Minimization of personnel traffic flow through the surgery area
- If possible, airflow should be away from the surgery area (e.g. positive room pressure, use of filtered, laminar flow air)
- Room surfaces should be non-porous and easily sanitized
- A regular room cleaning and disinfection schedule should be established (i.e., daily cleaning of floors and work surfaces, weekly to monthly cleaning of walls and cabinets)
- The surgery area should be free of all equipment and materials not necessary for the procedure. Storage items should be placed in cabinets or drawers.

Aseptic Technique

The goal of aseptic technique is to reduce the possibility of microbial contamination to the lowest practical level. No single technique, practice, germicide, or piece of equipment will achieve this objective. Rather, proper aseptic technique is dependent on numerous practices that require input and cooperation of all personnel within the operating area.

Components of successful aseptic technique include:

- Preparation of the animal
- Preparation of the surgeon
- Sterilization of instruments and supplies
- Appropriate surgical technique
- Anesthesia and intra-operative monitoring
- Antibiotic usage
- Post-surgical care
- Record keeping

Preparation of the animal

1. After the animal has been anesthetized, the eyes should be lubricated with a sterile ophthalmic ointment to prevent corneal drying.
2. Hair should be removed from the surgical site. This should be performed with electric clippers (# 40 blade) or depilatory rather than a razor.
3. The surgical area prepared should be approximately twice that needed for the incision, in the event a larger incision than planned may be required.
4. The skin should then be cleaned and disinfected with chlorhexidine or povidone iodine-based disinfectant followed by 70% alcohol. The site should be scrubbed by starting at the center of the site and working outward in a circular pattern. Typically, three scrubs with a disinfectant, followed by alcohol will suffice.
5. A sterile surgical drape should be used whenever possible to isolate the disinfected area from surrounding tissue and hair. To be effective, a drape should adhere tightly to the skin and be impermeable to moisture.

Preparation of the surgeon

1. Sterile gloves must be used for all procedures. Gloves must be replaced or sterilized with a cold sterilant between rodents if surgeries are being performed on multiple animals.
2. The surgeon must wear a clean lab coat, scrubs, or smock while performing surgery.
3. A surgical mask and a cap or bouffant cap are also recommended to reduce the risk of gross contamination of the surgical site.

Sterilization of instruments and supplies

All instruments and supplies that come in contact with the surgical site must be sterile. A number of options are available to sterilize surgical equipment and supplies.

1. **Autoclave**- Relies on pressurized steam, is extremely reliable and cost effective. However, instruments must be durable (e.g., stainless steel) and the process is relatively slow, from 15 to 60 minutes.
2. **Ethylene oxide**- A gaseous sterilant that requires specialized containment equipment. This is a good sterilization method for supplies that cannot tolerate high heat such as plastics and catheters. It is more costly than autoclaving and typically is performed overnight.
3. **Cold sterilant solutions (hypochlorite, glutaraldehyde, etc.)**- Generally, cold sterilants must have prolonged contact time (follow label recommendations) to sterilize surgical equipment. In addition, the instruments must be rinsed completely with a sterile solution like saline to prevent tissue irritation. **Note: Alcohol is not a sterilant.**
4. **Hot bead sterilizer**- This device is a small tabletop unit, approximately 6 x 6 x 8 inches. The appliance heats a small container of Pyrex beads to approximately 250 C and can sterilize the tips of metal surgical instruments in 10-20 seconds. It is very useful for sterilizing instruments between rodents when performing multiple surgeries.
5. **Pre-sterilized items**- Many instruments and supplies can be purchased in sterilized packaging. Such items must be used prior to the label expiration date.

Appropriate surgical technique

1. **Maintenance of the sterile field**- If a sterile instrument or gloved hand touches something outside the sterile field (area of the surgical drape and inside of the opened instrument pack), the instrument or glove must be replaced immediately with one that is sterile.
2. **Gentle tissue handling**- Minimize the use of toothed or crushing instruments. Hold the cut edge rather than grasping the middle of a tissue layer. When tying off vessels, include a minimum of surrounding tissues. Use electrocautery and electroscalpels sparingly as they cause tissue necrosis. Keep tissue moist during surgery.
3. **Minimize the duration of surgery**- Prolonged surgery times expose tissues to contaminants, dry out tissues, leading to an increased risk of necrosis, dehydration and postoperative infection.
4. **Supplemental heat**- Animals lose their ability to regulate body temperature while under general anesthesia and they should be provided a heat source during surgery such as a heat lamp or heating pad (avoid electric heating pads). Systems available that monitor body temperature and regulate supplementary heat as necessary are the most ideal. Regular body temperature must be maintained throughout the procedure and recovery period.

Anesthesia and intra-operative monitoring

The anesthetic agent, dose, and route of administration must be described in the approved IACUC protocol. Personnel involved with surgical procedures must be competent in administering and monitoring anesthetic depth in the animals. If needed, additional doses of anesthetic may be required during a surgical procedure.

General signs of inadequate surgical anesthesia include:

- Movement in response to painful stimuli (toe pinch, needle prick)
- Reflex activity: corneal reflex, foot withdrawal in response to toe pinch, etc.
- Increase in muscle tone, e.g., increased jaw tone as surgery progresses
- Increase in respiratory or heart rate; increase in blood pressure
- Movement, and/or vocalization during the surgery

Antibiotic usage

In general, antibiotics should not be needed for short procedures if proper aseptic technique is followed throughout the surgery and the surgeon is well trained or experienced.

General recommendations for the use of antibiotics:

- Antibiotics should be administered before the start of surgery so adequate tissues levels are present throughout the surgery.
- An appropriate antibiotic should be selected based on the species of surgical patient, spectrum of activity, dosing frequency and probable organism and sensitivity. Please contact Veterinary Services for assistance in selecting an appropriate antibiotic.
- Antibiotics should not be used in place of surgical asepsis and good tissue handling techniques.

Post-Surgical care

Appropriate post-surgical care will help to ensure timely attention to problems that may arise and provide the animal an uneventful recovery from anesthesia and surgery. Trained personnel and appropriate facilities and equipment must be available for post-surgical care.

Post-surgical care begins with completion of the surgery and recovery from anesthesia. The period may extend for days to weeks depending on post-surgical outcomes and study design. Post-surgical care includes after-hours and weekend care and is the responsibility of the investigator. Post-surgical care includes the following:

- Continuous observation to ensure uneventful recovery from anesthesia.
- Provision of supplemental heat during anesthetic recovery.
- Administration of fluids, analgesics, antibiotics, and other medications as indicated in the approved IACUC protocol.
- Consultation with an ARC Veterinary if animals experience complications or unmanageable pain or distress.
- Monitoring and management of chronic indwelling devices such as catheters or implants. For example, indwelling catheters typically require daily flushing with anti-coagulant solutions and chronic electrodes may require daily wound cleaning and debridement.
- Adequate care for surgical incisions, including removal of non-absorbable skin closures (sutures or staples) approximately 2 weeks after surgery.
- Maintenance of records of all care given, including all medications administered to the animal, times of all observations and assessment findings.

2. Survival Surgical Procedures for Large Animals (Rabbits, Swine, Sheep, Dogs, etc.)

Pre-surgical Planning

Appropriate planning is important for all successful surgical outcomes. Pre-surgical planning requires input from all members of the surgical team and includes the investigator, surgeon, anesthetist, technicians, veterinarians, and animal care staff. Investigators are encouraged to contact ULAR veterinarians for assistance in planning surgeries and post-surgical care.

A surgical plan should be developed for each procedure and identify the following:

- All necessary personnel, as well as their roles, experience and training needs
- Equipment and supplies required for the planned procedures

- Location and nature of the surgical facilities (shared facilities may require reservation)
- Preoperative animal health assessment criteria and a description of presurgical medications if needed
- Expected postoperative care and identification of all needed supplies

Personnel Training

Personnel involved with surgery in a research setting often have a wide range of educational backgrounds and may require various levels of training before performing surgery on animals. Personnel trained to perform surgery in humans may require additional training for interspecies variations in anatomy, physiology, and response to anesthetics and analgesics.

Regardless of an individual's responsibility or educational background, all personnel performing surgery must have thorough knowledge and understanding of the approved IACUC protocol procedures and possess knowledge and familiarity with the relevant anatomy of the species and the surgical site.

At a minimum, training of surgical personnel must include:

- A thorough knowledge of aseptic technique, including sterile gowning techniques.
- Administration and assessment of anesthesia
- Appropriate tissue handling (tissue trauma contributes to postoperative infections)
- Familiarity with possible adverse events and ability to properly manage such events (e.g., cardiac arrhythmias, bradycardia, etc.)
- Appropriate use of instruments
- Effective methods of hemostasis
- Correct use of sutures and/or skin staples
- Post-surgical care and monitoring, including the ability to recognize and alleviate pain and distress

Surgical Facilities

Animal welfare regulations require that survival surgery in large species be performed in a dedicated surgical facility. The facility must provide separate rooms for patient preparation, surgeon preparation, an operating room and an area for animal recovery. Non-survival surgery, and minor surgery can be performed in a dedicated work area. The IACUC reviews and approves all surgical areas at Dartmouth College. The Surgical Research Laboratories (SRL) maintains several large animal operating rooms (OR's). Investigators are encouraged to contact the SRL and ARC Veterinary Staff for more information.

Large animal surgical space must be designed and managed to achieve the following:

- Separation of the animal preparation, surgeon preparation, operating room and recovery areas
- Minimization of personnel traffic flow through the surgery area
- If possible, airflow should be away from the surgery area (e.g. positive room pressure, use of filtered, laminar flow air)
- Room surfaces should be non-porous and easily sanitized
- A regular room cleaning and disinfection schedule must be established (i.e., daily cleaning of floors and work surfaces, weekly to monthly cleaning of walls and cabinets)

- The surgery area should be free of all equipment and materials not necessary for the procedure. Storage items should be placed in cabinets or drawers.
- For shared surgery facilities, investigators are expected to clean the surgical suite after each use.

Aseptic Technique

The goal of aseptic technique is to reduce the possibility of microbial contamination to the lowest practical level. No single technique, practice, germicide or piece of equipment will achieve this objective. Rather, proper aseptic technique is dependent on numerous practices that require input and cooperation of all personnel within the operating area.

Components of successful, aseptic technique include:

- Preparation of the animal
- Preparation of the surgeon
- Sterilization of instruments and supplies
- Appropriate surgical technique
- Anesthesia and intra-operative monitoring
- Antibiotic usage
- Post-surgical care
- Record keeping

Preparation of the animal

1. After the animal has been anesthetized, the eyes should be lubricated with a sterile ophthalmic ointment to prevent corneal drying. Alternatively, the lids can be taped closed to prevent the corneas from drying.
2. Hair should be removed from the surgical site. This should be performed with electric clippers (# 40 blade) or depilatory rather than a razor.
3. The surgical area to be prepared should be approximately twice that needed for the incision, in the event a larger incision than planned may be required.
4. The skin should then be cleaned and disinfected with a chlorhexidine or povidone iodine based disinfectant followed by 70% alcohol. The site should be scrubbed by starting at the center of the site and working outward in a circular pattern using sterile gauze and instruments. Typically, three scrubs with a disinfectant, followed by alcohol will suffice.
5. A final application of disinfectant solution should be applied to the skin and allow to dry before the start of surgery.
6. A sterile surgical drape is required to isolate the disinfected area from surrounding tissue and hair. To be effective, a drape should adhere tightly to the skin and be impermeable to moisture. Self-adhesive drapes are available for this purpose.

Preparation of the surgeon

1. Sterile gloves must be used for all procedures. Prior to donning gloves, the surgeon must scrub the hands and forearms with a disinfectant soap for a minimum of 3 minutes, followed by drying with a sterile towel

2. A new pair of sterile gloves must be worn for each patient. The surgical gown must remain sterile between animals.
3. A cap, face mask, shoe covers and sterile gown must be worn for all survival large animal surgeries

Sterilization of instruments and supplies

All instruments and supplies that will come in contact with the surgical site must be sterile. A number of options are available to sterilize surgical equipment and supplies

1. **Autoclave** - Relies on pressurized steam, is extremely reliable, and cost effective. However, instruments must be durable (e.g., stainless steel) and the process is relatively slow, from 15 to 60 minutes. Instruments are typically wrapped or sealed in packs that are opened as needed on the day of surgery.
2. **Ethylene oxide** - A gaseous sterilant that requires specialized containment equipment. This is a good sterilization method for supplies that cannot tolerate high heat such as plastics and catheters. It is more costly than autoclaving and typically is performed overnight.
3. **Cold sterilant solutions (hypochlorite, glutaraldehyde, etc.)** - Generally, cold sterilants must have prolonged contact time (follow label recommendations) to sterilize surgical equipment. In addition, the instruments must be rinsed completely with a sterile solution like saline to prevent tissue irritation. **Note: Alcohol is not a sterilant.**
4. **Pre-sterilized items** - Many instruments and supplies can be purchased in sterilized packaging. Such items must be used prior to the label expiration date.

Appropriate surgical technique

1. **Maintenance of the sterile field** - If an instrument, gloved hand, or sterile gown touches something outside the sterile field (area of the surgical drape and inside of the opened instrument pack), the item must be replaced immediately.
2. **Gentle tissue handling** - Minimize the use of toothed or crushing instruments. Hold the cut edge rather than grasping the middle of a tissue layer. When tying off vessels, include a minimum of surrounding tissues. Use electrocautery and electroscalpels sparingly as they cause tissue necrosis. Keep tissue moist during surgery.
3. **Ablate all "dead space" during closure** - Any pockets or spaces remaining between tissue layers will fill with extracellular fluid or blood and increase the risk of developing abscesses.
4. **Minimize the duration of surgery** - Prolonged surgery times expose tissues to contaminants and dry out tissues and lead to increased risk of necrosis and postoperative infection.
5. **Supplemental fluids** - Prolonged surgeries require placement of an IV catheter and intra-operative fluid supplementation. Fluid administration may also be continued into the postoperative recovery period. Please contact Veterinary Services for assistance in developing a fluid therapy plan.
6. **Supplemental heat** - Animals lose their ability to regulate body temperature while under general anesthesia and they should be provided a heat source during surgery such as a heat lamp or heating blanket. Regular body temperature must be maintained throughout the procedure and recovery period.

Anesthesia and intra-operative monitoring

The anesthetic agent, dose, and route of administration must be described in the approved IACUC protocol. Personnel involved with surgical procedures must be competent in administering and monitoring anesthetic depth in the animals. If needed, additional doses of anesthetic may be required during a surgical procedure.

General signs of inadequate surgical anesthesia include:

- Movement in response to painful stimuli (toe pinch, needle prick)

- Reflex activity: corneal reflex, foot withdrawal in response to toe pinch, etc.
- Increase in muscle tone, e.g., increased jaw tone as surgery progresses
- Increase in respiratory or heart rate; increase in blood pressure
- Movement, and/or vocalization during the surgery

Antibiotic usage

In general, antibiotics should not be needed for short procedures if proper aseptic technique is followed throughout the surgery and the surgeon is well trained or experienced.

General recommendations for the use of antibiotics:

- Antibiotics should be administered before the start of surgery so adequate tissues levels are present throughout the surgery. For prolonged surgeries, additional intraoperative doses may be required.
- An appropriate antibiotic should be selected based on the species of surgical patient, spectrum of activity, dosing frequency and probable organism and sensitivity. Please contact Veterinary Services for assistance in selecting an appropriate antibiotic.
- Antibiotics should not be used in place of surgical asepsis and good tissue handling techniques.

Post-surgical care

Appropriate post-surgical care will help to ensure timely attention to problems that may arise and provide the animal an uneventful recovery from anesthesia and surgery. Trained personnel and appropriate facilities and equipment must be available for post-surgical care.

Post-surgical care begins with completion of the surgery and recovery from anesthesia. The period may extend for days to weeks depending on post-surgical outcomes and study design. Post-surgical care includes after-hours and weekend care and is the responsibility of the investigator.

Post-surgical care includes the following:

- Continuous observation to ensure uneventful recovery from anesthesia.
- Provision of supplemental heat during anesthetic recovery.
- Administration of fluids, analgesics, antibiotics, and other medications as indicated in the approved IACUC protocol.
- Consultation with Veterinary Services if animals experience complications or unmanageable pain or distress.
- Monitoring and management of chronic indwelling devices such as catheters or implants. For example, indwelling catheters typically require daily flushing with anticoagulant solutions and chronic electrodes may require daily wound cleaning and debridement.
- Adequate care for surgical incisions, including removal of non-absorbable skin closures (sutures or staples) approximately 2 weeks after surgery.
- Maintenance of records of all care given, including all medications administered to the animal, times of all observations and assessment findings.

3. Survival surgical procedures for non-mammalian species (fish, amphibia, reptiles, avian)

Pre-surgical Planning

Appropriate planning is important for all successful surgical outcomes. Pre-surgical planning requires input from all members of the surgical team and includes the investigator, surgeon, anesthetist, technicians, veterinarians and animal care staff. Investigators are encouraged to contact ULAR veterinarians for assistance in planning surgeries and post-surgical care.

A surgical plan should be developed for each procedure and identify the following:

- All necessary personnel, as well as their roles, experience and training needs
- Equipment and supplies required for the planned procedures
- Location and nature of the surgical facilities (shared facilities may require reservation)
- Preoperative animal health assessment criteria and a description of pre-surgical medications if needed.
- Expected postoperative care and identification of all needed supplies

Personnel Training

Personnel involved with surgery in a research setting often have a wide range of educational backgrounds and may require various levels of training before performing surgery on animals. Personnel trained to perform surgery in humans may require additional training for interspecies variations in anatomy, physiology, and response to anesthetics and analgesics.

Regardless of an individual's responsibility or educational background, all personnel performing surgery must have thorough knowledge and understanding of the approved IACUC protocol procedures and possess knowledge and familiarity with the relevant anatomy of the species and the surgical site.

At a minimum, training of surgical personnel must include:

- A thorough knowledge of aseptic technique
- Administration and assessment of anesthesia
- Appropriate tissue handling (tissue trauma contributes to postoperative infections)
- Appropriate use of instruments
- Effective methods of hemostasis
- Correct use of sutures and/or skin staples
- Post-surgical care and monitoring, including the ability to recognize and alleviate pain and distress

Surgical Facilities

A dedicated surgical suite is not required for the performance of surgical procedures on non-mammalian species. However, the space must be designated for surgery at the time of the procedure and appropriately managed to minimize contamination from other activities in the room during surgery.

Non-mammalian surgical space should be designed and managed to achieve the following:

- Separation of the animal preparation area from the surgery area whenever possible
- Minimization of personnel traffic flow through the surgery area

- If possible, airflow should be away from the surgery area (e.g. positive room pressure, use of filtered, laminar flow air)
- Tanks, cages and work surfaces should be non-porous and easily sanitized
- A regular room cleaning and disinfection schedule should be established (i.e., daily cleaning of floors and work surfaces, weekly to monthly cleaning of walls and cabinets)
- The surgery area should be free of all equipment and materials not necessary for the procedure. Storage items should be placed in cabinets or drawers.

Aseptic Technique

The goal of aseptic technique is to reduce the possibility of microbial contamination to the lowest practical level. No single technique, practice, germicide, or piece of equipment will achieve this objective. Rather, proper aseptic technique is dependent on numerous practices that require input and cooperation of all personnel within the operating area.

Components of successful aseptic technique include:

- Preparation of the animal
- Preparation of the surgeon
- Sterilization of instruments and supplies
- Appropriate surgical technique
- Anesthesia and intra-operative monitoring
- Antibiotic usage
- Postsurgical care
- Record Keeping

Preparation of the animal

Aquatic species

1. After the animal has been anesthetized, it should be transferred to the surgical station and placed on a moist substrate (shallow water, moist towel, etc.)
2. Surgical preparation of the incision site should minimize disruption of skin and mucus layer.
3. The skin at the incision site should be gently wiped with sterile gauze or cotton-tipped applicator to reduce gross contamination. If greater antimicrobial activity is wanted, the skin can be wiped with a dilute solution of povidone iodine (1:20) or chlorhexidine (1:40). Application of harsher chemical disinfectants and alcohol may irritate the skin and increase the risk of tissue damage and postoperative morbidity and mortality.
4. For larger fish species, removing large scales by extracting them caudally can facilitate a smooth incision.
5. A sterile clear plastic drape can be positioned over the animal to help isolate the incision site, create a sterile field and help retain moisture. A rim of petroleum jelly can be used to adhere the drape to the animal if desired.
6. The animal's skin should be kept moist throughout the surgery, with care taken to prevent irrigating the incision site with contaminated anesthetic or tank water.

Avian

1. After the animal has been anesthetized, the animal should be positioned to allow easy access to the surgical site.
2. The feathers at the surgical site should either be parted for small incisions or plucked to expose the intended incision site. The skin should be exposed to create a space approximately twice the size of the intended incision. Tape can be applied to surrounding feathers to prevent them from entering the sterile field during surgery.
3. The skin should then be cleaned and disinfected with a chlorhexidine or povidone iodine-based disinfectant followed by 70% alcohol. The site should be scrubbed by starting at the center of the site and working outward in a circular pattern. Typically, one scrub with a disinfectant, followed by alcohol will suffice.
4. If possible, the use of a sterile surgical drape is recommended to isolate the sterile field and reduce the risk of postoperative infection.

Reptiles

1. The animal should be anesthetized and restrained in a position that allows easy access to the surgical site.
2. The skin should then be cleaned and disinfected with a chlorhexidine or povidone iodine-based disinfectant followed by 70% alcohol. The site should be scrubbed by starting at the center of the site and working outward in a circular pattern. Reptiles harbor significant pathogens on the skin, such as Salmonella, and a prolonged vigorous scrub with multiple applications of disinfectant followed by an alcohol wipe is recommended.
3. A sterile surgical drape should be used whenever possible to isolate the disinfected area from surrounding skin. To be effective, a drape should adhere tightly to the skin and be impermeable to moisture. Self-adhesive drapes are available for this purpose.

Preparation of the surgeon

1. Sterile gloves must be used for all procedures. Gloves must be replaced or sterilized with a cold sterilant between animals if performing multiple surgeries.
2. The surgeon must wear a clean lab coat, scrubs or smock while performing surgery.
3. A surgical mask and a cap or hairnet is also recommended to reduce the risk of gross contamination of the surgical site.

Sterilization of instruments and supplies

All instruments and supplies that will come in contact with the surgical site must be sterile. A number of options are available to sterilize surgical equipment and supplies.

1. **Autoclave** - Relies on pressurized steam, is extremely reliable and cost effective. However, instruments must be durable (e.g., stainless steel) and the process is relatively slow, from 15 to 60 minutes. Instruments are typically wrapped or sealed in packs that are opened as needed on the day of surgery.
2. **Ethylene oxide** - A gaseous sterilant that requires specialized containment equipment. This is a good sterilization method for supplies that cannot tolerate high heat such as plastics and catheters. It is more costly than autoclaving and typically is performed overnight.
3. **Cold sterilant solutions (hypochlorite, glutaraldehyde, etc.)** - Generally, cold sterilants must have prolonged contact time (follow label recommendations) to sterilize surgical equipment. In addition, the instruments must be rinsed completely with a sterile solution like saline to prevent tissue irritation. **Note: Alcohol is not a sterilant.**
4. **Hot bead sterilizer** - This device is a small tabletop unit, approximately 6 x 6 x 8 inches. The appliance heats a small container of Pyrex beads to approximately 250 C and can sterilize the tips of metal surgical

instruments in 10-20 seconds. It is very useful for sterilizing instruments between animals when performing multiple surgeries.

5. **Pre-sterilized items** - Many instruments and supplies can be purchased in sterilized packaging. Such items must be used prior to the label expiration date.

Appropriate surgical technique

1. **Maintenance of the sterile field** - If an instrument or gloved hand touches something outside the sterile field (area of the surgical drape and inside of the opened instrument pack), the instrument or glove must be replaced or re-sterilized immediately.

2. **Gentle tissue handling** - Minimize the use of toothed or crushing instruments. Hold the cut edge rather than the grasping the middle of a tissue layer. When tying off vessels, include a minimum of surrounding tissues. Use electrocautery and electroscalpels sparingly as they cause tissue necrosis. Keep tissue moist during surgery.

3. **Minimize the duration of surgery** - Prolonged surgery times expose tissues to contaminants and dry out tissues and lead to increased risk of necrosis and postoperative infection.

4. **Supplemental heat** - Animals lose their ability to regulate body temperature while under general anesthesia and they should be provided a heat source during surgery such as a heat lamp (remembering that you can dry tissues and cause further damage if surgery is prolonged over 15 minutes) or heating blanket.

Anesthesia and intra-operative monitoring

The anesthetic agent, dose and route of administration must be described in the approved IACUC protocol. Personnel involved with surgical procedures must be competent in administering and monitoring anesthetic depth in the animals. If needed, additional doses of anesthetic may be required during a surgical procedure.

General signs of inadequate surgical anesthesia include:

- Movement in response to painful stimuli (toe pinch, needle prick)
- Reflex activity: corneal reflex, foot withdraw in response to toe pinch, etc.
- Increase in muscle tone, e.g., increased jaw tone as surgery progresses
- Increase in respiratory or heart rate; increase in blood pressure
- Movement, and/or vocalization during the surgery

Antibiotic usage

In general, antibiotics should not be needed for short procedures if proper aseptic technique is followed throughout the surgery and the surgeon is well trained or experienced.

General recommendations for the use of antibiotics:

- Antibiotics should be administered before the start of surgery so adequate tissue levels are present throughout the surgery.
- An appropriate antibiotic should be selected based on the species of surgical patient, spectrum of activity, dosing frequency and probable organism and sensitivity. Please contact Veterinary Services for assistance in: selecting an appropriate antibiotic.
- Antibiotics should not be used in place of surgical asepsis and good tissue handling techniques

Post-surgical care

Appropriate post-surgical care will help to ensure timely attention to problems that may arise and provide the animal an uneventful recovery from anesthesia and surgery. Trained personnel and appropriate facilities and equipment must be available for post-surgical care.

Post-surgical care begins with completion of the surgery and recovery from anesthesia. The period may extend for days to weeks depending on post-surgical outcomes and study design. Post-surgical care includes after-hours and weekend care and is the responsibility of the investigator.

Post-surgical care includes the following:

- Continuous observation to ensure" uneventful recovery from anesthesia
- Provision of supplemental heat during anesthetic recovery, if needed
- For aquatic species, salt-treated (1-3 g/l) water for several days to a week is well tolerated and reduces the osmotic gradient experienced by the animal
- Administration of fluids, analgesics, antibiotics, and other medications as indicated in the approved IACUC protocol
- Consultation the ARC Veterinary Staff if animals experience complications or unmanageable pain or distress
- Monitoring and management of chronic indwelling devices such as catheters or implants. For example, indwelling catheters typically require flushing with anticoagulant solutions and chronic electrodes may require daily wound cleaning and debridement.
- Adequate care for surgical incisions, including removal of non-absorbable skin closures (sutures or staples) after complete healing of the skin
- Maintenance of records of all care given, including all medications administered to the animal, times of all observations and assessment findings

C. Terminal Surgical Procedures

Terminal surgical procedures are defined as those procedures that the animal is not intended to recover from anesthesia after completion of the surgery. Although postoperative recovery and care is not an issue in terminal surgeries, many issues common to the performance of any surgery must be addressed for a successful outcome. Investigators are encouraged to contact the ARC Veterinary Staff for assistance in planning surgeries.

Pre-surgical Planning

Appropriate planning is important for all successful surgical outcomes. Pre-surgical planning requires input from all members of the surgical team and includes the investigator, surgeon, anesthetist, technician, and veterinarian.

A surgical plan should be developed for each procedure and identify the following:

- All necessary personnel, as well as their roles, experience and training needs
- Equipment and supplies required for the planned procedures
- Location and nature of the surgical facilities (shared facilities may require reservation)
- Preoperative animal health assessment criteria and a description of pre-surgical medications if needed.

Personnel Training

Personnel involved with surgery in a research setting often have a wide range of educational backgrounds and may require various levels of training before performing surgery on animals. Personnel trained to perform surgery in humans may require additional training for interspecies variations in anatomy, physiology, and response to anesthetics.

Regardless of an individual's responsibility or educational background, all personnel performing surgery must have thorough knowledge and understanding of the approved IACUC protocol procedures and possess knowledge and familiarity with the relevant anatomy of the species and the surgical site.

At a minimum, training of surgical personnel must include:

- Administration and assessment of anesthesia
- Appropriate tissue handling
- Appropriate use of instruments
- Effective methods of hemostasis
- Correct use of sutures, if indicated

Surgical Facilities

A dedicated surgical suite is not required for the performance of terminal surgical procedures. However, the surgery space must be designated for surgery at the time of procedure and appropriately managed to minimize contamination from other activities in the room during surgery.

The surgical space should be designed and managed to achieve the following:

- Separation of the animal preparation area from the surgery area whenever possible
- Minimization of personnel traffic flow through the surgery area
- If possible, airflow should be away from the surgery area (e.g. positive room pressure, use of filtered, laminar flow air)
- Work surfaces should be non-porous and easily sanitized
- A regular room cleaning and disinfection schedule should be established (i.e., daily cleaning of floors and work surfaces, weekly to monthly cleaning of walls and cabinets)
- The surgery area should be free of all equipment and materials not necessary for the procedure. Storage items should be placed in cabinets or drawers

Aseptic Technique

Aseptic technique is generally not required for the performance of terminal surgery. However, for studies in which local bacterial contamination of tissues, or sepsis, could influence study outcomes, standard aseptic technique is recommended. Such studies include long term (>8 hours) terminal surgeries and collection of samples for tissue or microbial culture.

Anesthesia and intra-operative monitoring

The anesthetic agent, dose, and route of administration must be described in the approved IACUC protocol. Personnel involved with surgical procedures must be competent in administering and monitoring anesthetic depth in the animals. If needed, additional doses of anesthetic may be required during a surgical procedure.

General signs of inadequate surgical anesthesia include:

- Movement in response to painful stimuli (toe pinch, needle prick)

- Reflex activity: corneal reflex, foot withdrawal in response to toe pinch, etc.
- Increase in muscle tone, e.g., increased jaw tone as surgery progresses
- Increase in respiratory or heart rate; increase in blood pressure
- Movement, and/or vocalization during the surgery

Personal Protective Equipment

It is recommended that laboratory personnel wear:

- Dedicated clothing (i.e., lab jacket, smock; surgical gown)
- Disposable gloves
- Face mask
- Safety goggles or glasses if performing procedures that generate aerosols
- Terminal perfusion must be performed in a chemical (fume) hood or on a down draft table

D. Record Keeping Requirements

Surgical records are both helpful and required by animal welfare regulations. Accurate records allow one to monitor trends and they can be helpful in refining and improving research projects. Complete records also assure compliance with accepted care standards and agreed-upon procedures approved by the IACUC. Records can also be helpful in interpreting research data.

Records are required for all animals undergoing terminal or survival surgery. For rodents, fish and reptiles; anesthesia, surgical and post-surgical records can be maintained in a laboratory notebook. This notebook needs to be maintained available at Dartmouth College for at least 3 years after completion of the IACUC Protocol. While these animals are maintained, post-operative records need to be maintained on the cage or in the room where the animals are housed, so that they are available to the ARC veterinary staff. For larger species such as rabbits, cats, swine, etc.; anesthesia (including surgical info) and postoperative records must accompany the animal in the vivarium to allow veterinary oversight and involvement in the care of the animal. In addition to the required records, this information may also (duplicate info) be maintained in laboratory notebooks.

Forms required for documentation of Surgery and Anesthesia, as well as Post-Operative Care are described and attached below. These forms must be used where indicated and are available from the ARC office and will be maintained in the Surgical Research Laboratories OR area.

Anesthesia Record (see attached form - “Laboratory Animal Anesthesia Record”)

Anesthesia records must include:

- Animal identification-- species and animal number or unique identifier
- Body weight--required for precise calculation of dosages (anesthetics, analgesics, antibiotics, etc.)
- Identification of the surgeon
- Brief description of surgical procedure. Any complications encountered during surgery can be documented as well.
- An itemization of all medications including anesthesia given to the animal; including dosage, route, and time of administration.

Post-surgical Records (see attached form - “Post-Operative Recovery Record”)

Post-operative care records **MUST** include:

- The plan for monitoring, and analgesic or antibiotic administration (e.g., record the body temperature every 12 hours; 0.05 ml buprenorphine every 12 hours).
- Any complications encountered-- e.g., delayed recovery from anesthesia, bleeding from incision site, etc.
- Dosage, route, and time of all medications or compounds administered to the animal.
- Itemization of all care and monitoring provided to the animal such as wound cleaning, bandage changes, flushing of indwelling catheters, body temperature, heart rate, etc. as described in the approved IACUC protocol.
- Contact information for all research staff responsible for daily assessment and care must be provided in the IACUC protocol.
- All postoperative record entries must include time, date, and clearly written initials of personnel performing the procedure

Terminal Procedures Records

The following records are required for terminal surgical procedures:

- **Anesthesia Record (see attached form - “Laboratory Animal Anesthesia Record”)**. Note that there is no post-operative care so that there is no requirement for a “Post-Operative Recovery Record”.

This form must include the following information:

- Animal identification -- species and animal number or unique identifier
- Body weight--required for precise calculation of dosages (anesthetics, analgesics, antibiotics, etc.)
- Identification of the surgeon
- Brief description of surgical procedure. Include any complications encountered during the surgery if possible.
- Itemization of all medications given to the animal, including dosage, routes and time of administration
- Euthanasia method, including appropriate doses and route of administration

E. Who to contact for help?

Contact the ARC Veterinary Staff during development of an IACUC protocol for help with anesthesia, surgery and post-operative care requirements and guidance. Also, contact the ARC Veterinary staff at any time there is a question in these areas.



| | | | | |
|--------------|-------------|------------------|------------|---|
| Investigator | Protocol # | Species | Animal ID# | Cage Card # |
| Surgeons | Anesthetist | Surgery Location | | Date of Surgery |
| Procedures: | | | | Survival Sx.: <input type="checkbox"/> Yes or <input type="checkbox"/> No |
| Age | Sex | Weight | Temp. | HR |
| | | | RR | M.M. Color |
| | | | | Pulse Quality |

| Pre-Anesthetic Drugs | | | |
|----------------------|-----------|-------|------|
| Drug | Dose (mg) | Route | Time |
| | | | |
| | | | |
| | | | |

| Anesthetic Induction | | | |
|----------------------|-----------|-------|------|
| Drug | Dose (mg) | Route | Time |
| | | | |
| | | | |
| | | | |

| Anesthesia Confirmation (Prior to start of any surgical procedures) | | |
|---|-------------------------------|---|
| Time: | Reflexes/parameters assessed: | Adequate anesthesia? <input type="checkbox"/> Yes or <input type="checkbox"/> No ⁺ |
| Time: | Reflexes/parameters assessed: | Adequate anesthesia? <input type="checkbox"/> Yes or <input type="checkbox"/> No ⁺ |

Record of anesthetic monitoring must occur at least every 15 minutes. These parameters are recommended for monitoring but all are not required.

| | :15 | :30 | :45 | :00 | :15 | :30 | :45 | :00 | :15 | :30 | :45 | :00 | :15 | :30 | :45 |
|--|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| O ₂ / N ₂ O L/min | | | | | | | | | | | | | | | |
| Isoflourane % | | | | | | | | | | | | | | | |
| ● Heart Rate | | | | | | | | | | | | | | | |
| ○ Resp. Rate | | | | | | | | | | | | | | | |
| Ø IPPV | | | | | | | | | | | | | | | |
| Blood Pressure | | | | | | | | | | | | | | | |
| ∨ syst. | | | | | | | | | | | | | | | |
| ^ diast. | | | | | | | | | | | | | | | |
| - mean | | | | | | | | | | | | | | | |
| Times Start Procedure 1 | | | | | | | | | | | | | | | |
| Start Procedure 2 | | | | | | | | | | | | | | | |
| End Surgery | | | | | | | | | | | | | | | |
| Extubation | | | | | | | | | | | | | | | |
| Temp. <input type="checkbox"/> Eso <input type="checkbox"/> Rectal | | | | | | | | | | | | | | | |
| ETCO ₂ | | | | | | | | | | | | | | | |
| MM Color / CRT | | | | | | | | | | | | | | | |
| FLUIDS | | | | | | | | | | | | | | | |
| Adequate anesthetic depth? ⁺ | Y/N | Y/N | Y/N | Y/N | Y/N | Y/N | Y/N | Y/N | Y/N | Y/N | Y/N | Y/N | Y/N | Y/N | Y/N |

⁺ If 'No', you must reassess and adjust anesthetic regimen or contact an ARC veterinarian for assistance. Document all anesthetic changes.

| |
|--|
| Comments / Complications: |
|--|

