



INDIANA UNIVERSITY

Office of Research Compliance (ORC) Institutional Animal Care and Use Committee (IACUC)

Surgery & Post-Operative Care

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Responsible University Office:
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Scope

This policy applies to all Indiana University faculty, staff, and students in the use of animals in biomedical research involving surgery (survival and non-survival). The IUSM IACUC is obligated to provide policies that notify and train personnel concerning the appropriate techniques, equipment, and agents for performing appropriate procedures to ensure humane care and use of laboratory animals.

Background

Surgery is defined as a procedure that uses instruments to incise tissue and penetrate a body cavity or subcutaneous fascia. Surgical procedures include, but are not limited to, resection of a nerve or vessel, implantation of a catheter or biomaterial substance, or other modification of tissues. Surgery is classified as either survival or non-survival. Survival surgery is defined as surgery after which the animal recovers from anesthesia (*Guide* p117).

Surgery Policy Statement

Nonsurvival Surgery:

Nonsurvival surgery is defined as any surgical intervention on an appropriately anesthetized animal from which the animal is euthanized before recovery from anesthesia (*Guide* p118). This includes any procedures which involve making incisions and dissections prior to death of the animal. However, this does not include procedures occurring after the death of an animal, such as post-mortem tissue harvest. All animal nonsurvival surgeries, whether rodent or non-rodent require that certain procedures are followed. For non-survival procedures of extended duration, attention to

aseptic technique may be more important in order to ensure stability of the model and a successful outcome. However, at a minimum, the surgical site should be clipped, the surgeon should wear gloves, and the instruments and surrounding area should be clean (Guide p118).

Aseptic Technique:

Aseptic technique is an important component of successful surgical outcome. Aseptic technique is used to reduce microbial contamination to the lowest possible practical level (Guide p118). No procedure, piece of equipment, or germicide alone can achieve that objective. Proper results can be achieved only when proper preparation of the animal, surgeon, and equipment are preformed and when proper technique is used. Research results can be altered significantly or invalidated due to local or systemic infections after improper aseptic technique. For non-survival procedures of extended duration, attention to aseptic technique may be more important in order to ensure stability of the model and a successful outcome (Guide p 118). The investigator should consider whether the use of aseptic technique may be warranted in their approved non-survival surgical procedures. At a minimum, for non-survival surgical procedures, the surgical site should be clipped, the surgeon should wear gloves, and the instruments and surrounding area should be clean (Guide p118).

Survival Surgery:

All vertebrate animals (USDA-covered and Non-USDA-covered): The IACUC requires that all survival surgery be performed using aseptic technique. The use of sterile technique is important for both animal welfare and scientific research validity.

The surgical site must be prepared by first removing the hair or fur. This can be accomplished with clippers fit with a surgical clipper blade (a number 40 blade is typically used) or with a depilatory. The surgical site then must be cleaned with an antiseptic soap or solution such as an iodophor (Betadine) or chlorhexidine gluconate (Hibiclens or Nolvasan). Skin antisepsis is maintained by both the mechanical action of scrubbing and chemical action. The surgical site is then wiped with either sterile saline or 70% alcohol (do not use alcohol if using cautery, this may result in fire/burns) in a spiral circular motion starting from the incision site out to the periphery. The scrub and rinse is repeated in an alternating fashion three times, with a fresh sterile gauze (large incision) or cotton tipped applicator (small incision) used for each repeat. A final betadine solution (not scrub) painting may be applied to the surgical incision. The surgery site is usually draped with a sterile drape (USDA or non-USDA covered vertebrate animals) or commercially available food storage plastic wrap (e.g., Glad Press 'n Seal) (non-USDA-covered vertebrate animals only) to protect the sterile site, instruments, and suture material from non-sterile areas.

Alcohol (any concentration of ethyl or isopropyl alcohol) is neither a sterilant nor a high-level disinfectant, but may be acceptable for some procedures if prolonged contact times are used (Guide p119). Sterilization indicators should be used to validate that materials have been properly sterilized (Guide p119). See IACUC Protocol Form Appendix 6 for recommended hard surface disinfectants, skin disinfectants and instrument sterilants (Tables 1 – 3).

Animals may not undergo more than one major operative procedure in which they recover from anesthesia, unless specifically approved by the IACUC. This approval will be based on scientific necessity (Animal Welfare Regulations § 2.31.d.x. and Guide p30). Multiple survival surgical procedures on a single animal are strongly discouraged. Under special circumstances, multiple surgical procedures on a single protocol may be permitted with the approval of the IACUC (e.g., if individual surgical procedures are essential related components of a research project), or if necessary for clinical reasons. Care must be taken to provide sufficient recovery time between surgeries. Cost savings alone is not an adequate reason for performing multiple survival surgeries.

USDA-covered animals: All survival surgery performed on animals covered under the Animal Welfare Act must be performed in a dedicated surgical suite (Animal Welfare Regulations § 2.31.d.ix.). All survival surgeries must be performed using aseptic procedures, which includes wearing sterile gloves, a surgical cap, sterile surgical gown, and a surgical mask, using sterile instruments, and using aseptic technique. All instruments must be sterilized by an appropriate method (e.g., autoclave, ethylene oxide) prior to use for each animal.

Non-USDA-covered vertebrate animals: Survival surgery on species not covered under the Animal Welfare Act (birds, mice of the genus *Mus*, and rats of the genus *Rattus* bred for use in research, and cold-blooded vertebrates) must be performed in a dedicated space or an area that provides separation from other activities in an appropriately equipped laboratory (Guide p116). Survival surgery must be performed using aseptic procedures, which includes wearing sterile gloves (nitrile gloves may be autoclaved, see LeMoine, 2015), a clean lab coat or scrubs, and a surgical mask, using sterile instruments, and using aseptic technique. The surgeon may opt to wear exam gloves for rodent surgery if a “tips-only”

technique is used. This requires that the surgeon uses only the sterile working ends of the surgical instruments to manipulate the surgical field. The gloved, but not sterile, hand must never touch the working end of the instruments, the suture, suture needle, or any part of the surgical field (Hoogstraten-Miller, 2008). If surgery is performed on multiple rodents using the same surgery pack, the IACUC requires using instruments sterilized by an autoclave to start a batch, then using a hot bead sterilizer or another approved method to sterilize instruments between animals (*Guide* p119). One pack used in this way may be used for up to 5 animals.

Non-survival surgery in rodents should follow the same guidelines as described above for non-survival surgery in USDA-covered animals.

IACUC - Pre-Surgical Preparation Procedures	
Species	Pre-surgical Preparation Description
Mouse and Rat	<p><u>Aseptic technique is used for Survival Surgery.</u> <u>Surgery Area:</u> The laboratory area is disinfected before use. A dedicated space for survival surgery is recommended. Sterile drapes are to be used to drape off the incision area if the abdomen or thorax are open. <u>Surgeon Prep:</u> The surgeon wears a mask, cap, sterile surgical gloves, and clean lab coat or gown. The surgeon performs a thorough hand scrubbing before donning gloves. <u>Patient Prep:</u> If hair is present over the incision site, it is removed with clippers, razor, or depilatory to include an area approximately twice as large as the intended surgery site. Skin is disinfected using three alternating rounds of surgical disinfectant scrub/solution (betadine, hibiclens, chlorhexadine, etc.) with 70% isopropyl alcohol or sterile saline rinses. Care is taken to avoid over-wetting fur outside of the surgical area as this will increase hypothermia. Consider application of disinfectant/alcohol using cotton-tip applicators rather than larger gauze squares as it may be harder to control application in small areas. A sterile surgery field is best maintained through the placement of a sterile drape over non-sterile parts of the animal and surrounding area. Sterile drapes must be used if the abdominal or thoracic cavities are opened. Ophthalmic ointment should be placed in both eyes of anesthetized rodents. <u>Instrument Prep:</u> Instruments, sutures, wound clips, and implanted devices are sterilized in an autoclave or ethylene oxide sterilizer prior to surgery and a sterile field is maintained during surgery. Instruments are re-sterilized between rodents using an autoclave or bead sterilizer. Instruments will not be used on more than 4-5 rodents prior to re-autoclaving.</p>
USDA-covered species (including Hamster, Guinea Pig, Dog, Pig, Rabbit Sheep, Cats, Ferrets and other covered rodents)	<p><u>Aseptic technique is used for Survival Surgery.</u> <u>Surgery Area:</u> Surgery is done in a dedicated operating room. <u>Surgeon Prep:</u> The surgeon wears a mask, cap, sterile surgical gloves, and sterile gown. The surgeon performs a thorough surgical hand scrubbing before donning gloves. <u>Patient Prep:</u> The hair over incision areas is removed in an area approximately twice as large as the intended surgical site. A sterile skin prep is done using at least three alternating rounds of surgical disinfectant scrub (betadine, hibiclens, chlorhexadine, etc.) with 70% isopropyl alcohol or sterile saline rinses. Sterile drapes must be used to protect the surgical field. Ophthalmic ointment should be placed in both eyes of anesthetized animals. <u>Instrument Prep:</u> Instruments, sutures, wound clips, biopsy devices, and implanted devices are sterile and a sterile field is maintained during the procedure. Instruments are autoclaved between animals.</p>

IACUC – Anesthetic Monitoring	
Species	Anesthesia Monitoring Procedures
Rat and Mouse	<p><u>Signs of adequate anesthesia:</u> Rodents will be unresponsive to surgical or procedural stimulation. They will not have withdrawal reflexes when their rear toes are pinched. Incisions will not be made until loss of reflex responses has occurred. Anesthetized animals have regular respiration rates. Their ears and feet remain pink, indicating that peripheral perfusion is adequate. A source of supplemental heat (recirculating hot water pad, microwaveable gel pack, hot water bottle, or heating pad) will be used to prevent hypothermia.</p>

	<p><u>Criteria for administration of additional anesthetic:</u> Respiration rate increases in response to surgical or procedural stimulation, withdrawal reflexes return. Animals should not be re-dosed with injectable anesthetics more than once. The LARC veterinary staff should be consulted if the selected anesthesia does not appear to be effective with the species or strain.</p> <p><u>Monitoring frequency during procedure:</u> Visually monitor every 2-3 minutes during the procedure. Because the surgeon and anesthesiologist are typically the same individual when working with these species, it is not necessary to document monitoring during the procedure. However, the fact that the animal was monitored should be described in a surgery log (e.g. surgery monitoring sheet, notation in lab notebook, etc).</p> <p><u>Anesthetic Monitoring records:</u> Records are kept by the PI. Records must be available for review by the IACUC during semi-annual inspections.</p>
Hamster, Guinea Pig, Dog, Pig, Rabbit (All USDA covered species)	<p><u>Signs of adequate anesthesia:</u> The animal is unconscious with good muscle relaxation and absent jaw tone. There is no limb withdrawal reflex when the toes are pinched. The palpebral reflex is absent. The respiration and heart rates are stable, and these do not increase in response to surgical or procedural stimulation. The mucous membrane color is pink, and the capillary refill time is 1-3 seconds. A source of supplemental heat (recirculating hot water pad, microwaveable gel pack, hot water bottle, or heating pad) will be used to prevent hypothermia.</p> <p><u>Criteria for administration of additional anesthetic:</u> Respiration and/or heart rates increase in response to surgical or procedural stimulation, jaw tone returns, withdrawal reflexes return.</p> <p><u>Monitoring frequency during procedure:</u> Visually monitor and record observations of heart rate, respiration rate, and % gas anesthetic (if being used) on a permanent anesthesia record form approximately every 10 – 15 minutes. Additional parameters that may be of use include body temperature, blood pressure, and oxygenation (capillary refill time or pulse oximetry).</p> <p><u>Anesthetic Monitoring records:</u> This anesthetic record form must be turned into the LARC and be kept with the animal's medical record.</p>
Amphibians & Zebrafish	<p><u>Signs of adequate anesthesia:</u> Loss of righting ability, decreased abdominal respiratory movement (frogs); lack of response to surgical or procedural stimulation.</p> <p><u>Criteria for administration of additional anesthetic:</u> Return of responses during procedural stimulation; increased abdominal respiratory movement (frogs).</p> <p><u>Monitoring frequency:</u> Visually monitor every 2-3 minutes during the procedure. Because the surgeon and anesthesiologist are typically the same individual when working with these species, it is not necessary to document monitoring during the procedure. However, the fact that the animal was monitored should be described in a surgery log (e.g. surgery monitoring sheet, notation in lab notebook, etc).</p> <p><u>Anesthetic Monitoring records:</u> Records are kept by the PI. Records must be available for review by the IACUC during semi-annual inspections.</p>

Post-Operative Care Policy Statement

Appropriate post-operative care must be provided to all animals following surgery (*Guide* p119- 120). Animals should be placed in a clean, dry recovery area where they can be monitored by trained personnel frequently until the animals are fully recovered (responsive to touch and manipulation, have regained a righting reflex and are ambulatory). Attention should be given to thermoregulation. This may include a supplemental heat source to maintain body temperature, such as a lamp (250 W) or circulating water blanket set on medium (37-38C) (Hendrick, 2009). Electric blankets, thermogenic gel packs and reflective foil are NOT recommended due to risk for burns. Icepacks may be used to lower the body temperature if necessary. Attention should also be given to cardiovascular and respiratory function. Animals should be placed on a solid substrate (not bedding) that will not interfere or block nose or mouth. No other awake animals should be in the same enclosure or cage as an anesthetized animal. Post-operative pain or discomfort should be monitored and treated if present. Failure to appropriately monitor animals after a surgical procedure may be a reportable offense of non-compliance to the IACUC and/or OLAW.

IACUC Anesthetic Recovery	
Species	Recovery Monitoring Description

Rodents	<p><u>Monitoring frequency during recovery period:</u> Rodents will be directly observed at least once every 15 minutes until they can respond to gentle manipulation and have regained a righting reflex. They must not be left unattended. They will be kept in a warmed recovery cage that contains a solid substrate. They will not be returned to the animal room until they are fully recovered from anesthesia. This is necessary to ensure that the PI can intervene if there are problems with the surgical site (e.g. incision opens when animal begins moving), to ensure that post-procedural pain has been alleviated, and to minimize communication errors with the LARC staff (e.g. anesthetized animals reported as ill).</p> <p><u>Anesthetic Recovery Monitoring records:</u> The fact that the animal was monitored should be described in a surgery log (e.g. surgery monitoring sheet, notation in lab notebook, etc). Records are kept by the PI. Records must be available for review by the IACUC during semi-annual inspections.)</p>
Hamster, Guinea Pig and other rodents covered by the USDA	<p><u>Monitoring frequency during recovery period:</u> Monitor every 10-15 minutes until they are awake enough to maintain sternal recumbency. They will be turned over at least once every 30 minutes until they can maintain themselves in sternal recumbence. They will be kept in a warmed recovery cage that contains no bedding.</p> <p><u>Anesthetic Recovery Monitoring records:</u> Records are kept with the individual animal's record in the LARC for three years past protocol expiration.</p>
Cat, Dog, Pig, Sheep, Rabbit, Ferret	<p><u>Monitoring procedures during the recovery period:</u> Animals will be monitored at least once every 10-15 minutes and vital signs (such as temperature, heart rate, respiratory rate and capillary refill time) will be assessed and recorded on the permanent anesthesia record form or in the chart. Animals recover under direct observation of trained personnel. Monitoring continues until animals can maintain themselves in voluntary sternal recumbence. Animals are to be kept warm during recovery. Animals may be returned to regular housing areas as approved to do so by the Attending DVM.</p> <p><u>Anesthetic Recovery Monitoring records:</u> This anesthetic recovery record must be turned into the LARC and be kept with the animal's medical record for three years past protocol expiration.</p>
Amphibians & Fish	<p><u>Monitoring procedures during the recovery period:</u> Transfer to a clean tank of conditioned water, monitor until swimming and righting ability are regained.</p> <p><u>Anesthetic Recovery Monitoring records:</u> The fact that the animal was monitored should be described in a surgery log (e.g. surgery monitoring sheet, notation in lab notebook, etc). Records are kept by the PI. Records must be available for review by the IACUC during semi-annual inspections.</p>

IACUC Post-Operative Care	
Species	Post-Operative Care Description
Mouse and Rat	<p>Incisions are checked daily to ensure they are intact and not infected (heat, swelling, pain, discharge, redness) until they are healed (at least 4 days). Appetite, water consumption, general body condition, attitude, and mobility are checked daily (at least 4 days). Sutures and wound clips are removed 7-10 days after surgery. Additional analgesic drugs are given for pain control. Wet feed is given to encourage animals to eat. Monitoring the animal's body weight can be helpful to determine if the appetite is adequate.</p> <p>Records are kept by the PI to document post-surgical care. Records must be available for the IACUC during semi-annual inspections and veterinary staff when requested. Records must be maintained for three years past protocol expiration.</p>
USDA-covered species (including Hamster, Guinea Pig, Dog, Pig, Rabbit Sheep,	<p>Incisions are checked at least once daily to ensure they are intact and not infected (heat, swelling, pain, discharge, redness) until sutures are removed approximately 10-14 post-op. Appetite, water consumption, general body condition, attitude, and mobility are checked daily (at least 7 days). Additional analgesic drugs are given for pain control.</p>

Cats, Ferrets and other covered rodents)	Records to document post-surgical care are kept with the individual animal's record in LARC for three years past protocol expiration.
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Surgical and Post-Operative Records Policy Statement

Survival surgical/anesthetic monitoring and post-operative monitoring records must be documented and maintained according to IACUC Protocol Form Appendices 3 (Anesthetic Monitoring), 4 (Anesthetic Recover) and 7 (Post-Operative Care).

Non-survival surgical records shall follow the same policy as described in Appendix 3 (Anesthetic Monitoring), and must be kept by the PI for three years past protocol expiration (non-USDA covered vertebrate species) or in the individual animal's record in LARC for three years past protocol expiration (USDA covered vertebrate species).

Storage of Sterile Instruments

Durable instruments and supplies may be autoclaved. This an extremely reliable and cost-effective method for sterilization. Normally a wrapped "pack" of instruments is prepared and is opened the day of surgery. Packs may be stored if they are kept away from moisture, see information below on the proper storage per the CDC.

A preparation/autoclave date should be put on each prepared pack and packs should not be used if they are more than 12 months old.

Storage:

Per the CDC, "Sterile items should be stored in an enclosed storage area (e.g., cabinet or drawer). Instruments should not be stored under sinks or in other locations where they might become wet. Sterilized items should remain wrapped until they are needed for use. Sterilized instruments should be stored in a manner that preserves the integrity of the packaging material. An event-related storage approach recognizes that the product should remain sterile until some event causes the item to become contaminated (e.g., a package becomes torn or wet). The quality of the packaging material, the conditions under which items are stored and transported, and the amount that they are handled all affect the chances that the package and its contents will remain sterile. All packages containing sterile items should be inspected before use to verify barrier integrity and dryness. Any package that is wet, torn, dropped on the floor, or damaged in any way should not be used. The instruments should be re-cleaned, packaged in new wrap, and sterilized again."

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