
1. PURPOSE

The intent of this Standard Operating Procedure (SOP) is to describe procedures for survival rodent surgery.

2. RESPONSIBILITY

Principal investigators (PI) and their staff, veterinary care staff or any individual performing surgery on rodents, or assisting in those procedures.

3. MATERIALS

- 3.1. Sterile isotonic solution for injection (e.g. 0.9% saline)
- 3.2. Analgesics
- 3.3. Anesthetics
- 3.4. Sterile ophthalmic ointment
- 3.5. Electric clipper or depilatory cream
- 3.6. Gauze
- 3.7. Antiseptic solution for skin (e.g., chlorhexidine 2% solution or povidone-iodine solution used alternatively with 70% alcohol, or 2% chlorhexidine in 70% alcohol solution)
- 3.8. Heating disc, warming pad or warm-water circulating pad. Do not use electric heating pads unless specifically designed for use with laboratory rodents.
- 3.9. Sterile surgical drapes or Glad® Press'n Seal® wrap
- 3.10. Sterile surgical instruments
- 3.11. Sterile gauze
- 3.12. Suture material or wound clips (Autoclips)
- 3.13. Dry bead sterilizer or cold sterilization agents (e.g. glutaraldehyde) and 70% alcohol (as a rinsing agent)
- 3.14. Rodent Procedure Log and Post-Procedure cage cards

4. PROCEDURES

- 4.1. Document the details of the surgical procedure in the Rodent Procedure Log.
- 4.2. Perform pre-operative procedures at a safe distance from the surgical environment in order to prevent contamination with hair.
- 4.3. Pre-operative Care:
 - 4.3.1. Administer general analgesic according to Rodent Analgesia SOP.
 - 4.3.2. Anesthetize the animal according to Rodent Anesthesia SOP.
 - 4.3.3. Apply ophthalmic ointment in both eyes to prevent corneal desiccation. Reapply as needed.
 - 4.3.4. Administer from 0.2 to 0.5mL/10g body weight of isotonic fluids, subcutaneously.
 - 4.3.5. Remove hair over the surgical area with a clipper, depilatory cream or by plucking, allowing a perimeter of at least 1cm around surgical site. Remove loose hair with gauze.
 - 4.3.6. Wash the surgical site with 2% chlorhexidine solution or povidone-iodine solution. Be careful not to wet the animal.
 - 4.3.7. Bring animal into surgical area.

- 4.3.8. Preparation of the surgical site:
 - 4.3.8.1. Apply 70% alcohol with gauze or swabs in a circular motion, from the center of the surgical site to the exterior. Be careful not to wet a large area on the animal as the evaporation of alcohol will lead to heat loss.
 - 4.3.8.2. Apply 2% chlorhexidine solution or povidone-iodine solution with gauze or swabs in a circular motion, from the center of the surgical site to the exterior.
 - 4.3.8.3. Repeat steps 4.3.8.2 and 4.3.8.3 two more times.
 - 4.3.8.4. Alternatively, use a 2% chlorhexidine in 70% alcohol solution. Apply 3 times with gauze or swabs in a circular motion, from the center of the surgical site to the exterior. Be careful not to wet a large area on the animal as the evaporation of the solution will lead to heat loss.
- 4.3.9. Surgeon's preparation:
 - 4.3.9.1. Wash hands.
 - 4.3.9.2. Wear a surgical mask, bonnet, and clean gown.
 - 4.3.9.3. Use aseptic technique.
 - 4.3.9.4. Wear sterile or alcohol-asepticized gloves.
 - 4.3.9.5. The surgeon must avoid touching non-sterile surfaces.
- 4.3.10. Cover the animal with a sterile drape.
 - 4.3.10.1. For minor surgical interventions, the drape can be placed only when suturing the wound to prevent sutures from coming into contact with hair and skin around the surgical area.
 - 4.3.10.2. Surgical drapes must be sterile for the first animal, and may then be transferred to the following animal during serial surgeries. The top surface of the drape must never come in contact with non-aseptic areas, and must not be soiled.
 - 4.3.10.3. Glad® Press'n Seal® wrap can be used as a surgical drape to cover the animal. As it is transparent, it allows for easier monitoring of the animal.
- 4.4. Surgical Principles/Aseptic technique:
 - 4.4.1. Ensure that all the available materials are at hand.
 - 4.4.2. Begin surgery with clean and sterile surgical instruments, handle instruments aseptically.
 - 4.4.3. Designate a sterile area on the working surface for the sterile material (instruments, suture material, drapes, gauze, etc).
 - 4.4.4. Prior to surgery, verify depth of anesthesia by loss of animal's pedal withdrawal (toe pinch) reflex using smooth-tipped/non-toothed forceps.
 - 4.4.5. Use a scalpel blade or scissors to make the smallest possible incision.
 - 4.4.6. Avoid contact of tissues with fingers by using the tip of instruments.
 - 4.4.7. Disinfect the instruments between each animal by dipping them in a hot glass bead sterilizer for approximately 30 seconds after removing any blood and debris (let cool completely) or in liquid sterilizing solution (e.g., glutaraldehyde or equivalent) for a few minutes (>5 minutes) and then rinsed with 70% alcohol. For liquid sterilization, it is recommended to use two alternating surgical kits in order to increase contact time with the solution.
 - 4.4.8. Infiltrate the wound with a local anesthetic, e.g., mixture of lidocaine and bupivacaine, prior to closing the skin. Refer to Rodent Analgesia SOP.
 - 4.4.9. Close the different tissue layers separately, such as peritoneum/abdominal muscles layer together then subcutaneous tissue and finally skin. For some surgeries, subcutaneous tissue may need to be sutured independently from the skin to prevent dead space.
 - 4.4.10. Keep suture material in 70% alcohol between each animal.
 - 4.4.11. Recommended suture and wound closure:

TISSUE	SUTURE MATERIAL	SIZE	NEEDLE
Peritoneum/abdominal	Vicryl PDS Polypropylene	3-0, 4-0, 5-0, 6-0	Taper
Subcutaneous tissues	Vicryl PDS	5-0, 6-0	Cutting Reverse-cutting
Skin*	PDS Vicryl Polyamide-nylon	3-0, 4-0, 5-0, 6-0	Cutting Reverse-cutting
	Wound clips (Autoclips)	7mm or 9mm	

* Silk suture should be avoided for skin closure as it may cause a local inflammatory tissue reaction and is associated with a higher incidence of wound infection.

4.5. Surgical Monitoring and Supportive Care:

- 4.5.1. Provide a contact heat source to prevent hypothermia.
- 4.5.2. Frequently monitor the presence of reflexes, the respiratory rate and breathing pattern, and when available, the heart rate.
- 4.5.3. Adjust the depth of anesthesia according to monitored parameters (presence of reflexes, respiratory rate and breathing pattern, heart rate).
- 4.5.4. In the case of respiratory arrest, stop anesthesia, administer oxygen and compress the thorax rapidly between thumb and index at a frequency of 80-120/min.

4.6. Post-operative Care:

- 4.6.1. Post-operative care begins immediately following surgery, lasts a minimum of 3 days, and extends for up to 10 days.
- 4.6.2. Post-operative animals should be identified with a Post-Procedure cage card.
- 4.6.3. Do not return animals that have not completely recovered to an animal housing room.
- 4.6.4. Observe the animal until it regains righting reflexes; do not leave recovering animal unattended. Observe respiration and coloration of the eyes (for albinos), mucous membranes, and skin.
- 4.6.5. Prevent heat loss and maintain the animal in contact with a heat source or inside a heated cabinet until it regains righting reflexes.
- 4.6.6. Administer oxygen if necessary.
- 4.6.7. For surgeries exceeding 60 minutes, or if there has been significant blood loss, administer an additional 0.2 to 0.5mL/10 g body weight of isotonic fluids, subcutaneously.
- 4.6.8. Monitor animals daily for at least the first 3 days following the surgery. Continue daily monitoring and contact veterinary care staff if recovery is prolonged beyond 3 days. Record supportive care provided on the Post-Procedure cage card.
 - 4.6.8.1. Repeat analgesics post-surgically according to Rodent Analgesia SOP 101.
 - 4.6.8.2. Provide moistened food at the bottom of the cage.
 - 4.6.8.3. Administer from 0.2 to 0.5mL/10g body weight of isotonic fluids, subcutaneously.
 - 4.6.8.4. Examine the wound daily for signs of inflammation or infection such as redness, swelling or purulent discharge.
 - 4.6.8.5. Ensure adequate wound closure, presence of sutures or wound clips.
 - 4.6.8.6. Measure body weight.
- 4.6.9. Remove skin sutures or wound clips after 7 to 10 days.

SOP REVISION HISTORY

DATE	NEW VERSION
2015.09.20	4.3.12.3 Glad® Press'n Seal® wrap can be used as a surgical drape to cover the animal. As it is transparent, it allows for easier monitoring of the animal.
2020.05.20	3.5. Electric clipper razor or depilatory cream
2020.05.20	3.9. Heating disc, warming pad or warm-water circulating pad. Do not use electric heating pads unless specifically designed for use with laboratory rodents.
2020.05.20	3.10. Sterile surgical drapes or Glad® Press'n Seal® wrap
2020.05.20	3.15. Rodent Procedure Log and Post-Procedure cage cards
2020.05.20	4.3.8. Preparation of the surgical site: 4.3.8.1. Apply 70% alcohol with gauze or swabs in a circular motion, from the center of the surgical site to the exterior. Be careful not to wet a large area on the animal as the evaporation of alcohol will lead to heat loss. 4.3.8.2. Apply 2% chlorhexidine solution or povidone-iodine solution with gauze or swabs in a circular motion, from the center of the surgical site to the exterior. 4.3.8.3. Repeat steps 4.3.8.2 and 4.3.8.3 twice two more times. 4.3.8.4. Alternatively, use a 2% chlorhexidine in 70% alcohol solution. Apply 3 times with gauze or swabs in a circular motion, from the center of the surgical site to the exterior. Be careful not to wet a large area on the animal as the evaporation of the solution will lead to heat loss.
2020.05.20	4.3.10. Cover the animal with a sterile drape for surgeries involving long incisions or prolonged surgical time, and, to prevent sutures from coming into contact with hair and skin around the surgical area. 4.3.10.1. For minor incisions surgical interventions , the drape can be placed only when suturing the wound to prevent sutures from coming into contact with hair and skin around the surgical area.
2020.05.20	4.4.4. Prior to surgery, verify depth of anesthesia by loss of animal's pedal withdrawal (toe pinch) reflex using smooth-tipped/non-toothed forceps.
2020.05.20	4.4.10. Keep Dip suture material in 70% alcohol between each animal.
2020.05.20	4.4.11. Recommended suture and wound closure: (Coverted to table) Tissue: Peritoneum/abdominal; Suture: Catgut , Vicryl, PDS, or Polypropylene; Size 3-0, 4-0 , 5-0, 6-0 ; Needle: Taper Tissue: Subcutaneous tissues; Suture: Catgut , Vicryl, PDS; size 5-0, 6-0; Needle: Cutting, Reverse-cutting Tissue: Skin; Suture: Polyamide-nylon, PDS, Vicryl; size: 3-0, 4-0 , 5-0, 6-0 , Wound clips (Autoclips); 7mm or 9mm; Needle: Cutting, Reverse-cutting * Silk suture should be avoided for skin closure as it may cause a local inflammatory tissue reaction and is associated with a higher incidence of wound infection.
2020.05.20	4.5.1. Surgeries exceeding 15 minutes for mice or 30 minutes for rats require Provide a contact heat source to prevent hypothermia.
2020.05.20	4.5.2. Frequently monitor the presence of reflexes, the respiratory rate and breathing pattern, and when available, the heart rate.
2020.05.20	4.6.1. Post-operative care begins immediately following surgery, lasts a minimum of 3 days , and extends for up to 10 days.
2020.05.20	4.6.3. Do not return animals that have not completely recovered to an animal housing room.
2020.05.20	4.6.4. Observe the animal until it regains righting reflexes; do not leave recovering animal unattended. Observe respiration and coloration of the eyes (for albinos), mucous membranes, and skin.
2020.05.20	4.6.5. Prevent heat loss and maintain the animal in contact with a heat source or inside a heated cabinet until it regains righting reflexes.
	4.6.7. For surgeries exceeding 60 minutes, or if there has been significant blood loss , administer again between an additional 0.2 and to 0.2 and to 0.5mL/10 g body weight of isotonic fluids, subcutaneously, immediately. Fluids can be administered on the following days, to maintain the preoperative body weight.
2020.05.20	4.6.8. Monitor animals daily for at least the first 3 days following the surgery. Continue daily monitoring and contact veterinary care staff if recovery is prolonged beyond 3 days. Record supportive care provided on the Post-Procedure cage card. 4.6.8.1. Repeat analgesics post-surgically and for the next few days (usually for 72 hours) , according to Rodent Analgesia SOP 101. 4.6.8.2. Moist food can be provided Provide moistened food at the bottom of the cage during the post-operative period. 4.6.8.3. Administer from 0.2 to 0.5mL/10g body weight of isotonic fluids, subcutaneously. 4.6.8.4. Examine the wound daily for signs of inflammation or infection such as redness, swelling or purulent discharge. 4.6.8.5. Ensure adequate wound closure, presence of sutures or wound clips. 4.6.8.6. For invasive surgeries, Measure body weight.
2020.05.20	4.6.9. Remove the skin sutures or staples wound clips after 7 to 10 days.
2020.11.17	3.7. Antiseptic solution for skin (e.g., chlorhexidine 2% solution or povidone-iodine solution used alternatively with 70% alcohol solution) 3.8. 70% Alcohol 3.9. Chlorhexidine 2% solution or povidone-iodine solution 3.10. 2% chlorhexidine in 70% alcohol solution
2021.01.13	In the case of respiratory arrest, stop anesthesia, administer oxygen and compress the thorax rapidly between thumb and index at a frequency of 80-120/min.

Investigator:	Protocol:
Procedure:	Performed by:

Instructions: complete this log for rodent procedures requiring anesthesia, analgesia or post-procedure care (ex. surgeries, experimental infection). Keep the log in the housing room while active and in your files for 3 years for future review by the Quality Assistant and/or the FACC.

ANALGESIA

- carprofen: mouse: 20mg/kg, rat: 5-10 mg/kg, SC, every 24 hrs
- buprenorphine: mouse: 0.1mg/kg SC or IP every 4-8 hrs;
rat: 0.05mg/kg, SC or IP, every 8-12 hrs
- buprenorphine slow release 1 mg/kg
- lidocaine/bupivacaine (local analgesic)

ANESTHESIA

- isoflurane 2-2.5%
- ketamine/xylazine/acepromazine*:
mouse: 100 mg/kg (K)- 10 mg/kg (X)- 3 mg/kg (A) IP
rat: 50 mg/kg (K)- 5 mg/kg (X)- 1 mg/kg (A); IP or IM
- other: _____

OTHER AGENTS ADMINISTERED

- _____
- _____
- _____

Animal ID	Date	Anesthesia		Analgesia		Other		Heat Source Provided		Recovery time	Comments/observations	Initials
		dose	time	dose	time	dose	time	procedure	recovery			
1								<input type="checkbox"/>	<input type="checkbox"/>			
2								<input type="checkbox"/>	<input type="checkbox"/>			
3								<input type="checkbox"/>	<input type="checkbox"/>			
4								<input type="checkbox"/>	<input type="checkbox"/>			
5								<input type="checkbox"/>	<input type="checkbox"/>			
6								<input type="checkbox"/>	<input type="checkbox"/>			
7								<input type="checkbox"/>	<input type="checkbox"/>			
8								<input type="checkbox"/>	<input type="checkbox"/>			
9								<input type="checkbox"/>	<input type="checkbox"/>			
10								<input type="checkbox"/>	<input type="checkbox"/>			
11								<input type="checkbox"/>	<input type="checkbox"/>			
12								<input type="checkbox"/>	<input type="checkbox"/>			
13								<input type="checkbox"/>	<input type="checkbox"/>			
14								<input type="checkbox"/>	<input type="checkbox"/>			

Comments/footnotes:

*Dose can vary with the sex, the age, the strain, and the body condition of the animal.

ANALGESIA

- carprofen: mouse: 20mg/kg, rat: 5-10 mg/kg, SC, every 24 hrs
- buprenorphine: mouse: 0.1mg/kg SC or IP every 4-8 hrs; rat: 0.05mg/kg, SC or IP, every 8-12 hrs
- buprenorphine slow release 1 mg/kg

Initial the appropriate boxes when completed

Animal ID	Date	Analgesia			SC fluids			Wet food			Time			Remove Sutures (Day 7-10)
		Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	
1														
2														
3														
4														
5														
6														
7														
8														
9														
10														
11														
12														
13														
14														
Comments/footnotes:														