Rodent Surgery

The intent of this Standard Operating Procedure (SOP) is to describe procedures for rodent surgery. This SOP is intended for use by qualified personnel on an IACUC approved protocol who will be performing surgery on rodents, or assisting in those procedures. This procedure is approved by the NUS Institutional Animal Care and Use Committee (IACUC). Any deviation must be approved by the IACUC prior to its implementation.

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1. INTRODUCTION

This SOP outlines the procedures performed when doing surgical procedures on rodents. For the purposes of this SOP, rodents are defined as rats, mice and guinea pigs.

Definitions

A. Survival Surgery
Any surgical procedure from which an animal regains consciousness for any period of time. **All survival surgeries are required to follow aseptic technique**.

i. Major Survival Surgery
Refers to any survival surgical procedure that
a) Penetrates and exposes a body cavity.
b) Requires the use of more than a single application of a short-term anaesthetic.
c) Produces substantial impairment of physical or physiologic functions.
Examples of major surgery include: laparotomy, castration, ovariectomy, thoracotomy, craniotomy, joint replacement, spinal transection, and limb amputation.

ii. Multiple Survival Surgeries
Multiple survival surgical procedures are not permitted on animals unless scientifically justified and approved by NUS Institutional Animal Care & Use Committee (IACUC).

B. Non-survival Surgery
Any surgical procedure from which an animal is euthanized before recovery from anaesthesia.

C. Aseptic Technique

Aseptic technique is used to reduce microbial contamination to the lowest possible practical level and includes:

i. Preparation of the animal, such as hair removal and disinfection of the operative site
ii. Preparation of the surgeon, such as the provision of clean surgical attire, surgical scrub, and sterile surgical gloves
iii. Sterilisation of instruments and supplies including implants
iv. The use of operative techniques to reduce the likelihood of infection

Aseptic technique is required for all survival surgery and for prolonged non-survival surgeries or for certain types of downstream *in vitro* work.

2. MATERIALS

A. Animal Support and Preparation
   i. Sterile isotonic solution for injection (e.g. 0.9% saline, lactated ringer's solution)
   ii. Needles and syringes
   iii. Analgesics
   iv. Anaesthetics (gaseous or injectable)
   v. Electric clipper or hydroxide-based hair removal cream e.g. Veet® or Nair® sensitive hair removal cream
   vi. Eye lubricant e.g. Duratears® eye ointment
   vii. Scrub solution (e.g. povidone iodine, chlorhexidine S)
   viii. 70% Alcohol
   ix. Supplemental heat source
      1. **Recommended external heat sources** includes e.g. water circulating warmer, far infrared (FIR) warming pad, selected air-activated heat packs like Hothands® hand warmer
      2. **Heat lamps and electric blanket warmers are not allowed due to uneven heating and the tendency to overheat and cause burns.**

      Refer to SOP #101 Anaesthetic Regimens for Mice, Rats and Guinea Pigs for more details.
   x. Physiological monitoring equipment e.g. rodent pulse oximeter
   xi. Contact CM veterinary staff for more information including borrowing of heating and monitoring equipment.

B. Surgical Supplies
   i. Sterile surgical instruments
   ii. Sterilisation equipment/ material (e.g., bead sterilizer, ethylene oxide, cold sterilants)
   iii. Sterile gauze, cotton swabs, cotton Q-tips, elastic wraps, drapes
   iv. Sterile gloves
   v. Suture material and/or skin staples
   vi. Sterile implants (when applicable)

NOTE: Sterile supplies in sealed pouches e.g. Viewpacks, are valid for use within 12 months from date of sterilisation, provided that they have been appropriately stored e.g. seal is intact, never gotten wet, kept in a closed cabinet.
Sterile supplies wrapped in a veterinary surgical pack i.e. drapes, are valid for use

within 6 months from date of sterilisation, with similar provisions as above.
All sterilised pouches or packs must indicate the expiry date.

3. PROCEDURES

A. Instrument Preparation
   i. Sterilise surgical instruments prior to the start of surgery e.g. autoclaved, gas sterilised with ethylene oxide.
   ii. When performing the same surgical procedure on multiple animals on the same day, more than one set of sterile instruments should be utilised.
   iii. Alternatively,
      1. A dry bead steriliser should be used:
         a. Remove gross dried blood and debris from instruments with a sterile saline-moistened gauze.
         b. Place the tips of the instruments in a dry heat bead sterilizer for approximately 10-15 seconds.
         c. Note that only the tips of instruments are considered sterile and care must be taken not to contaminate them. If required, they can be re-sterilised.
         d. Ensure that all instruments have completely cooled before using.
      2. Or, at minimum, cold sterilants should be used with sufficient contact time:
         a. Always follow the manufacturer's recommendations.
         b. As a guide:
            i. 2% glutaraldehyde e.g. Cidex®: soak instruments for 10 hours. The shelf life of the solution is 14 days after opening and activation.
            ii. 8% formaldehyde plus 70% alcohol: soak instruments for 18 hours.
            iii. Chlorine dioxide 1:5 solution e.g. Clidox®: soak instruments for 6 hours. This must be freshly prepared daily.
            iv. 1.37% sodium hypochlorite e.g. Alcide: soak instruments for 6 hours. The shelf life of the solution is 14 days.
         c. Thoroughly rinse instruments with sterile water or saline before use.

B. Surgery Area Preparation
   i. Use a dedicated area for rodent surgery.
      1. The space must be free of clutter and excessive equipment.
      2. CM facilities have procedure rooms equipped with BSCs that can be used.
   ii. The use of an investigator's laboratory for rodent survival surgery is acceptable if it is scientifically justified, and inspected, licensed and approved by IACUC and AVA.
   iii. Ensure that all required materials are ready and at hand prior to the commencement of surgery.
   iv. Clean and disinfect all surfaces in the surgical area.

v. Dedicate a sterile area (typically a sterile drape) on the surgical work surface for the sterile materials (instruments, suture material, drapes, gauze, etc.).
    1. Maintain aseptic conditions throughout the procedures.
    2. Once an instrument comes into contact with a non-sterile surface, it cannot be used again until it is sterilised.

C. Animal Preparation
i. Perform pre-operative evaluations to ensure that the animals are healthy, and disease-free.
    1. This includes a visual inspection, physical examination and assessment of the behavioural status of the animal.
    2. The animal should be alert and behave normally, have a smooth coat and clear eyes.
    3. Physical or behavioural abnormalities must be brought to the attention of the Comparative Medicine (CM) veterinary staff.
ii. Do not withhold food or water unless scientifically justified and approved by IACUC.
    1. When it is approved, the duration and time period e.g. fasting from 8 p.m. to 6 a.m., must be clearly indicated on the cages.
iii. **It is recommended to initiate analgesic treatment prior to surgery.**
iv. Antibiotics can be provided depending on the type of procedure performed.
    1. **However, the routine use of antibiotics should never be considered a replacement for proper aseptic surgical techniques.**
    Contact a CM veterinarian for more information.
v. Administer analgesics according to *SOP #104 Rodent Analgesia*, and antibiotics as approved in the protocol.
vi. Anaesthetise the animal according to *SOP #101 Anaesthetic Regimens for Mice, Rats and Guinea Pigs* or as approved in the protocol.
vii. Apply bland ophthalmic ointment to both eyes to prevent corneal drying. This can be reapplied whenever necessary.
viii. Administer 0.1 mL/10g body weight of isotonic fluids e.g. Lactated Ringer's or 0.9% saline, subcutaneously for surgeries exceeding 30 minutes.
ix. Remove hair from the surgical area e.g. with an electric clipper or hair removal cream, with a generous border (at least 1 cm) to avoid contaminating the incision site, and remove loose hair and debris from the animal. Care must be taken when using hair removal cream:
    1. Apply a consistent, thick layer of cream over the desired area.
    2. A dry cotton swab or edge of gauze can be used to test if the hair is easily removed every 30 seconds.
    3. In general, the duration of contact time is 2-3 minutes. Longer durations may cause skin irritation and burns.
    4. Wipe cream off completely with a saline or sterile water moistened gauze. Repeat until there is no residual cream left.
x. Place the animal in the surgical area.
xi. Perform three surgical scrubs:
    1. Scrub surgical site with a povidone iodine or chlorhexidine S soaked gauze/cotton swab/cotton Q-tip.
        a. Start at the centre of the surgical site and move towards the outside of the prepared area in a circular manner.
        b. Do not overlap areas that have been previously scrubbed

with the same piece of gauze/swab/Q-tip. Discard.

2. Use a 70% alcohol-soaked gauze/swab/Q-tip to rinse the area using in the same circular outward-moving pattern.
    a. Do not excessively wet the animal.
3. Repeat scrub and rinse process twice, for a total of three rounds.
    a. Use a new gauze/swab/Q-tip after each round.
4. If the animal is excessively dirty, repeat the surgical scrub until the prepared area is clean.

D. Surgeon and Surgery Assistant Preparation

i. Don a surgical mask and clean laboratory coat/gown, and any additional PPE as required for the area where surgery is performed.

ii. Put on sterile gloves using aseptic technique:
    1. Do not touch non-sterile surfaces.

iii. Discard and re-glove if the gloves come into contact with a non-sterile surface, Drape the animal with a sterile, impermeable covering to isolate the disinfected area.
    1. Draping is performed by the gloved and gowned surgeon, in order to prevent contamination of the surgical field.
    2. The choice of drapes must allow isolation of the surgical site while allowing sufficient monitoring of the anaesthetized patient. Tegaderm™ transparent film dressing, OpSite incise drape and other similar sterile transparent surgical drapes are recommended.
    3. If using cloth drapes, no fur must be visible through the window.

E. Non-survival Procedures

i. For non-survival procedures, asepsis is recommended. Aseptic techniques must be used depending on the type of downstream procedures to be conducted e.g. cell culture, and for long procedures.

ii. If non-aseptic technique is approved in the protocol by the IACUC,
    1. At a minimum:
        a. The surgical area must be clipped or free of fur.
        b. The surgeon must wear clean gloves.
        c. The instruments must be clean.
    2. The surgery area must be free of clutter and excessive equipment, and be clean.

F. General Principles

i. Anaesthesia and Monitoring
    1. Refer to *SOP #101 Anaesthetic Regimens for Mice, Rats and Guinea Pigs* for details on monitoring requirements and procedures, and body temperature maintenance.
    2. Verify the depth of anaesthesia prior to start of surgery e.g. the loss of withdrawal reflex in the hind paws. Re-check animal's anaesthetic depth every 10 - 15 minutes throughout surgery.
    3. If the animal makes any kind of movement in response to the incision or manipulation of organs, surgery must be temporarily stopped and anaesthesia supplemented.
    4. Surgery cannot continue until the animal is sufficiently anaesthetised.
    5. Never leave anaesthetised animals unattended.

6. In the case of respiratory arrest, stop surgery, turn off the gaseous anaesthesia or administer the reversal anaesthetic agent, and contact a CM veterinarian or veterinary technician immediately.

ii. Surgery Techniques

1. Efficient surgical planning will decrease surgical time, tissue contamination, and tissue damage.
2. Handle tissues gently.
3. Use a scalpel blade or scissors to make the smallest possible incisions.
4. 'I' shaped instead of 'T' shaped incisions are recommended.
5. Use the tip of instruments to handle tissues. Avoid handling tissues with fingers.
6. Do not contaminate suture materials on non-sterile surfaces.

iii. Tissue closure

1. Close tissue layers separately i.e. peritoneum/abdominal muscle layer, then subcutaneous tissue, then skin.
    a. The subcutaneous tissue and skin can be closed in one layer if there is minimal dead space.
2. Suture Materials: Refer to Table 1 and Table 2 for recommendations.
    a. Alternatively, skin staples can be placed. 7 mm staples for mice, 9 mm staples for rats and guinea pigs. Care must be taken to ensure that only the skin is stapled and no underlying tissue is caught.
    b. Training is required to ensure the proper placement of staples.
3. Tissue glue e.g. Vetbond™ can be used as secondary method of securing closure by applying over the entire length of the incision after sutures or staples are placed.
    a. Draw up in a 1 ml syringe and use a 23G needle to reduce excessive application.
    b. Only veterinary/ medical grade tissue glue made of Butyl-2-cyanoacrylate or 2-Octyl cyanoacrylate (e.g. VetBond™, Vet Glu, Dermabond®, Surgiseal, Histoacryl®) can be used. Check with CM Pharmacy for this supply.
    c. **The use of glue made of Ethyl-2-cyanoacrylate (such as KrazyGlue, or SuperGlue) is not allowed**.
4. Sutures or staples must be removed after 10 - 14 days.

Table 1. Recommended Suture Materials

Suture	Characteristics and Frequent Uses
Vicryl®, Dexon®	Absorbable; 60-90 days. Suitable for internal wound closure. Recommended for skin closure if buried.
PDS®, Maxon®	Absorbable; 6 months. Suitable for internal wound closure where extended wound support is desirable.
Prolene®	Non-absorbable. Suitable for skin closure.
Nylon	Non-absorbable. Suitable for skin closure.
Stainless Steel Wound Clips, Staples	Non-absorbable. Suitable for skin closure. Recommended for skin closure if exposed. Requires instrument for removal from skin.

Table 2. Recommended Suture Gauges /Sizes

Species	Location or Function
Mouse	Abdominal or peritoneal area: 4-0 to 5-0 Skin (subcuticular): 5-0 to 6-0 Skin (external): 4-0 to 6-0
Rat	Abdominal area: 4-0 to 5-0 Skin (subcuticular): 4-0 to 6-0 Skin (external): 3-0 to 5-0
Guinea Pig	Abdominal area: 4-0 to 5-0 Skin (subcuticular): 4-0 to 6-0 Skin (external): 3-0 to 5-0

G. Post-operative Care

i. Post-operative care begins immediately following surgery and extends up to 14 days or until the incision has healed.

ii. Place the animal in a clean cage; provide a quiet environment for recovery. If recovering the animal in a cage, place the animal on a clean paper towel in order to prevent aspiration of bedding material.

iii. Do not place anaesthetised animals in a cage with conscious animals.

iv. Until the animal regains righting reflexes, observe and monitor respiratory rate, mucous membrane colour, and skin tent for hydration status.

v. Keep the animal warm and dry in order to prevent hypothermia. Supplemental heating is to be provided. It is best to provide heating to only half of the cage, where the animal is recovering. This allows the animal to move off the heating elements when it awakes and is too warm.

vi. See MATERIALS section on types of acceptable and unacceptable supplemental heating sources.

vii. Provide analgesia and antibiotics according to the approved protocol and fill in the post-operative cards (Appendix A). For any deviations or extensions, please consult a CM veterinarian.

viii. Examine the wound/incision daily for at least 5 days.

ix. For any complications or emergencies contact the CM Veterinary staff.

H. Record Keeping

i. Record keeping is an essential component of all surgical procedures according to the National Advisory Committee for Laboratory Animal Research (NACLAR) guidelines and the Guide for the Care and Use of Laboratory Animals.

ii. Documentation of animal well-being, anaesthesia, and surgical care on the **Rodent Surgical Record** (Appendix B) is required for all survival and terminal surgical procedures. The form can be downloaded from CM website: https://cm.nus.edu.sg/corp/attachments/NUS_CM_Rodent_Surgical_Record.docx

iii. Investigative personnel using their own rodent surgical record are to ensure that the parameters listed in the Rodent Surgical Record Checklist (Appendix B) have been included in their record. The form can be downloaded from CM website: https://cm.nus.edu.sg/corp/attachments/Rodent_Surgical_Record_Checklist.docx

iv. All procedures must also be recorded on the cage cards and dated. Post-operative cards must be kept with the cage at least for the duration of post-operative care.

v. All the records must be maintained in the user's laboratory and available for review upon request for at least 3 years from the completion of the protocol.

4. REFERENCES

- SOP #104 Rodent Analgesia
- SOP #101 Anaesthetic Regimens for Mice, Rats and Guinea Pigs
- SOP #612 Attending Veterinary Clinical Cases and Follow-up
- Cornell University http://www.research.cornell.edu/care/documents/ACUPs/ACUP201.pdf
- Boston University http://www.bu.edu/orccommittees/iacuc/policies-and-guidelines/rodent-surgery-guidelines/
- NIH OACU http://oacu.od.nih.gov/ARAC/documents/Rodent_Surgery.pdf
- National Academy of Sciences, 2011; The Guide for the Care and Use of Animals, Eight Edition.
- NUS IACUC http://www.nus.edu.sg/iacuc/policies%20and%20guidelines/Guidelines%20for%20Rodent%20and%20Bird%20survival%20surgery
- Marcel I. Perret-Gentil, Principles of Veterinary Suturing. The University of Texas at San Antonio https://research.utsa.edu/_files/pdfs/compliance-integrity-pdf-folder/larc-documents/Principles-of-Rodent-Aseptic-Surgery-Perioperative-Care.pdf
- Johns Hopkins University http://web.jhu.edu/animalcare/procedures/survival-rodents.html
- Bhumisirikul W, Bhumisirikul P, Pongchairerks P. Long-term storage of small surgical instruments in autoclaved packages. Asian J Surg. 2003. Oct;26(4):202-4.
- Butt WE, Bradley DV Jr, Mayhew RB, Schwartz RS. Evaluation of the shelf life of sterile instrument packs. Oral Surg Oral Med Oral Pathol. 1991. Dec;72(6):650-4.
- CDC Guideline for Disinfection and Sterilization in Healthcare Facilities. 2008. https://www.cdc.gov/infectioncontrol/guidelines/disinfection/sterilization/sterilizing-practices.html

- Guidelines on the Care and Use of Animals for Scientific Purposes. National Advisory Committee for Laboratory Animal Research (NACLAR). Second Edition. 2022.

Revision #	Author	IACUC Approval/Effective Date	SOP #:
.01	Anna Acuna	17 September 2012	201.01
.02	Yasmina Paramastri	26 September 2016	201.02
Revision .02: Removed alcohol as sterilant for surgical instrument			
.03	Jassia Pang	19 March 2018	201.03
Revision .03: Addition of hair removal and monitoring options, drape, surgical instrument preparation, surgery area preparation, fasting, non-survival surgery requirements, external heating, and skin closure options. Alignment with other revised SOPs.			
.04	Jassia Pang	21 May 2018	201.04
Revision .04: Validity of sterile supplies included, references added.			
.05	Jassia Pang	18 January 2021	201.05
Revision .05: Updated validity of sterile supplies depending on packing, to include expiry dates on all sterile supplies.			
.06	Grace Lim/Jasmin Wu	28 February 2025	201.06
Revision .06: Updated record keeping for all surgical procedure, added Rodent Surgical Record and checklist in Appendix B, updated the references.			
.07	Grace Lim/Jasmin Wu	16 March 2026	201.07
Revision .07: Updated Section (H) Record keeping specifying that Rodent Surgical Record and Checklist is required for all survival and terminal surgical procedures. The updated Rodent Surgical Record and Checklist is provided in Appendix B.			

5. APPENDIX

Appendix A- Post-operative Card

Date:
Procedure:
Analgesic(s)/dose:
Antibiotic/dose:

Date	Time	√ as appropriate	Initial
		☐ Analgesic ☐ Antibiotic	
		☐ Analgesic ☐ Antibiotic	
		☐ Analgesic ☐ Antibiotic	
		☐ Analgesic ☐ Antibiotic	
		☐ Analgesic ☐ Antibiotic	
		☐ Analgesic ☐ Antibiotic	
		☐ Analgesic ☐ Antibiotic	
		☐ Analgesic ☐ Antibiotic	
		☐ Analgesic ☐ Antibiotic	
		☐ Analgesic ☐ Antibiotic	

Suture removal date:
Comments:

These cards are to be placed by the research staff to notify CM that animals have had a procedure (e.g. anaesthetic episode, surgery). The analgesic or antibiotic should include the drug name (e.g. buprenorphine, carprofen, ketoprofen, etc.; or Baytril, etc.), dose expressed as mg/kg (w/w), and frequency of administration E.g. once a day (SID) or twice per day (BID). Tick (√) as appropriate and initial in the space provided after each treatment.

Appendix B – Rodent Surgical Record and Checklist

The Rodent Surgical Record and Rodent Surgical Record Checklist are available for download on the CM website

Rodent Surgical Record for Survival and Terminal Surgical Procedures

Investigative personnel are responsible for maintaining a record of pre-operative anesthetic and analgesic use.
Records must be kept in the user's laboratory and produced upon request.
Ensure that all medications and procedures listed on this sheet are as stated in the approved IACUC protocol.

Housing Facility and Room:	**Date of Procedure:**
Principal Investigator:	**IACUC Protocol #:**
Surgeon:	**Procedure:**
Species:	

			Pre-Operative Evaluation^		
No.	**Rack/Cage Location:**	**Animal ID**	**Body Wt (g)**	**Condition⁺**	**Anesthetic and Surgical Complications^^**
#1					
#2					
#3					
#4					
#5					

^ To be completed pre-surgery
^^ To be completed post-surgery

⁺Scores below are used to evaluate pre-operative condition of the rodent.
1 = Animal is bright, alert, responsive & active
2 = Animal is quiet, alert, responsive & less active
3 = Animal is lethargic & less responsive*
*Contact Veterinary Staff if Score 3 is chosen for any animal

Surgical Drugs Administered*	**Drug Type** (Check Applicable Box)					**Time Administered** Enter time under the appropriate header corresponding to the **Animal ID #** above or under **"All"** if applicable					
Drug Name	**Anesthetic**	**Pre-emptive Analgesic**	**Other:** (e.g. SQ fluid)	**Dose (mg/kg)**	**Route**	**#1**	**#2**	**#3**	**#4**	**#5**	**All**
	☐	☐	☐								
	☐	☐	☐								
	☐	☐	☐								
	☐	☐	☐								
	☐	☐	☐								

		Pre-Operative Check List (Check Applicable Box)		**Peri-Operative Checklist** (Check Applicable Box if the Parameter was Monitored During Surgery)			
No.	**Animal ID**	**Ophthalmic Ointment**	**Heat Source** Type:	**Respiration Movement/ Depth/ Rate**	**Mucous Membrane Color**	**Pinch Response**	**Others:**
#1		☐	☐	☐	☐	☐	☐
#2		☐	☐	☐	☐	☐	☐
#3		☐	☐	☐	☐	☐	☐
#4		☐	☐	☐	☐	☐	☐
#5		☐	☐	☐	☐	☐	☐

Monitor respiratory rate/depth, mucous membrane color and depth of anesthesia at least every 15 minutes during anesthesia.
Please ensure all drugs, supportive care, and monitoring is in accordance with the approved protocol and CM SOPs.
Please ensure all post-operative treatments have been documented on the post-operative cards.

Rodent Surgical Record Checklist for Survival and Terminal Surgical Procedures

Investigative personnel using their own rodent surgical record are to ensure that the parameters listed in the rodent surgical record checklist have been included in their record.

- Investigative personnel are responsible for maintaining a record of pre-operative anesthetic and analgesic use.
- Records must be kept in the user's laboratory and produced upon request.
- Ensure that all medications and procedures listed on this sheet are as stated in the approved IACUC protocol.

1. Pre-Surgical Evaluation

No.	For each animal, please record the following,	Check Boxes
1	IACUC Protocol No.	☐
2	Date of Procedure	☐
3	Name of Procedure	☐
4	Name of Surgeon	☐
5	Species	☐
6	Housing facility, Rack, Cage Location & Animal ID	☐
7	Body Weight	☐
8	Condition^	☐

^Scores below are used to evaluate pre-operative condition of the rodent.
1 = Animal is bright, alert, responsive & active
2 = Animal is quiet, alert, responsive & less active
3 = Animal is lethargic & less responsive*
*Contact Veterinary Staff if Score 3 is chosen for any animal

2. Drugs Administered

No.	For each animal, please record the following,	Check Boxes
1	Anaesthetic Agent Administered	☐
2	Pre-emptive Analgesia	☐
3	Any other Drugs/Chemicals	☐

For all drugs administered, please ensure that the time, dose & route of admin has been recorded.

3. Pre Operative Check List

No.	For each animal, please record the following,	Check Boxes
1	Ophthalmic Ointment	☐
2	Heat Source	☐

4. Peri and Post Operative Check List

No.	For each animal, please record the following,	Check Boxes
1	Respiratory Movement/Depth/Rate	☐
2	Mucous Membrane Colour	☐
3	Pinch/Pedal Reflex	☐
4	Anaesthetic & Surgical Complications	☐
5	Others:	☐

Monitor respiratory rate/depth, mucous membrane color and depth of anesthesia at least every 15 minutes during anesthesia.