LAB_099 Mammary Fat Pad Resection Surgery in Mice (Expires February 2026)

I. OBJECTIVE

To describe a standard methodology, and standard set of consideration, when performing surgical mastectomy in mice following tumour implantation into the associated mammary fat pad.

II. II. DEFINITIONS

Competent: "the consistent application of knowledge and skill to the standard of performance required regarding the care and use of animals. It embodies the ability to transfer and apply knowledge and skill to new situations and environments."[1]

Mammary fat pad: a general anatomical term used to describe the soft tissue (fat, glandular tissue, connective tissue) associated with a mammary gland

Mammary gland: an anatomical term indicating the exocrine glandular tissue within a mammary fat pad

III. COMMENTS / RECOMMENDATIONS

- Relative to animal ethics applications, when using this SOP, the following must be described in the individual ethics application: any experimental compounds or medications administered (including analgesia protocol), tumours size expectations at the time of mastectomy, any complications that may be expected to occur with the model, and any intended variation to this SOP.
- Analgesia protocol, implemented for this procedure, should be consistent with classification "2": "Moderate" pain, as per Guideline – Rodent Analgesia (Procedure Specific).
- Monitoring records, which includes surgical and anaesthesia records, must be maintained (example templates can be obtained by contacting the UQBR Veterinarians or Animal Ethics Unit Veterinary Officer).
- Hygienic practices must be applied when performing surgery, wherever practicable, aseptic surgical technique must be performed, LAB_001 Aseptic Technique for Laboratory Animal Surgery. Where this is not possible, clean surgical technique must be practiced, LAB_002 Clean Technique for Laboratory Animal Surgery.
- In the event of equipment failure, or anaesthetic recovery mid-surgery, "alleviating unanticipated pain and distress must take precedence over an individual animal reaching the planned endpoint of the project, or the continuation or completion of the project. If necessary, animals must be humanely killed without delay"[2]
- It can be difficult to achieve successful wound closure and healing in rodents undergoing mastectomy if the tumour is quite large, has infiltrated the dermis, or is in an area that is difficult to resect. If difficulty is being experienced, contact a UQBR Veterinarian, and consider the following:
    - Tumour size: large tumours tend to infiltrate other tissues and require more radical surgery for removal. Difficulty resecting large tumours can be helped by performing the mastectomy at an earlier experimental timepoint, effectively reducing the tumour size.
    - Accurate tumour placement: Tumours that infiltrate proximal soft tissues, particularly fascia and dermis, can be difficult to accurately appose the surgical margins. Performing "open", as compared to "closed" tumour inoculation can help to improve accuracy of the tumour placement, and subsequently help surgical resection. Refer to LAB_096 Injections - Mammary Fat Pad for both techniques.

[1] Australian code for the care and use of animals for scientific purposes, 8th Edn., 2013, National Health and Medical Research Council (NHMRC).
[2] Clause 2.4.18, Australian code for the care and use of animals for scientific purposes, 8th Edn., 2013, NHMRC.

  - Tumour location: The 3rd and 4th mammary fat pads are commonly used due to their relatively large size and their positioning relative to other soft tissue structures. The other fat pads are often too small and too awkwardly located within the very "mobile" areas[3] of the forelimbs (1st and 2nd fat pads), or the hindlimbs (5th fat pad) (Katsuta et al., 2016).
- Personal Protective Equipment (PPE) is facility and procedure dependent (e.g., handling potential zoonoses or carcinogens). Generally, PPE should include at least disposable gloves, long sleeved lab gown, face mask, safety glasses, hair bonnet, closed in shoes.

IV. EQUIPMENT

- PPE, as required
- Disinfectants: surface disinfectant (e.g. 70% ethanol) and skin disinfectants (e.g. chlorhexidine based). Refer to LAB_001 Aseptic Technique for Laboratory Animal Surgery and LAB_002 Clean Technique for Laboratory Animal Surgery for options.
- Clean recovery boxes – standard housing boxes including feed, water, appropriate nesting materials (to aid thermal support) and environmental enrichment.
- Active heating equipment (e.g. fit-for-purpose heat mats, Aria Ventilated Cabinets®)
- Anaesthetic agents – as per AEC approved protocol
- Analgesic agents – as per AEC approved protocol
- Ophthalmic lubricant (non-medicated, viscous and pH neutral: e.g. Refresh "Lacri-lube"©, Visco-tears© gel)
- Electric clippers or depilatory cream (e.g. Nair hair removal cream©)
- Gentle tape (e.g. silicone tape, or Micropore™)
- Sterile surgical instruments
  - Including: scalpel, fine surgical scissors, wound clip applicator (if using wound clips)
- Sterile surgical consumables
  - Including: gauze, cotton tips, absorbable monofilament suture (size: 4-0, 5-0 or 6-0), warmed normal (0.9%) saline (sterile), 7mm or 9mm wound clips, and or tissue glue (tissue glue should only be used as a supplement, not a primary means of closure).

V. PREPARATION

1. Prepare yourself and the work station as per LAB_001 Aseptic Technique for Laboratory Animal Surgery / LAB_002 Clean Technique for Laboratory Animal Surgery, including a heat mat at the surgical site.
2. Prepare clean, warm recovery boxes (e.g. resting on a heat mat).
3. Plan each surgery. Dependent on various factors, including tumour size and location, mastectomy can be difficult to achieve successful wound closure and healing. A surgical plan needs to be considered prior to commencing surgery with each animal. This includes consideration of the incision site and size, surgical approach, intraoperative procedures, closure technique and potential complications. The following points should be considered as guiding principles:
   - keep the surgical incision as small as possible,
   - the incision should be made in the least invasive location possible (usually this is parasagittal, and medial to the tumour), with consideration of the skin's natural plains of tension,

[3] Soft tissue structures of the axilla or "armpit" of the forelimb, and the "flank" of the hindlimbs are very mobile. In rodents, because the limbs cannot effectively be immobilised post operative, surgery in these areas can be difficult to manage to ensure appropriate wound healing. This is especially true for large surgical wounds (e.g. large tumour removal) at these sites.

- when removing the tumour avoid resecting skin, wherever possible,
- larger surgical wounds (>15mm), and wounds where it is difficult to accurately appose the surgical margins, should be closed using skin sutures, in preference to wound clips (as they are generally more secure and provide better tissue apposition for irregular wounds)
- for this procedure, tissue glue should not be used as the primary method of surgical closure (as the surgical wounds are often too large and mobile); tissue glue may be used to supplement closure,
- if a large tumour has been removed and there is "dead space", the subcutaneous space should be closed using sutures (to obliterate the dead space), in addition to closure of the skin

VI. PROCEDURE

1. Collect the mouse from its home cage and induce anaesthesia, as per AEC approved protocol.
2. Apply ophthalmic lubricant to both eyes, using a sterile cotton tip.
3. Prepare the animal for surgery, including removal of fur and skin prep, as per LAB_001 Aseptic Technique for Laboratory Animal Surgery / LAB_002 Clean Technique for Laboratory Animal Surgery.
1. Using gentle tape, position the limbs (and body) in such a way optimises access to the surgical site. Generally, this will mean the mouse is positioned between dorsal and lateral recumbence (i.e. approx. 45° angel) with the tumour bearing site facing up.

   Tape should only be used with very gentle traction on the limbs. Excessive tension on limbs will cause neuromuscular and vascular injury, as well as restrictions to respiration.
2. Check the animal is at an appropriate anaesthetic depth (e.g. check for the absence of a withdrawal reflex as per LAB_060 Rodent Anaesthesia – Isoflurane or LAB_025 Rodent Anaesthesia - Injectable Agents. If reflexes are present, the animal is not sufficiently anaesthetised and anaesthetic depth needs to be increased prior to proceeding.

   If movement of skeletal muscle, or withdrawal reflexes are present at any point throughout the procedure, activity must stop and only resume once sufficient anaesthetic depth is regained. If you are having difficulty maintaining appropriate anaesthetic depth consult a UQBR veterinarian (once the animal has recovered, and before proceeding to anesthetise any more animals).
3. Perform a skin incision adjacent to the tumour which enables subcutaneous access to the tumour. The incision is made with one hand, while the other hand manipulates the skin, applying gentle traction, to assist precision. The incision should be:
   - made using a small, fine scalpel blade (e.g. #10 or 11) or fine surgical scissors,
   - linear and full thickness,
   - as small as possible (generally, this is less than ≤1cm),
   - located para-sagittal, and medial to the tumour (to minimise complications from subsequent limb movement),
   - made in a relatively cranio-caudal direction (to minimise complications from the skin's natural plains of tension).

   See figure 1, which identifies potential incision site locations for access to the 3rd and 4th mammary fat pads.
4. Using the sterile surgical instruments perform a combination of sharp and blunt dissection to expose and isolate the tumour for resection. Usually, the tumour will need to be exteriorised through the incision site.

5. Once the tumour is isolated, use the fine surgical scissors to excise the tumour (i.e. sharp dissection). Observe the site for haemorrhage and ensure haemostasis before proceeding to close.
   - Initially any blood should be mopped up from the site with a sterile cotton tip;
   - Any areas of bleeding should undergo gentle pressure with a sterile cotton tip for ~60 seconds;
   - Vessels that continue to bleed require further intervention: clamping (surgical clamping with fine surgical forceps), ligation, cauterisation, or gel foam sponge implants (a commercial porcine skin gelatine product).
6. If there is significant dead space within the surgical site, consideration must be made for the placement of subcutaneous sutures to obliterate the dead space. The appropriate pattern and number of sutures will vary dependent upon the wound's configuration. If there is uncertainty in assessing a wounds' suitability for subcutaneous sutures, then advise should be sought from a UQBR veterinarian.
7. Skin closure is then performed using either skin sutures or wound clips (with tissue glue only as an option to supplement these).
8. The surgical site is then gently cleaned with gauze, or cotton tips, moistened with aqueous skin disinfectant (not alcohol), ensuring that any blood contamination is removed from around the surgical site.
9. Place the animal into a recovery box maintained on a heat mat and continue monitoring until recovered from anaesthesia*. Once recovered, the mouse may be returned to its cage mates, within a clean home cage.

   ** a mouse has "recovered" from anaesthesia when it has regained its physiological reflexes, is normally responsive to external stimuli, and is able to ambulate, eat, and drink and toilet normally.*
10. Clean and disinfect all equipment before proceeding to the next animal.
11. At the end of the procedure home cages/recovery boxes containing post operative animals may be placed into a climate controlled, Ventilated Cabinets® for ~12 hours recovery.
12. Post operatively mice should be reassessed within 6 hours post recovery, then at least daily for the following 2 days. Ongoing monitoring is as per the approved AEC protocol.
13. Remove skin sutures or surgical wound clips between 10-14 days post-operatively.

VII. REFERENCE MATERIAL

Table 1. Murine mammary fat pad number and associated anatomical name.

Mammary fat pad	Associated mammary gland
1st	Cervical mammary gland
2nd	Cranial thoracic mammary gland
3rd	Caudal thoracic mammary gland
4th	Abdominal mammary gland
5th	Inguinal mammary gland

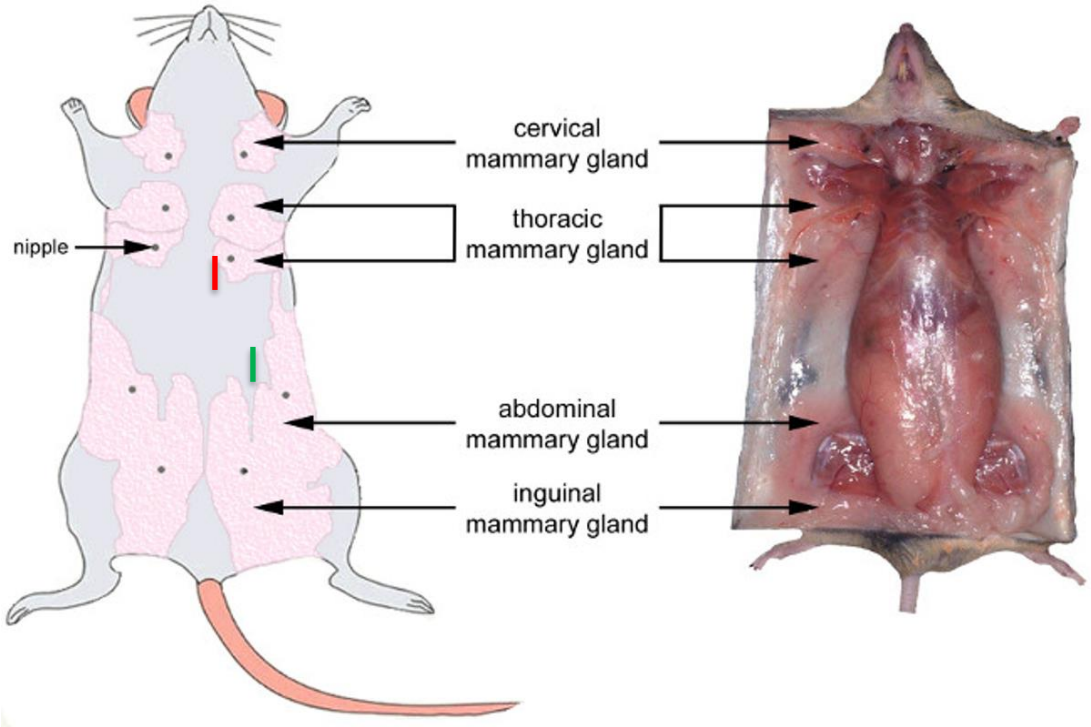

Figure 1. Murine mammary glands and associated potential locations for skin incisions (to effect mastectomy). LEFT: ventral view schematic of normal anatomy, with potential incision sites for access to the 3rd mammary gland indicated by a **RED** line (parasagittal and caudo-medial to the 3rd mammary fat pad) and the 4th mammary gland indicated by a **GREEN** line (parasagittal and cranio-medial to the 4th mammary fat pad); RIGHT: ventral view photograph with skin reflected to demonstrate the subcutaneous locations of the mammary fat pads. Image adapted from: Honvo-Houéto, E., & Truchet, S., 2015.

VIII. BIBLIOGRAPHY

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Version #	**Reviewing AEC** (note: all other relevant AECs ratify the approval)	AEC Review Date	Approved Until
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