LAB_098 Subcutaneous (tumour) Implant Surgery (Expiry: February 2026)

I. OBJECTIVE

To describe a standard methodology, and set of consideration, when performing surgical implantation of tumour cells into the subcutaneous space over the parasagittal/lateral dorsal pelvic area (i.e. the "dorsal flank") in rodents.

Note: this procedure has distinct differences to LAB_097 Subcutaneous Implant Surgery, and LAB_096 Mammary Fat Pad Injections.

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]

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, and their expected impacts or symptoms that may be observed in the animals (for tumours this includes consideration of the cell type, how rapidly they will divide and grow, the risk of local inflammation, or metastasis), any complications that may be expected to occur, and any intended variation to this SOP.
- Subcutaneous (SC) injection of tumour cells may be a viable alternative to performing this procedure. If SC injection is a viable option for the model, it should always be performed in preference to surgical implantation (as described in this document).
- Analgesia protocol, implemented for this procedure, should be consistent with classification "1": "Mild" 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 Research Ethics and Integrity 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]
- Tumours should only be implanted in locations that are likely to result in optimal wound healing and as little discomfort for the rodent as possible (when compared to other potential anatomical locations). The parasagittal/ lateral dorsal pelvic area (i.e. the "dorsal flank") is considered standard, and is described in this procedure. Alternatively, implantation off-centre along the back line (i.e. latero-dorsal lumbar and caudal-thoracic areas) are also appropriate locations for implantation.

[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.

  - Highly mobile soft tissue structures, such as that of the axilla of the forelimb, and the "flank" of the hindlimbs, should be avoided as they are more likely to result in delayed wound healing, wound dehiscence and discomfort for the animal (post implantation).
  - Dorsal mid-line should be avoided, because as the tumour grows it may cause complications for the handler when trying to restrain the rodent; they may not be able to appropriately scruff the animal.
  - Any sites involving special senses should be avoided.
- Standard humane endpoints relative to solid tumours have been established at UQ and are communicated via score sheets on the animal ethics webpage. Specifically see, *Tumours - score sheet for mice with tumours*.
- 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
- Tumour cells for implantation (<250mm^3)

V. PREPARATION

1. Ensure the tumour cells are ready for implantation, including any required vehicles/ diluents.
2. 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.
3. Prepare clean, warm recovery boxes (e.g. resting on a heat mat).
4. Ensure you are well familiar with your surgical plan, including incision site and size, surgical approach, intraoperative procedures, closure technique and management options for any potential complications.

   It can help to mentally run through each of the steps before commencing surgery.

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.
4. Position the rodent in lateral recumbence with the "surgery side" facing up. Use gentle tape, as required, to position limbs in such a way that optimises access to the surgical site (i.e. the "dorsal flank").

   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.

1. 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).

2. Perform a skin incision in the parasagittal/ lateral dorsal pelvic area (i.e. the "dorsal flank"). 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 (~5mm),
   - made parallel to the skin's natural plains of tension.
3. Using the sterile surgical instruments perform blunt dissection to create a small subcutaneous pocket (<1cm deep).

   With a small subcutaneous pocket (e.g. <1cm) there should be no need to place subcutaneous sutures (to obliterate the associated dead space)

4. Observe the site for haemorrhage and ensure haemostasis before proceeding.
   - Initially any blood should be mopped up from the site with a sterile cotton tip;
   - Local 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).
5. Implant the tumour within the subcutaneous pocket.
6. Close the skin layer using either skin sutures, wound clips, or tissue glue.
7. Gently cleaned the surgical site with gauze, or cotton tips, moistened with aqueous skin disinfectant or saline (not alcohol), to remove any blood contamination from the skin around the surgical wound.

   Figure 1 displays an image of a nude mouse, recovering from surgery that has had subcutaneous tumour implantation. The wound is clean, intact, and dry.

8. Place the animal into a recovery box maintained on a heat mat and continue monitoring until recovered from anaesthesia*.

** 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.*

9. Once recovered, the mouse may be returned to its cage mates, within a clean home cage.
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 4-6 hours after their surgery, then at least daily for the following 2 days. Ongoing monitoring is as per the approved AEC protocol.
13. If skin sutures or surgical wound clips were used, these must be removed between 10-14 days post-operative.

VII. REFERENCE MATERIAL

Figure 1. A photograph depicting a nude mouse immediately post operative (following subcutaneous implantation of tumour cells into the right "dorsal flank"). Skin sutures have been used to close the surgical wound. Please note: ear tag-identifiers (as displayed in this image) are generally not considered appropriate for use in mice at UQ, as there are other, more refined, methods of individual identification available. Image sourced from: Schmidt, *et al.* (2016).

Figure 2. A photograph depicting how a nude mouse may be expected to appear several weeks post-surgical subcutaneous tumour implantation into the left "dorsal flank"/lumbar region. The area of tumour development is outlined by a black dashed line. Image sourced from: Wang, *et al.* (2018).

VIII. BIBLIOGRAPHY

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Version #	**Reviewing AEC** (note: all other relevant AECs ratify the approval)	**AEC Review Date**	**Approved Until**
1		February 2023	

Conditions:

- Investigators named in an animal ethics application, relative to this SOP, must be competent to implement the SOP
- Any variation to this SOP must be described in the relevant animal ethics application
- If this SOP has not been reviewed and approved by a UQ AEC within the last three years it is no longer valid and cannot be used in animal ethics applications until reapproved (see "AEC Reviewed/Approved" date in this document's header).