LAB_096 Injections - Mammary Fat Pad

I. OBJECTIVE

To perform safe and humane experimental injection of compounds into the mammary fat pad of rodents.

II. COMMENTS / RECOMMENDATIONS

- Users must keep monitoring records, which includes surgical records (example templates can be obtained by contacting the UQBR Veterinarians or Animal Ethics Unit Veterinary Officer).
- Any associated experimental compounds or medications (including your anaesthetic protocol) must be detailed within the Animal Ethics Committee (AEC) application.
- PPE is facility dependent, however, this should at least include disposable gloves, long sleeved lab gown, face mask, safety glasses, hair bonnet, closed in shoes.
- Wherever possible, active heating (e.g. a heat mat) must be used at all times.
- Clean surgical technique must be practiced, as per LAB_002 Clean Technique for Laboratory Animal Surgery
- Wherever practicable, aseptic surgical technique must be practiced, as per LAB_001 Aseptic 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" (Clause 2.4.18, Australian code for the care and use of animals for scientific purposes 8th Edn., 2013)
- The time from induction of anaesthesia to the completion of surgery **should not exceed 10 minutes**
- The closed or open method of performing this procedure should be chosen prior to commencing
- Generally mature females are used in this technique to increase accuracy of injection due to improved ability to identify the fat pad

III. EQUIPMENT

- 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 sterile 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
- Experimental compounds for injection – 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©)
- Sterile surgical instruments
  - Including: scalpel, fine surgical scissors, curved forceps
- Sterile surgical consumable
  - Including: gauze, cotton tips and surgical glue

IV. PROCEDURE

Preparation of Injection Substance

- Confirm the concentration and volume with the approved AEC protocol
  A maximum volume of 0.03ml (30uL) per site is recommended for mammary fat pad injections. Any volume larger than this should be clearly cited and justified in the AEC application as larger volumes have a greater risk of mis-injection and/or leakage from the site.

  Consider temperature, pH, injection of cells, hazardous substances (cytotoxic, radioactive, infectious), and highly viscous liquids to improve success of the procedure. These considerations can impact safety and animal welfare, refer to Reference Information below for information about these variables.

- It is the responsibility of the researcher to convey all risks associated with compounds and materials to be used. This may include lab specific risk assessments and SDS and other OHS obligations.
  If substances to be used are experimental or off label (i.e. no Safety Data Sheet is available), the laboratory is responsible for conveying all of the risks to workers involved in the project. This includes risk of performing the procedure as well as the risks associated with animal husbandry such as waste management of cage bedding and cadavers that UQBR staff may be exposed to. Exposure maybe acute or chronic.

- Prepare the solution for injection
  Ensure there are no air bubbles present in the syringe, these can be removed by pulling up and down on the plunger drawing the solution back and forward slowly. The needle should be uncapped and placed appropriate location until used as per Needle Use and Sharps Safety training.

  If injecting cells, a 25G needle is recommended to prevent damage to the cells. If you are injecting cells you may need to keep the syringe on ice to ensure cell viability. A new needle should be used for every animal to reduce discomfort from bluntness.

Mammary Fat Pad Injection Procedure

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
2. Prepare clean, warm recovery boxes (e.g. resting on a heat mat).
3. Anaesthetise the animal, as per AEC approved protocol.
4. Apply ophthalmic lubricant to both eyes, using a sterile cotton tip.
5. Prepare the animal for surgery in dorsal recumbence and remove fur, as per LAB_001 Aseptic Technique for Laboratory Animal Surgery / LAB_002 Clean Technique for Laboratory Animal Surgery.

   The skin is gently pulled taught to avoid cutting the skin when using clippers. Remove fur from the mid abdomen to the 5th teat. Fur can be wiped away using a dampened swab.

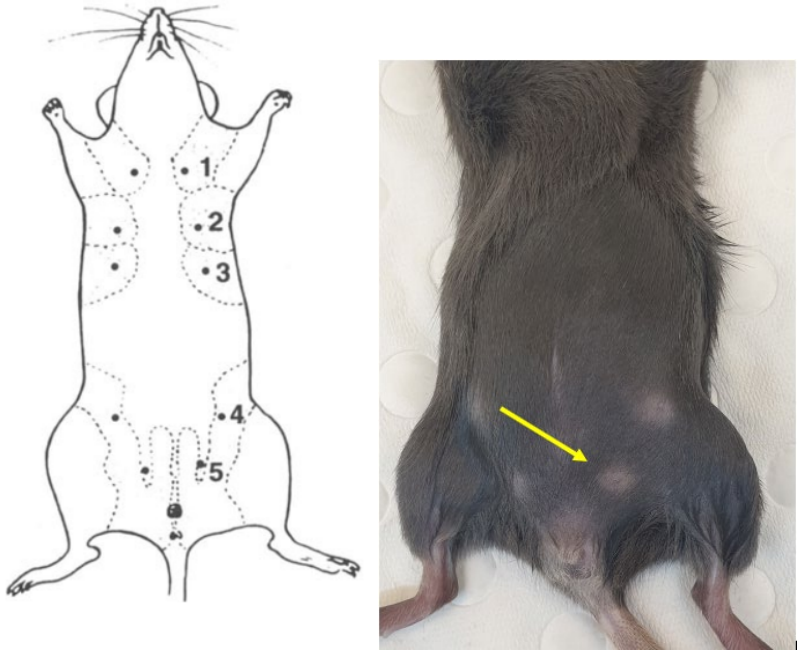

Figure 1 Diagram of mouse mammary gland location, black dots represent the nipples (Ip & Asch 2012, UQBR 2021).

6. Check for the absence of a withdrawal reflex. If a withdrawal reflex is 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 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).

7. Closed Injection Method

   The closed injection method into the mammary fat pad is less accurate – the injectate may miss the fat pad and sit subcutaneously next to it instead. Use of labelled cell lines and imaging technology might be required to confirm the location of injected material. It is recommended that unless you are frequently performing this injection, that you practice this on several cadavers with dye solution before commencing your experiment to see where the injection material is typically located after injection.

   a. Using curved forceps gently pinch the skin above the gland and lift the skin to make a ‘tent’
   b. Holding the syringe in your dominant hand, bevel facing up, align the syringe toward the middle of the tented skin facing away from the head
   c. Insert the needle parallel to the skin at a depth of ~2-3mm into the raised tent directly into the centre of the mammary fat pad.
   d. Release the skin from the forceps to remove any tension on the tissue

e. Inject pre-determined volume slowly, waiting 3-5 seconds then slowly and smoothly remove the needle.

The animal's skin should be free of blood and injection fluid, ensure there are no cuts or scratches around the injection site. If you do see blood a small amount of pressure should be applied with clean gauze until the bleeding ceases. If there is leakage of the substance immediately stop the injection and alter injection site slightly. Make sure to note any leakage as this may affect the success/variability of experimental outcomes.

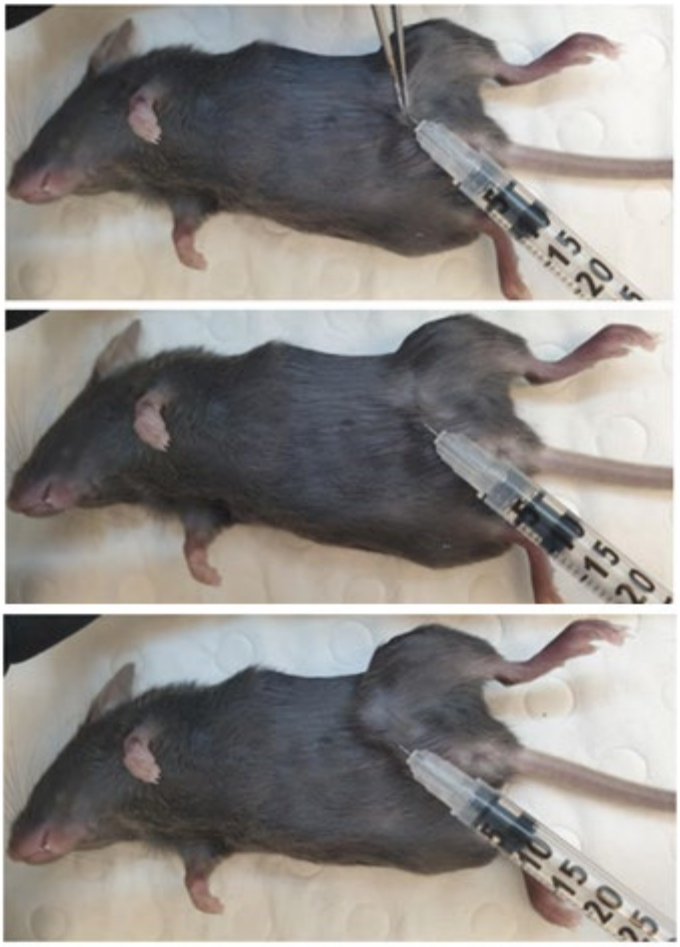

Figure 2 Closed method position and insertion of the needle (UQBR 2021)

Open Method

This method is a direct approach though a small surgical incision. Accuracy is better than the closed method due to the direct visualisation of the mammary fat pad prior to injection.

a. Make a small 2-4mm incision approximately 5mm caudal to the teat to locate the mammary fat pad

   Ensure that the incision made in a location that minimises tension at the wound site. Lift the skin to make a tent and angle scissors perpendicular to the skin when making a small shallow incision through the skin. Take care not to damage the abdominal muscle by accidentally cutting too deep.

Figure 3 Incision into the skin layer (UQBR 2021).

b. Locate and expose the fat pad by making a pocket at the incision site with a cotton swab moistened with saline. Use a tweezer to expose the mammary far pad, use a second tweezer to gently squeeze the fat pad at the base to fully expose the far pad.
   The mammary fat pad should be a visible shiny white mass located outside the peritoneal cavity. To avoid the fat pad from drying out, the injection should be done relatively quickly or use sterile saline to keep the area moist.

c. Holding the syringe in your dominant hand, insert the needle and inject pre-determined volume slowly, waiting 3-5 seconds then slowly and smoothly remove the needle.

   Once the solution has been injected directly into the mammary fat pad it should form a small bubble within the fat pad. If there is leakage of the substance immediately stop the injection and alter injection site slightly. If leakage is a recurring problem review the volume and thickness of the substance being injected.

d. Close the wound using the chosen method in the AEC approved project.

Figure 4 Injection and closure of the incision (UQBR 2021)

8. The surgical site is then gently cleaned with gauze or a cotton tip moistened with skin disinfectant to remove any blood contamination.
9. Place the animal into a recovery box, maintained on a heat mat until fully ambulatory. If available, recovery boxes may then be placed into a climate controlled, Ventilated Cabinets® for ~12 hours recovery.
10. Clean and disinfect all equipment between each animal.
11. Continuously monitor all mice during surgery and throughout the recovery phase until fully ambulatory. Rodents should be reassessed within 6 hours post recovery, then at least daily for the following 2 days. Ongoing monitoring is as described by the approved AEC activity.

V. REFERENCES

1. Ip. M & Asch, 2012, *Methods in Mammary Gland Biology and Breast Cancer Research. Springer.*
2. National Health and Medical Research Council (NHMRC) 2008, *Guidelines to promote the wellbeing of animals used for scientific purpose,* viewed 13 October 2021, https://www.nhmrc.gov.au/about-us/publications/guidelines-promote-wellbeing-animals-used-scientific-purposes
3. Office of the Gene Technology Regulator (OGTR) n.d., viewed 13 October 2021, http://www.ogtr.gov.au/
4. University of Queensland n.d., *Health, safety and wellbeing,* viewed 13 October 2021, https://staff.uq.edu.au/information-and-services/health-safety-wellbeing
5. University of Queensland n.d., *Health and safety risk assessments,* viewed 13 October 2021, https://staff.uq.edu.au/information-and-services/health-safety-wellbeing/health-safety-workplace/risk/assessments
6. UQ Biological Resources, 2021 Mammary Fat Pad Injections.

VI. REFERENCE INFORMATION

UQBR Training Consideration
For UQBR training purposes animals may remain for a number of days to monitor. Adverse effects may take time to develop and can assist with the assessment of competency.

Injection Considerations
Temperature – Consider if the substance has been stored in the fridge, if possible allow it to reach room temperature before injecting into the animal due to comfort and possible impact on body temperature.

Experimental Substances – A need for increased monitoring is generally required for experimental substances

Cells – When injecting cells, a larger gauge needle may need to be used. In a mouse a 25g needle will safely inject most cells. Depending on the research there may be a need to handle the needle and syringe in a specific manner for successful cell delivery.

Non-biological pH – There are mechanisms to improve pH of a substance for injection. For example, increasing the dilution, change of delivery vehicle, or anaesthetising the animal. This can decrease the risk of internal tissue necrosis and improve procedure outcomes.

If the substance is not a neutral pH of ~7, it may be acidic or alkaline, replace the needle that was used to drawn up the solution before injection to decrease any pain on entry to the animal.

Radioactive Substances – Additional approvals and safety precautions are required and will be included in the risk assessment. It is common to require safety goggles, additional gloves and shielding. You may also be required to work under a licensed person.

Infectious – Additional approvals and safety precautions are required and will be included in the risk assessment. Additional training may be required to ensure containment of infectious agents and waste management to protect other research projects and human health.

Cytotoxic – Additional approvals and safety precautions are required and will be included in the risk assessment. Additional training may be required to ensure containment of cytotoxic agents and waste management to protect other research projects and human health.

Non-TGA approved and off label substance use – If substances are experimental there may not be an SDS available. Ensure the risk assessment for the use and management of the substance includes excretion of the substance from the animal, chronic versus acute exposure, waste management of bedding/cage handling.

Injecting Schedule 7, 8 or 9's – The use and possession of these scheduled drugs requires special QLD Health Approval. Please ensure you have QLD Health 'Researcher Approval to 'possess', 'use' and 'dispose' of these drugs during project planning. Seek further advice about this from UQBR or your local area Drugs Officer.

Version #	**Reviewing AEC** (note: all other relevant AECs ratify the approval)	**AEC Review Date**	**Approved Until**
2	LBM	16/02/2022	16/02/2025