LAB_040 Injections – Intra-femoral in Mice (Expiry: March 2026)

I. OBJECTIVE

To effect safe and humane experimental injection of compounds into the intra-femoral bone 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)

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, Bair-hugger device, 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.
- Sterile surgical consumable
  - Including: gauze, cotton tips, absorbable suture (size: 5-0 or 6-0 or surgical glue), Needles (25 and 30G) .

IV. PROCEDURE

Preparation of Injection Substance

- Confirm the concentration and volume with the approved AEC protocol
  A maximum of 0.02ml (20uL) per site is recommended for intra-femoral 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, leakage, and even bone fracture.

  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 26-30G low-volume syringe is recommended to prevent fracturing the bone and pressure of liquid built up in the bone cavity, causing leakage. 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.

Intra-femoral 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 and provide pain relief, 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 from the knee joint, clean and disinfect skin as per LAB_001 Aseptic Technique for Laboratory Animal Surgery / LAB_002 Clean Technique for Laboratory Animal Surgery

Figure 1 Removal of fur from the knee joint (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. Position the animal in dorsal recumbency, place the leg horizontally to access the skin over the knee. Using forceps grasp the skin over the knee to make a small horizontal keyhole incision into the skin approximately 1-2mm.

   The incision should be small to keep the incision site minimal and straight to help with closure of the site.

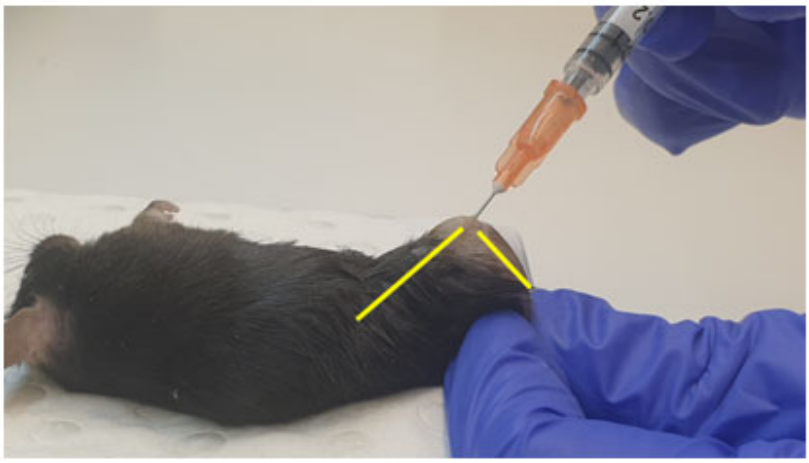
Figure 2 and 3, grasping the skin and resulting incision (UQBR 2021)

8. Position the animal of a heated raised surface, keeping the leg at a 90 degree angle, position the knee over your index finger, grasping the tibia with your thumb stabilising movement. The leg should naturally flex revealing the patella at the incision site.

The leg will need to be supported for movement, please be mindful that you're not grasping the tibia or foot tightly and cutting off circulation.

9. Using an empty 25G needle attached to an empty 1mL syringe, insert through the halfway point of the patella tendon and into the femoral bone marrow cavity.

   The purpose of inserting a larger gauge needle first is to bore a small hole in the femoral bone marrow to create space for the material to be injected. Performing a slight rotation of the needle/twisting movement while applying a small amount of pressure ensures enough space has been made to inject the substance. The angle of the needle should follow the femoral bone to ensure accurate insertion, the mouse femur is relativity straight with mild bowing. If the angle is incorrect the needle will push through the bone and into the muscle, or shatter the bone.



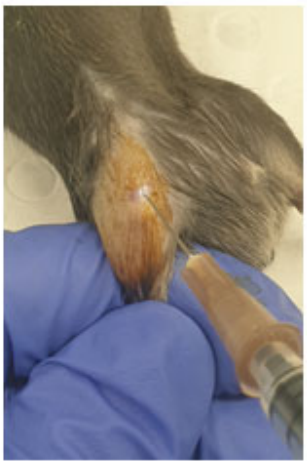


Figure 4 and 5, Location of needle insertion (UQBR 2021).

10. Continue to insert the needle while rotating the needle 2/3 into the femur, then slowly withdraw the needle and place into a sharps bin. It is critical to ensure the leg remains still at this point.

    A half rotation back and forth while gently applying pressure to progress the needle 1/3 of the way into the bone, too much friction/force can cause the bone to splinter or mis injection causing the needle to go through the patella and into the thigh muscle. To remove the needle, continue to rotate slowly back and forth whilst the needle is retracted out of the bone. The needle hub will be blocked with marrow so this needle cannot be used for injection.

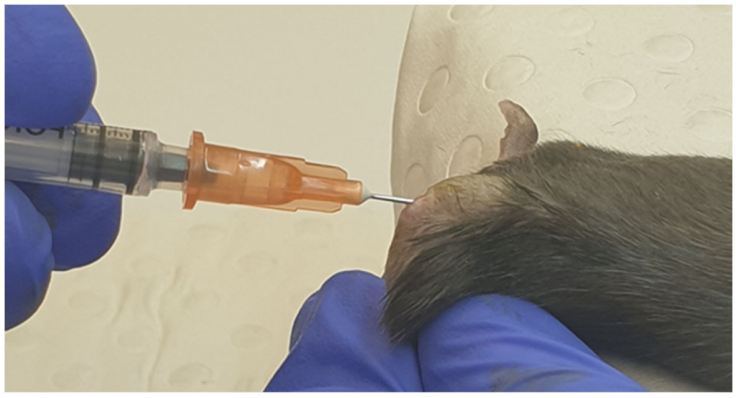


Figure 6 Example of needle depth (UQBR 2021)

11. Using the 2nd smaller 30G needle and syringe containing the substance for injection, insert into the site created at the above step.

    The 2nd needle needs to be inserted into the same hole that was created with exactly the same positioning/angle, this can be very challenging. Avoid twisting or boring with the needle again as this can cause blockages or mis injections.

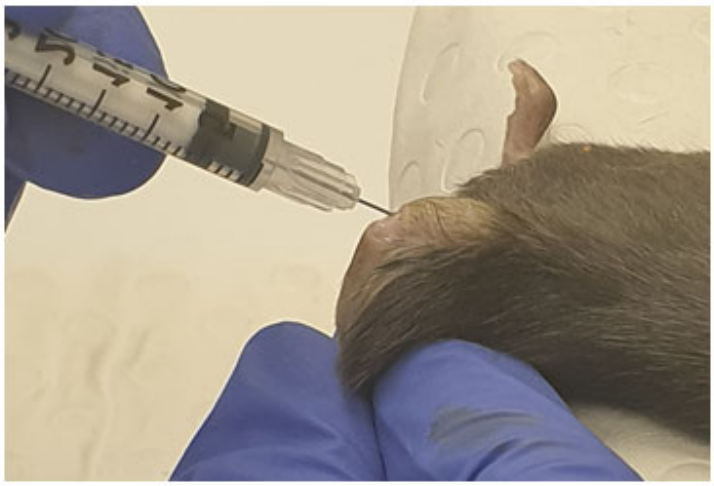


Figure 7 Insertion of 2nd smaller gauge needle at the same site/cavity (UQBR 2021)

12. To confirm the 2nd needle's placement, move the whole syringe gently to see if it is fixed inside the bone cavity. Observe if the leg and hip raise slightly off the surface.

    This will indicate you have correct placement, if misplacement has occurred the needle will be more easy to move within soft tissues and could be seen protruding into/from the thigh muscle.

13. Gently and slowly push on the plunger to inject the solution, then slowly remove the needle and dispose into a sharps bin.

The small amount injected should remain in the bone cavity, if there was too large a volume there might be some leakage at the injection site. If any swelling is observed in the muscle or skin layers or the pressure is too great to inject the injection was unsuccessful.

Figure 8 and 9, Dissected femoral bone, the left is natural in colour and was not injected, the right demonstrates successful confirmed by placement of the coloured injectable substance (UQBR 2021).

Figure 10 Example of leakage following injection (UQBR 2021).

14. The incision site is then gently cleaned with gauze or a cotton tip to remove any blood contamination and closed with tissue glue.

When closing the incision site ensure the skin can move freely when the leg is flexed.

15. Place the animal into a recovery box, maintained on a heat mat until awake and fully ambulatory. If available, recovery boxes may then be placed into a climate controlled, Ventilated Cabinets® for ~12 hours recovery.
16. Monitor the animals' movements once awake for any lameness or injury caused during the procedure. If any abnormalities are noted seek veterinary advice.
17. Clean and disinfect all equipment between each animal.
18. Continuously monitor all mice during surgery and throughout the recovery phase until fully ambulatory. Mice should be reassessed within 6 hours post recovery, then at least daily for the following 2 days. Perform ongoing monitoring and provide pain relief as described by the approved AEC activity.

V. REFERENCES

1. 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
2. Office of the Gene Technology Regulator (OGTR) n.d., viewed 13 October 2021, http://www.ogtr.gov.au/
3. University of Queensland n.d., *Health, safety and wellbeing,* viewed 13 October 2021, https://staff.uq.edu.au/information-and-services/health-safety-wellbeing
4. 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
5. UQ Biological Resources, 2021 Intra-femoral 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