LAB_029 Injections - Intramuscular (IM) in Mice and Rats (Expiry: March 2026)

I. OBJECTIVE

To describe the intra-muscular (IM) injection procedure in mice and rats used within the UQBR facilities.

NB: The use of (*) indicates this statement is dependent on the facility procedures
NB: The use of (**) indicates this statement is dependent on AEC Approvals

II. SAFETY

1. This procedure has the risk of needle stick or mouse bite injury – take appropriate care.
2. This procedure has a risk of causing musculoskeletal injury when performed regularly – consider suitable ergonomic design whenever possible.
3. In the event of a spill follow the facility emergency spill procedure.
4. Ensure you are familiar with the SDS for the substance to be injected should exposure or spills occur
5. Splash back into the face or eyes are a risk of performing injections. Protective visors or safety goggles should be worn at all times during the procedure

III. COMMENTS / RECOMMENDATIONS

- IM injections should be considered painful in rodents. Given their relatively small body muscle mass, the maximum injection volumes must be strictly observed (see table 1, in Reference Information)
- General anaesthesia is a reasonable inclusion to this procedure. If performing anaesthesia, because IM injection is a very short procedure injectable anaesthesia should not be used, instead gaseous anaesthesia (LAB_060 Rodent Anaesthesia – Isoflurane) should be used.
- When performing IM injection in mice, only one leg should be injected IM at any given time, then a rest period permitted between subsequent IM injections (4 to 7 days is considered sufficient)
- Oil adjuvants should not be used for IM injections in rodents, because they can cause painful and potentially fatal emboli (note: intravenous and intraperitoneal routes should also not be used for oil adjuvants)

IV. EQUIPMENT

- PPE (*)
- Disinfectant (*)
- Sharps Container
- Syringe
- Needle (**)
- Appropriate restraint device
- Substance for Injection(**)
- Change station/Bio-safety cabinet (*)
- Anaesthetic (*)(**)

V. PREPARATION OF EQUIPMENT

1. Check AEC approvals to ensure that the correct procedure and personnel are approved for the planned work
   Deviations can occur between approved procedures listed versus what is planned with the animal – check that these match and that the relevant personnel are approved.
2. Set up equipment items
3. Turn on Change station or Biosafety Cabinet (*)
4. Wipe surfaces with disinfectant
   Ensure equipment is operating as required.
5. Prepare for anaesthesia(**)

Anaesthesia Procedure

UQ Biological Resources offers anaesthetic training courses to all staff and researchers. For more information email uqbrtraincomp@uq.edu.au.

Aseptic Technique

Use an aseptic technique when performing procedures, this will minimise contamination from pathogens and subsequently infection in research animals.

VI. PROCEDURE

Preparation of Injection Substance

Refer to UQBR Online Module for Needle Use and Preparation.

- Confirm the concentration and volume with the approved AEC protocol
  Unless specific directions are provided in the AEC approved project, refer to NHMRC Guidelines for recommended maximum injectable volumes and recommended needle gauge.

  Consider temperature, pH, injection of cells, hazardous substances (cytotoxic, radioactive, infectious), and highly viscous liquids to improve success of 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.
  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.

Mouse Restraint for IM Injection

Refer to LAB_006 Handling and Restraint of Mice and Rats

1. Use a restraint device to hold the animal securely but comfortably
   Ensure the device you are using has a large enough gap to free the mouse's leg without discomfort.
2. Carefully hold the mouse's tail and leg that you are going to inject and guide the mouse into the restraint device so that the animals head is facing the opening.

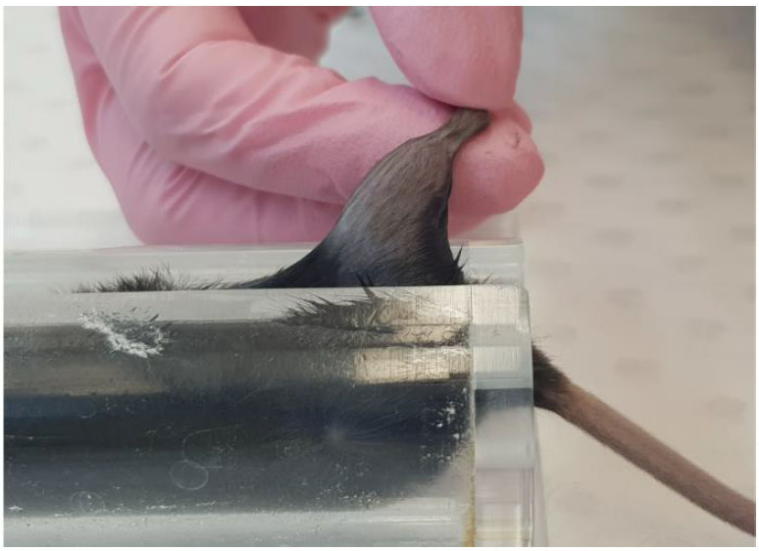

Figure 1 Restraint Technique in the Mouse (UQBR 2020).

Rat Restraint for IM Injection

There are various restraint techniques depending on the muscle group to be injected. Refer to the AEU Veterinary Officer or AEC for appropriate method and approval.

If you are not using an anaesthetic agent when completing IM injections on rat a second person may be required to hold the restrained rat firmly.

IM Injection Procedure

1. Have your needle ready with the solution you need to inject drawn up.

   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 in the appropriate location until used as per Needle Use and Sharps Safety training. If you are injecting cells you may put the syringe on ice.

2. Identify the animal to be injected

   Check animal's identification marks

3. Restrain the rodent based on the species-specific technique.

Anaesthetise rodent if required by your facility or ethics protocol (**)

4. Isolate the muscle area to be injected

If the animal is moving during the procedure this can cause tissue damage or potential needle stick injuries, ensure your rodent is secure but as comfortable as possible and the leg is accessible but isolated and extended. Identify the femur and note that the sciatic nerve runs along the length of this bone, it is critical to avoid this area and damage to this nerve is extremely painful and can result in the rodent becoming paralysed.

5. Wet the fur down with ethanol to help visualize the correct point of injection.

Use ethanol swabs or a tissue/gauze soaked in ethanol to do this, do not spray ethanol directly onto the animal as this can spray in eyes or completely soak the fur.

6. Holding the syringe in your dominate hand, insert the needle at a 90°C angle into the thigh muscle.

This is best accomplished by pointing the needle, caudally (posteriorly) rather than cranially, into the caudal thigh muscles. For a 20-25 g mouse the depth of entry will be approximately 0.5cm. On entry point it is the most likely time for the animals to kick or bite. Be sure to have a steady hand as moving the needle will be painful.

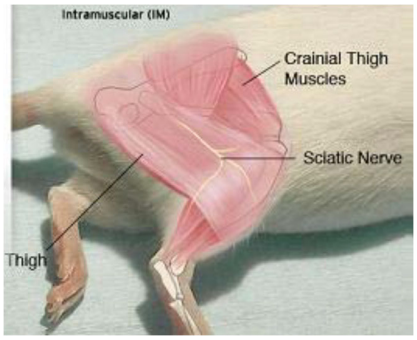

Figure 2 Anatomical guide in the mouse (NIH n.d.)

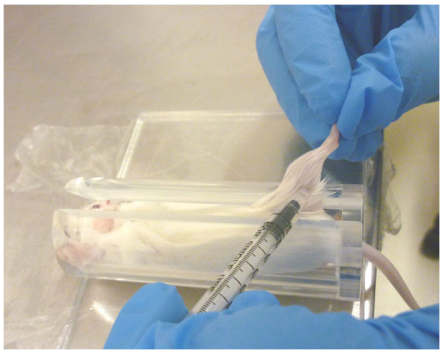

Figure 3 Injection (UQBR 2020)

7. Inject pre-determined volume, pushing slowly on the plunger to inject the solution

Refer to approved ethics protocol for volumes, inject the solution at a consistent, steady pace.

8. Pause for a couple of seconds to eliminate the risk of leakage and then remove the needle slowly

Ensure there is no injection fluid or blood sweeping out from around the injection site, if there is any liquid immediately stop the injection. This could be an indication that the needle is not deep enough or you have hit a blood vessel. If there is any blood around the area you may put a small amount of pressure onto the injection site using a clean tissue or gauze.

9. Place rodent into holding cage and continue to monitor health

The animal may hold up the injected limb or avoid bearing weight however this should return to normal in the following 15 minutes. If discomfort is observed longer than 15 minutes seek veterinary advice, refer to the UQBR SOP 22 Veterinary Care Program

10. Place needle into sharps container and syringe into clinical waste bin

Always use the specialised needle remover located on the lid of the sharps bin, if this cannot be located place the needle and syringe in the sharps bin as one unit. A new needle should be used for each animal.

11. Complete record keeping requirements – note procedure, date and initials on cage card, log procedure on relevant AEC animal monitoring paperwork and the relevant research sample collection labelling/records.

Injection procedures should also include the substance and volume injected. Records need to be clear and legible on each record to allow others to read and understand.

12. Repeat these steps for the next animal or if finished, pack and clean up equipment and space.

VII. REFERENCE INFORMATION

Table 1. Recommended values for Mice and Rat IM Injections (NHMRC 2008)

Values	Mouse	Rat
Needle Gauge	26-30G	25-30G
Needle Length	13mm	13-25mm
Max Injection Volume	0.05mL/site	0.2mL/site

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.

VIII. REFERENCES

1. National Health and Medical Research Council (NHMRC) 2013, *Australian code for the care and use of animals for scientific purposes,* viewed 11 April 2019, https://www.nhmrc.gov.au/about-us/publications/australian-code-care-and-use-animals-scientific-purposes
2. National Health and Medical Research Council (NHMRC) 2008, *Guidelines to promote the wellbeing of animals used for scientific purpose,* viewed 11 April 2019, https://www.nhmrc.gov.au/about-us/publications/guidelines-promote-wellbeing-animals-used-scientific-purposes
3. National Institutes of Health (NIH) n.d., Injections, viewed 23 January 2020, https://theodora.com/rodent_laboratory/injections.html
4. Office of the Gene Technology Regulator (OGTR) n.d., viewed 11 April 2019, http://www.ogtr.gov.au/
5. University of Queensland n.d., *Health, safety and wellbeing,* viewed 11 April 2019, https://staff.uq.edu.au/information-and-services/health-safety-wellbeing
6. UQ Biological Resources n.d., *UQBR SOP's, viewed 11 April 2019, https://biological-resources.uq.edu.au/*

Version #	Reviewing AEC (note: all other relevant AECs ratify the approval)	AEC Review Date	Approval To Date
~~#3~~	~~ABS~~	~~13/02/2020~~	~~13/02/2023~~ superseded
#4	ABS	13/04/2022	13/04/2025

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).