LAB_041 Injections - Intradermal Injection in Mice and Rats

(Expires May 2027)

I. OBJECTIVE

To describe the standard intradermal (ID) injection procedure used mice and rats across UQ research projects, also reflecting the procedure used to train workers across UQ by UQBR.

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

NOTE; When citing this SOP you must also describe your chosen anaesthetic technique (or quote the relevant SOP you will be following)

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]
Intradermal – between the epidermis and the subcutaneous layer

III. COMMENTS / RECOMMENDATIONS

- Intradermal injection under anaesthesia must be performed by appropriately trained personnel who have been deemed to be competent in the procedures.
- Intradermal injections are often used to administer experimental material such as allergens
- The **volume** of injection depends on the site location of the skin and viscosity of the substance pending AEC approval. Distension of the skin is painful, so the amount of fluid injected should be limited to volumes that will not overly stretch the skin. Minimise this by using multiple sites (up to 6 per session). See guidelines for maximum volume for injection and needle size in Table 1.
- **Aseptic technique** should be used in making up solutions, dilution of substances, drawing up the substance and injecting the animal. This includes using a new needle for each animal.
- **Clean technique** should be used in preparing the skin i.e., make sure skin is clean and dry. Wipe with 70% ethanol or similar if this is appropriate for the substance being injected.

[1] NHMRC, 2013, *Australian code for the care and use of animals for scientific purposes*, National Health and Medical Research Council (NHMRC).

- **Aseptic technique** should be used to prepare the skin if there is a risk of infection. For example, when injecting tumour cells, biologicals or jells. For example, clip the hair, clean with antiseptic such as chlorhexidine or betadine, wipe with 70% ethanol.
- After drawing up a substance, a new needle should be used to inject the animal. This is to ensure a sharp needle and minimise contamination.

Table 1. Recommended values for Intradermal Injections in Rodents (NHMRC 2008).

Values	Values for use – Mice	Values for use – Rats
Needle Gauge	27-30 G	27-30 G
Needle Length	13mm	13mm
Max Injection Volume	0.05 – 0.1 mL/site, the volumes depend on the thickness of the skin (maximum number of 6 sites)	0.05 – 0.1 mL/site, the volumes depend on the thickness of the skin (maximum number of 6 sites)
Attempt allowances	No more than 3 attempts per site. If unsuccessful allow another trained and competent person on the project to complete.	
Procedure frequency	No maximum recommendations identified. As approved by the AEC for each individual project. Provide justification for frequency	

IV. SAFETY AND COMPLIANCE

1. The person undertaking this task must ensure all relevant approvals are in place, training has been undertaken and risk assessments have been performed. If unsure, consult your supervisor.
2. Facility protocols should be followed.
3. Possible risks include mouse bite injury, needle stick injury, spills, exposure to infectious agents, repetitive task musculoskeletal injury and psychosocial harm.

V. TRAINING CONSIDERATIONS

- All unsupervised animal injections must be performed by appropriately trained personnel who have been deemed to be competent in the procedure.
- Training for intradermal injections must be undertaken on models or cadaver animals initially.
- Further training should be undertaken on animals under general anaesthesia.
- For UQBR training purposes animals, 0.05% of body weight (about 7% of blood volume) in blood volume will be collected, e.g. 10µl for a 20g mouse). 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.

VI. EQUIPMENT

- PPE *
  Minimum PPE is gloves and gown, additional PPE may be required based on facility or additional risk e.g. working with infectious animals.
- Disinfectant *
- Sharps Container
- Syringe
- Needle (27-30G x 13mm) **
- Substance for Injection**
- Change station/Bio-safety cabinet *
- Anaesthetic equipment **
- Forceps
- Inert pliable substance (Putty/Blu tac)
- Hair removal clippers
- Swabs
- Tissue (Kimwipes)

VII. PREPARATION

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. Prepare equipment items
   There should be no contamination of needles or substance for injection during this process.
3. Turn on Change station or Biosafety Cabinet *
4. Wipe surfaces with disinfectant
   Ensure equipment is operating as required.
5. Prepare for anaesthesia**

VIII. 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
  *The NHMRC Guidelines for intradermal injections recommend 0.05-0.1 mls ** maximum volume to be injected per site. In rodents this is up to a maximum of 6 sites. Any volume larger than this should be clearly cited and justified in the AEC application. Refer to the Reference Information above for recommended needle gauge and lengths.*
  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.

Unless specific directions are provided in the AEC approved project, refer to the table above (based on the NHMRC Guidelines) for recommended maximum injectable volumes and recommended needle gauge.

- 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 may be acute or chronic.

Preparation for Restraint

Rodent Restraint for ID Injection Refer to LAB_039 Handling and Restraint in Rats and Neonates and LAB_006 Handling and Restraint in Mice and Neonates

1. Generally the rodent is anaesthetised and a restraint device is not required. Injections without anaesthesia would require specific justification and AEC approval.

Intradermal Injection Procedure

1. Have your syringe and needle ready with the solution 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 appropriate location until used as per Needle Safety training.

2. Identify animal to be injected - *check animal's identification marks*
3. Anaesthetise the rodent

   Gaseous anaesthesia is preferred over injectable anaesthesia due to fast recovery time, this technique should be completed within the minimum amount of time that is reasonable to undertake the procedure from start to finish. The rodent is to be placed on a mask / nose cone unit once inducted. Test for pedal and tail reflexes before starting injection process.

4. Check depth of anaesthesia and remove hair at the injection site.

   Hair may need to be removed if injecting into a site other than the ear. Ensure the clippers are free from fur, gently pull the skin taught to avoid cutting the skin if using clippers. Shave over the top of an empty cage base to collect loose hair. Consider how the animal is positioned and do not hold too firmly as this can affect breathing. Remove any excess hair with an ethanol dampened swab or tissue.

5. Use forceps or thumb and index finger to stabile the area for injection on an elevated platform.

   Forceps, Blu tack / putty and thimbles can be used to stabilise and provide extra protection to the operator avoiding needle stick injury depending on injection site. Elevation of the ear can be achieved with the use of a sterile swab that has been rolled and placed under the ear to create a platform for ease of injection.

6. Hold the syringe in your dominant hand, with the needle pointing towards the tail of the rodent bevel facing up. Insert the needle parallel to the skin at a shallow depth of ~1mm into the skin layer until 3-4mm of the tip of the needle is within the skin layer into:

   Be sure to have a steady hand as moving the needle can cause tissue damage by inserting the needle through the skin. Forceps can be used to lightly grasp the skin. Ensure the forceps are not pinching the skin, the needle can be lifted slightly to create a shallow 'tent'. The injection into the dermal layer is extremely difficult there is a high chance of piercing through skin layers misinjection. A small bleb should appear with no leakage at the injection site.

   a. The loose skin of the shoulder blade
   b. The loose skin of the flank (Figure 1)
   c. The dermis of the ear tissue (Figure 2)

      The top or underside of the ear tissue may be injected. Lightly pull taught with forceps or rest the ear over your finger supported by putty/Blu tack, this will create a flat tacky surface for injection. Extra care should be taken to avoid needle stick injury or injury to the mouse's ear tissue as this is a delicate area. Forceps can be used on an elevated platform such as a rolled swab to avoid close contact with needle. Again do not put too much pressure on the ear tissue with the forceps that may tissue damage. The bleb will remain only for a short amount of time before the substance will disperse.

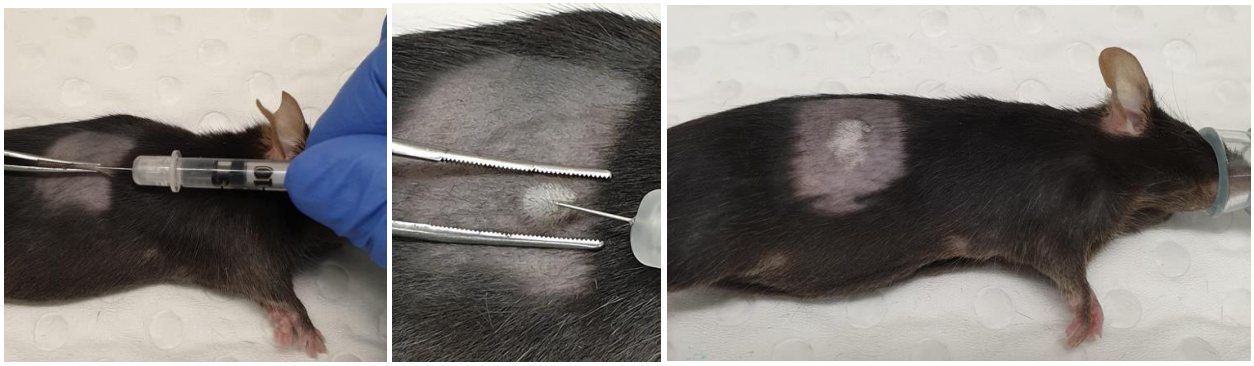

Figure 1 Mouse and needle positioning with 'bleb' following successful injection into the flank (UQBR 2020).

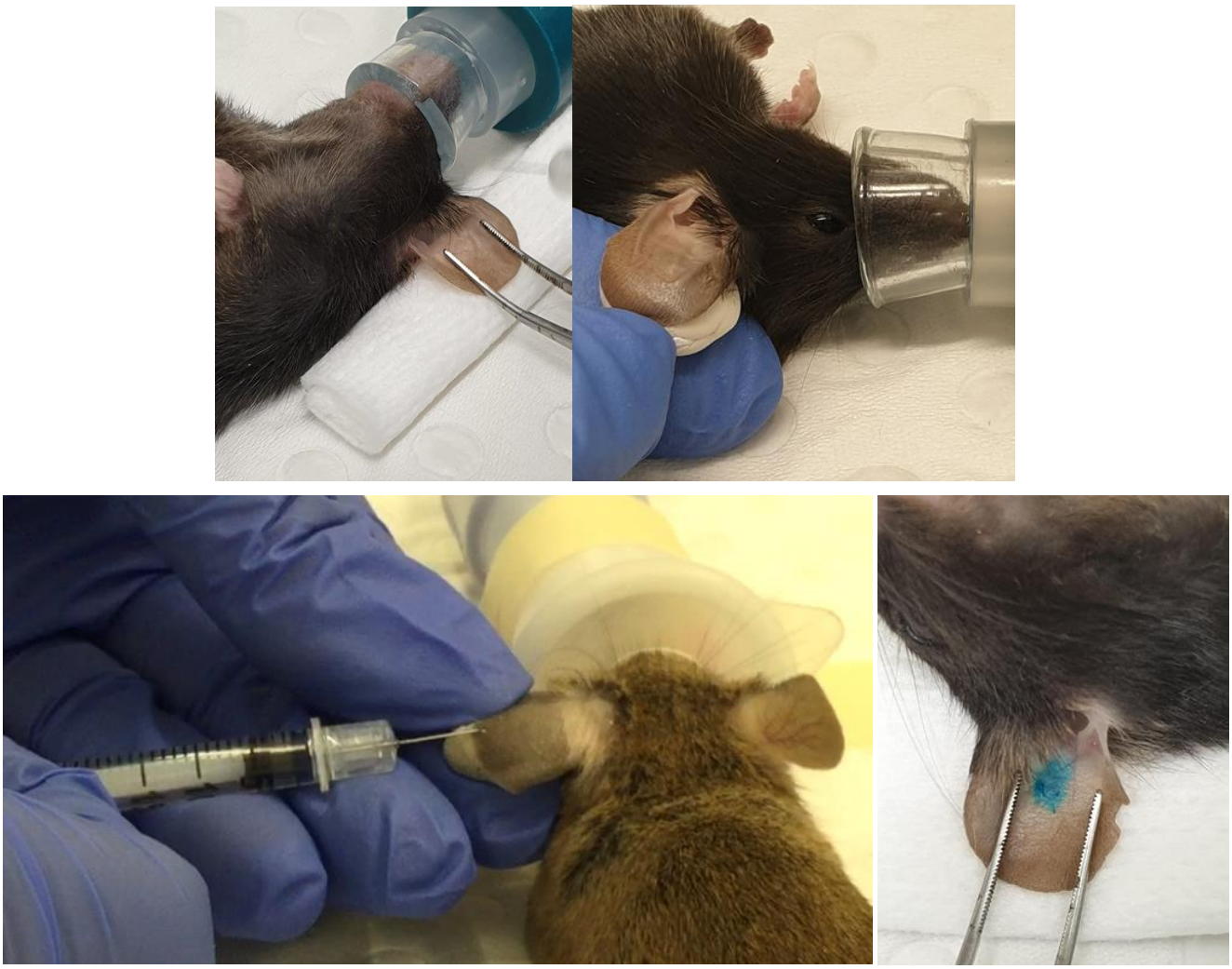

Figure 2 Mouse and ear positioning, successful intradermal injection of the ear using a coloured dye.

7. Inject pre-determined volume slowly**

   Inject the substance at a consistent and steady pace. A translucent bleb should form in the skin, if this does not occur it could be an indication you have injected too deep and the injection has been unsuccessful.

8. Wait 3 to 5 seconds after to injection is complete, then slowly and smoothly remove the needle.

   This will stop potential leakage of the solution. The skin should be free of blood and injection fluid, ensure there are not 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, the tip of the needle may not be within the skin layer. Do not be alarmed if there is a bleb at the injection site, this is expected and is an indication of a successful injection.

9. Release the rodent into holding cage and continue to monitor for recovery and health

   Following the procedure, the animal should return to normal movement once placed back in the cage. If you see the animal behaving abnormally once in their home cage or excessive cleaning of the area this could be an indication of discomfort. Seek veterinary advice. If discomfort is observed refer to the LAB_022 Program of Veterinary Care.

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.

IX. REFERENCE INFORMATION

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 draw 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.

X. BIBLIOGRAPHY

1. NHMRC, 2013, *Australian code for the care and use of animals for scientific purposes*, National Health and Medical Research Council (NHMRC).
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

Version #	Reviewing AEC (note: all other relevant AECs ratify the approval)	AEC Review Date	Approval To Date
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