LAB_041 Injections - Intradermal Injection in Mice and Rats

I. OBJECTIVE

To describe the intradermal (ID) injection method 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 rodent 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. 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) **
- Substance for Injection**
- Change station/Bio-safety cabinet *
- Anaesthetic equipment **
- Thimble *
- Forceps
- Inert pliable substance (Putty/Blu tac)
- Hair removal clippers
- Swabs
- Tissue (Kimwipes)

IV. 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. 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**
6. Place thimble onto index finger before gloving*

Anaesthesia Procedure

UQ Biological Resources offers anaesthetic training courses to all staff and researchers. It is highly recommended that anaesthesia training is completed before anesthetising rodents. 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.

V. 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 below 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 NHMRC Guidelines for recommended maximum injectable volumes and recommended needle gauge.
  The maximum needle gauge is outlined in the NHMRC Guidelines. Refer to Reference information below for guidance.

- 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.

Rodent Restraint for ID Injection

Refer to LAB_006 Handling and Restraint of Mice and Rats

1. There are various restraint techniques depending on the site to be injected. A restraint device may be used to hold the animal securely but comfortably. Generally the rodent is anaesthetised and a restraint device is not required. Refer to the AEU Veterinary Officer or AEC for appropriate method and approval.

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

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 Use and Sharps Safety training.

2. Identify animal to be injected - *check animal's identification marks*

3. Anaesthetise rodent **

   Gaseous anaesthesia is preferred over injectable anaesthesia due to fast recovery time, this technique should be completed in under 10 minutes 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.

   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.

   Movement of the animal during the procedure can cause needle stick injuries or misplaced injection. 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 driving 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.

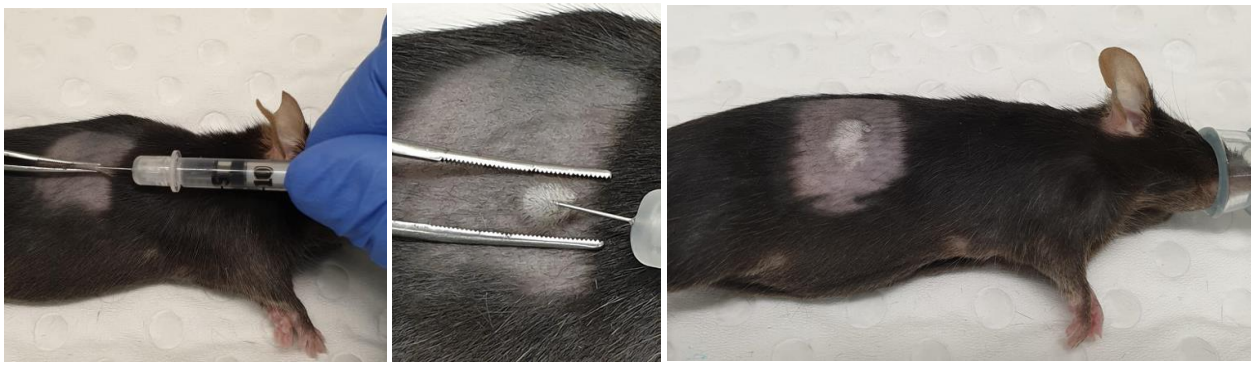

Figure 1 Mouse and needle positioning with 'bleb' following successful injection into the flank (UQBR 2020).

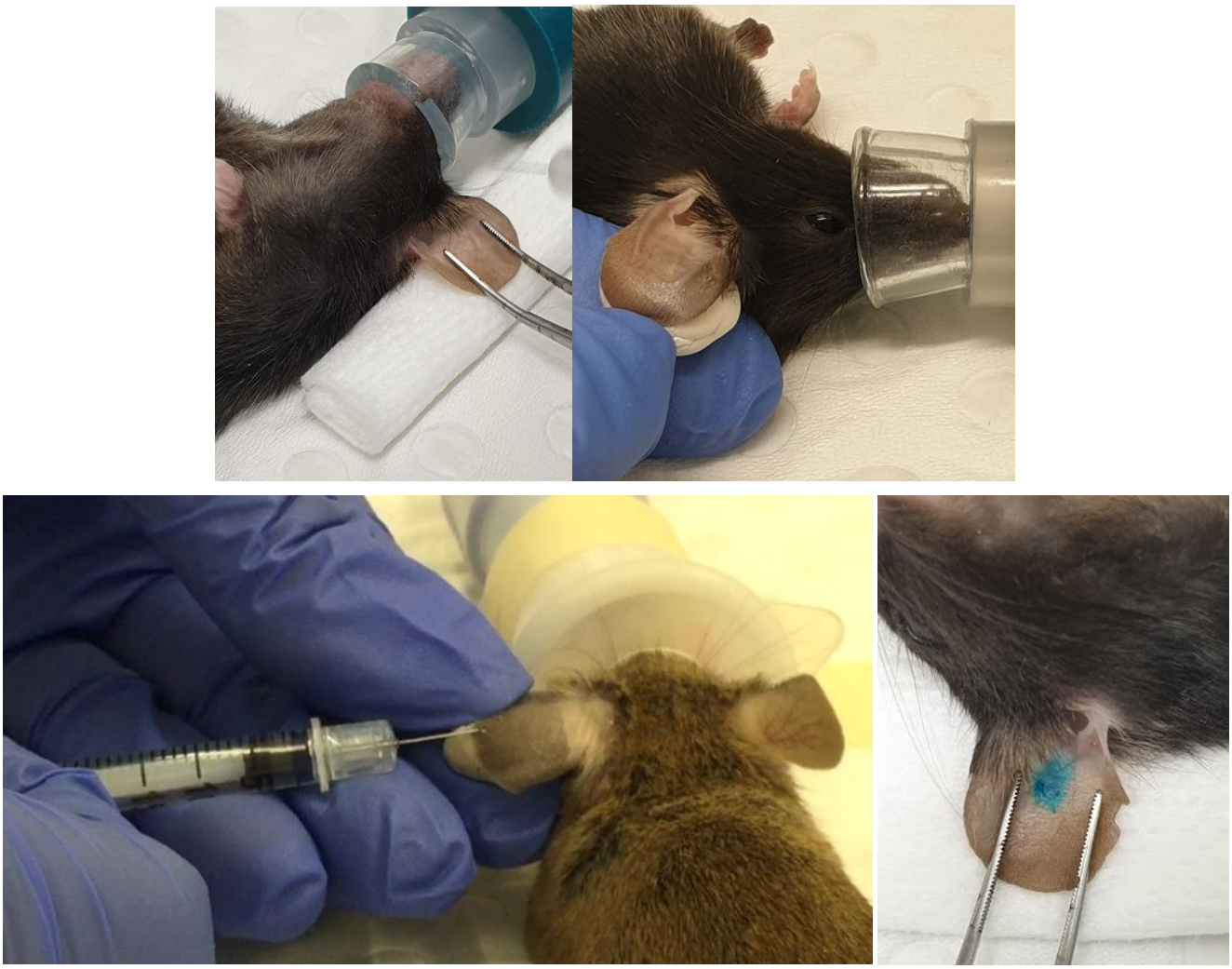

Figure 2 Mouse and ear positioning, successful intradermal injection of the ear using a coloured dye (UQBR 2020).

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 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.

Post Injection Monitoring

If discomfort is observed refer to the UQBR SOP 22 Veterinary Care Program

VI. REFERENCE INFORMATION

VII. 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)

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) 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-usedscientific-purposes
2. Office of the Gene Technology Regulator (OGTR) n.d., viewed 11 April 2019, http://www.ogtr.gov.au/

3. University of Queensland n.d., *Health, safety and wellbeing,* viewed 11 April 2019, https://staff.uq.edu.au/information-and-services/health-safety-wellbeing
4. University of Queensland n.d., *Incidents, injuries and hazard,* viewed 11 April 2019, https://staff.uq.edu.au/information-and-services/health-safety-wellbeing/health-safetyworkplace/incidents-injuries-hazards
5. UQ Biological Resources n.d., *UQBR SOP's, viewed 11 April 2019, https://biologicalresources.uq.edu.au/secure/reference-information#SOP's*
6. UQ Biological Resources, 2020 *UQBR Image Library.*