 <p>THE UNIVERSITY OF QUEENSLAND AUSTRALIA CREATE CHANGE</p>	<p>UQ Animal Ethics Committee - Standard Operating Procedure LAB_017 Injections - Subcutaneous (SC) Injection in Mice and Rats Institutional author: UQ Biological Resources AEC Reviewed & Approved: 04/08/2023</p>	Version 4
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LAB_017 Injections - Subcutaneous (SC) Injection in Mice and Rats

I. OBJECTIVE

To describe the SC injection procedure in mice and rats that is used within 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. 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

Subcutaneous Injection: Injection under the skin into the subcutaneous space


III. COMMENTS / RECOMMENDATIONS

- **Subcutaneous injections** are often used to administer substances (e.g. anaesthetics, pain relief, experimental material etc.) or to administer fluids for hydration. Absorption from this route is slow.
- The **volume** of injections depends on the looseness of the skin. 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 4 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.
- **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.

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

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IV. 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.

V. SAFETY- Individuals performing this task are responsible for identifying and managing the risks associated with this task

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 any potential risks associated with the substance to be injected, in case 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

VI. EQUIPMENT


- PPE *
- Disinfectant *
- Sharps Container
- Syringe
- Needle **
- Substance for Injection**
- Change station/Bio-safety cabinet *
- Restraint device for rats e.g. clean towel, pillow case or muslin bag
- Anaesthetic (*)(**)

VII. 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
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 if required**

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VIII. PROCEDURE

Preparation of Injection Substance

Refer to UQBR Online Module for Needle Use and Preparation.

1. Confirm the concentration and volume with the approved AEC protocol

Within mice, 3mL is considered the maximum SC injection volume, and injection volumes >1mL must be dispersed over multiple SC sites (up to 4 sites per session). Within rats, 5mL is considered the maximum SC injection volume, and injection volumes >2mL must be dispersed over multiple SC sites (up to 4 sites per session). See the NHMRC guidelines (2008), for further clarification.

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 above for information about these variables.

2. Unless specific directions are provided in the AEC approved project, refer to the table below (based on the NHMRC Guidelines) for recommended maximum injectable volumes and recommended needle gauge.
3. 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.

Preparation for Restraint

Mouse Restraint for SC Injection

Refer to LAB_006 Handling and restraint of mice and neonates

1. Identify the Animal/Animals that are to be injected and remove from the home cage

Check animal's identification marks, the mouse can either be placed on the bench coat or the wire of a cage.

2. Using your non dominate hand, with your thumb and forefinger pinch the skin firmly but not too hard and lift away from the body of the animal making a 'tent' with loose skin at the back of the neck and shoulders.

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Figure 1 Mouse restraint example for SC Injection at the shoulders with a 'tent' and the flank (UQBR 2020)

Rat Restraint for SC Injection

Refer to LAB_039 Handling and Restraint of rats and neonates

1. Remove the correct animal from its home cage and it on the bench, cover the animals head with the restraint device

Ensure that the animal can breathe normally and does not appear stressed

2. Using your non-dominant hand, with your thumb and forefinger pinch the skin firmly but not too hard and lift away from the body of the animal making a 'tent' with the loose skin at the back of the neck and shoulders.

Preparation of Injection Site

Clipping of fur or using hair removal cream to make the site more visible is considered an additional procedure that must be specified in the original Ethics Approval or requested as an Amendment. Refer to Online Module for Hair Removal.

SC Injection Procedure

1. Have your syringe and needle ready with the solution you require to be injected 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.

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

2. Identify the animal/animals that are to be injected *Check animal's identification marks*
3. Restrain the rodent based on the species specific technique listed above

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Be sure to hold enough skin so the animal cannot bite or kick. Movement of the animal during the procedure can cause needle stick injuries or misplaced injection.

4. Holding the syringe in your dominant hand with the bevel of the needle facing up, align the syringe and either place
 - a) Into the loose skin of the shoulders, towards the middle of the tented skin facing inferior position or away from the head.
 - b) Into loose skin of the flank, parallel to the mouse, facing cranially

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 around can cause tissue damage. If injecting into the flank lift the needle to create a 'tent'.

5. Inject pre-determined volume

Refer to approved ethics protocol for volumes, inject the solution at a consistent, steady pace. If the skin does not easily lift it could be an indication you have gone too deep or into the muscle mass, withdraw the slightly until you can see a tenting of the skin.

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

The animal's fur 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. Do not be alarmed if there is a lump at the injection site, this is expected and is an indication of a successful injection.

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

Following the procedure, the animal should recover to normal movement once placed back in the cage. If discomfort is observed refer to the UQBR SOP 22 Veterinary Care Program.

8. 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.

9. 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.

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

Substance 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

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- **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, it is very important to 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 need to be included in the relevant 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.


IX. REFERENCE MATERIAL

Table 1. Recommended Needle Gauge and Length (NHMRC 2008)

Values	Mouse	Rat
Needle Gauge	25-26G	23-26G
Needle Length	13-25mm	13-25mm
Max Injection Volume	3 mL over Multiple sites, volumes depends on looseness of skin (up to 4 sites per session)	5 mL over Multiple sites, volumes depends on looseness of skin (up to 4 sites per session)

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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.

X. 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 *SC Injections*.

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