

LAB_027 Injections - Retro-Orbital Injection in Mice

I. OBJECTIVE

To describe the retro-orbital injection method in mice 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. 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 **
- Substance for Injection**
- Change station/Bio-safety cabinet *
- Anaesthesia equipment **

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. 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

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Ensure equipment is operating as required.

5. Prepare for anaesthesia**

Anaesthesia Procedure

UQ Biological Resources offers anaesthetic training courses to all staff and researchers. It is highly recommended that anaesthesia training is completed before anaesthetising 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 a bolus injection volume is 1% of total body weight, any volume larger than this should be clearly cited and justified in the AEC application. The injectable solution is limited to 1% of total body or under 200µL in adults. A needle size of 28 G or smaller is recommended.
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 maybe acute or chronic.

Retro-Orbital Injection Procedure

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

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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.

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

- Anaesthetise rodent **

Gaseous is the preferred delivery anesthesia 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.

- Place the animal in lateral recumbency, use the index finger of the non-dominant hand to draw back the skin above the eye and the thumb to draw back the skin below the eye. The eye will protrude slightly.

A right-handed person will find it easiest to administer the injection into the right retro-orbital sinus of the mouse. The mouse is placed in left lateral recumbency with its head facing to the right. The opposite will apply for left handed workers.

Both the trachea and the ventral cervical (neck) vessels (the jugular veins and carotid arteries) run along the ventral cervical area. The veins drain the head area and the arteries supply this area.

Care must be taken not to apply excessive pressure to the ventral cervical vessels, because this could impede blood flow and impede the injection. It is also important not to apply pressure to the trachea because this could cause collapse resulting in the mouse not being able to breathe.

- Insert the needle bevel down at 30 ° angle into the medial canthus (see Figure 1 below), into the retro-bulbar space (the region behind the globe of the eye) then use the needle to follow the edge of the eyeball down until the needle tip is at the base of the eye.

There is a slight amount of resistance which causes the eye to regress back into the sinus, until the needle passes through the conjunctival membrane. The needle will be positioned behind the globe of the eye.

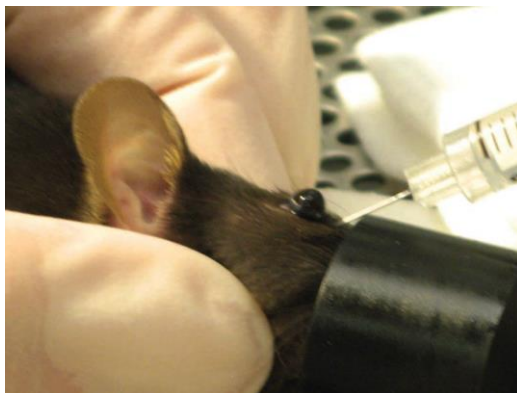


Figure 1 Placement of needle for retro-orbital injection

- Inject pre-determined volume slowly

Watch the eye closely for any swelling of the eyeball or clouding of the eye, if this happens immediately stop the injection as you are most likely in the retina or penetrated the eyeball, call your facility veterinarian for advice. In

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albino animals you may see a slight darkening of the eye, this is normal as a small amount of blood may build up in the retro-bulbar space.

If you see injection fluid coming from the nose of the animal you have penetrated the nasal sinus and your injection has been unsuccessful, this usually won't have a long term negative effect on the animal but further injection that day is not recommended and advice from your facility manager or veterinarian may be required.

7. 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. A small amount of blood may be seen on removal of the needle; this should not be flowing or persistent.

8. Release skin.

Close the eyelids and apply mild pressure to the injection site with clean gauze or tissue. If bleeding continues longer than 5 seconds seek advice from your facility manager or veterinarian.

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, the eye should not be weeping or closed. 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

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VI. REFERENCE INFORMATION

Table 1. Recommended values for Retro-orbital Injections in Mice (NHMRC 2008)

	Values for use (in mice)
Needle Gauge	28G
Needle Length	13mm
Max Injection Volume	1% of total body weight (no greater than 200uL)

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

Tumorigenic compounds - are never to be injected using this route as this will result in brain tumour development. This technique is only suitable for non-abrasive solutions and the PH should be identified and considered appropriate.

Accuracy - Injection failure when performing retro-orbital injection is possible, however, it should be considered a rare occurrence. In some circumstances retro-orbital injection may be considered more accurate and reliable than other methods of IV access, such as lateral tail-vein injection (Schoch *et al.*, 2014).

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.

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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.

VII. 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. Schoch, A., Thorey, I., Engert, J. *et al.* Comparison of the lateral tail vein and the retro-orbital venous sinus routes of antibody administration in pharmacokinetic studies. *Lab Anim* **43**, 95–99 (2014). <https://doi-org.ezproxy.library.uq.edu.au/10.1038/labam.481>
4. University of Queensland n.d., *Health, safety and wellbeing*, viewed 11 April 2019, <https://staff.uq.edu.au/information-and-services/health-safety-wellbeing>
5. 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>
6. UQ Biological Resources n.d., *UQBR SOP's*, viewed 11 April 2019, <https://biologicalresources.uq.edu.au/secure/reference-information#SOP's>
7. UQ Biological Resources, 2019 *IM Injections*.

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