LAB_027 Injections - Retro-Orbital Injection in Mice under anaesthesia (Expiry: May 2027)

I. OBJECTIVE

To describe the standard retro-orbital injection method used in UQ research projects, also reflecting the procedure used to train workers across UQ by UQBR.

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

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"

Retro-Orbital – situated or occurring behind the orbit of the eye

III. CONDITIONS FOR USING THIS SOP

- **It must be performed under anaesthesia.** When citing this SOP, you must also describe your chosen anaesthetic technique (or quote the relevant SOP you will be following)

- **You must log all complications of this technique.** Because of the potential complications of this technique, you are required to keep a log of any complications associated with retro-orbital blood collection in this project. Severe complications should be reported to the AEC as an unexpected adverse event

- **This can only be performed a maximum of 2 times per eye (a total of 4 injections per mouse)**
- **You must state the volume to be injected at each timepoint in your ethics application**
- **This technique cannot be used for tumour cells**

IV. COMMENTS / RECOMMENDATIONS

- **Aseptic technique** should be used in making up solutions, dilution of substances, drawing up the substance and injecting the animal.
- 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
- Retro-orbital injection under anaesthesia must be performed by appropriately trained personnel who have been deemed to be competent in the procedures.

- It is important that people undertaking this procedure, as a recovery technique, ensure they are competent and perform the procedure with great care.
- Signs of a failed attempt include a bulging/leaking eye, lens collapse, fluid coming from the nose. Guidelines on injection volumes are below.

Table 1. Recommended values for Retro-orbital Injections in Mice

Values	Values for use (in Mice)
Needle Gauge	27-30G or insulin syringe
Needle Length	13mm
Max Injection Volume	1% of total body weight (or no greater than 100uL) whichever is less volume. The volume to inject must be that approved in your ethics approval.
Procedure frequency	2 weeks apart with a minimum of 4 weeks apart per eye
Procedure number	maximum of 2 times per eye (a total of 4 injections per mouse)
Attempt allowance	No more than one attempt per eye.

Monitoring

- Mice should be monitored for a minimum of 2 days following this procedure. Signs to check for include corneal opacity (cloudy eye), swelling or bulging near the eye, less active mouse.

Possible adverse effects

- Retro-orbital haemorrhage resulting in haematoma and excessive pressure on the eye
- Corneal ulceration, keratitis, pannus formation, rupture of the globe and micro-ophthalmia caused by proptosis of the globe
- Damage to the optic nerve and other intra-orbital structures which can lead to deficits in vision and blindness
- Fracture of the fragile bones of the orbit and neural damage by the micro-pipette or needle
- Penetration of the eye globe itself with a loss of vitreous humour

V. 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.

VI. TRAINING CONSIDERATIONS

- All unsupervised injections must be performed by appropriately trained personnel who have been deemed competent in the procedure.
- Training in retro-orbital 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 may remain for a number of days to monitor. Adverse effects may take time to develop and can assist with the assessment of competency.

VII. 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 or insulin syringe
- Substance for Injection**
- Change station/Bio-safety cabinet *
- Anaesthesia equipment **

VIII. PREPARATION OF EQUIPMENT

1. Check AEC approvals to ensure 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**

IX. PROCEDURE

Preparation of Injection Substance

Refer to UQBR Online Module for Needle Safety

- Confirm the concentration and volume with the approved AEC protocol

The injectable solution is limited to 1% of total body weight or under 100µL in adults.
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. 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.

   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 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, remember you will need temporary access to the head. Test for pedal and tail reflexes before starting injection process. Refer to LAB_060 Rodent Anaesthesia – Isoflurane.

4. 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.

5. Insert the needle bevel down at 30 ° angle from the nose to the whiskers. (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.

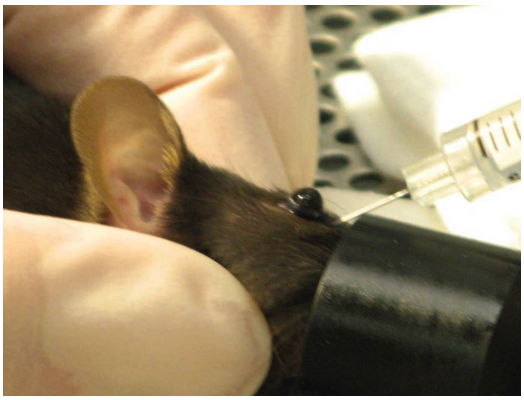

Figure 1 Placement of needle for retro-orbital injection

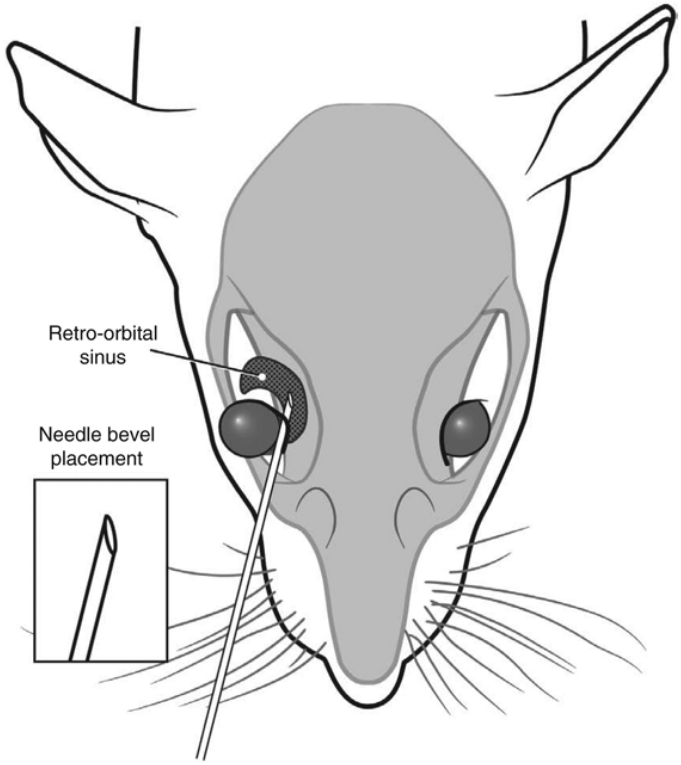

Figure 2 Insert needle, bevel down (Yardeni *et al.* 2011)

6. Inject pre-determined volume in a controlled manner

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 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 the 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 LAB_022.

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.

Monitoring

- Mice should be monitored for a minimum of 2 days following this procedure. Signs to check for include corneal opacity (cloudy eye), swelling or bulging near the eye, less active mouse.

X. 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 approval. Please ensure you have UQ Substance Management Plan approval, refer to your local area Drugs Officer.

XI. BIBLIOGRAPHY

1. Huizing, M., Morris, H. D., Yardeni, T., Eckhaus, M., & Hoogstraten-Miller, S. (2011). Retro-orbital injections in mice. *Lab Animal*, *40*(5), 155–160. https://doi.org/10.1038/laban0511-155

Version #	Reviewing AEC (note: all other relevant AECs ratify the approval)	AEC Review Date	Approval To Date
[#]		[DD/MM/YYYY]	

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).