LAB_030 Injections - Intravenous (IV) Tail Vein Injection in Mice and Rats

(Expires May 2027)

I. OBJECTIVE

To describe the standard IV injection procedure in mice and rats used 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

II. CONDITIONS FOR USING THIS SOP

- This procedure can be performed either under general anaesthesia or with the mouse carefully restrained. When citing this SOP in your ethics application, you must also describe your chosen restraint or anaesthetic technique (or quote the relevant SOP you will be following)
- You must state the volume you will be injecting in your ethics application. Maximum volumes are outlined in the table below.
- The minimum number possible should be performed. If more than 3 total IV injections per mouse are requested, provide justification to the AEC in your application
- Frequency should be as low as possible. If more than one IV injection per week is requested, provide justification and measures to reduce complications to the AEC in your application
- It is ideal to not use tail vein for blood collections if tail vein injections are being used

III. 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]
Intravenous – taking place within or administered into a vein.

IV. COMMENTS / RECOMMENDATIONS

- **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.
- Intravenous injections 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 swelling (blister or bubble) and resistance to injection.
- You must state the volume to be injected at each timepoint in your ethics application**
- If injecting cells, it is important to monitor for instances of embolism in capillaries after injection.

Calculation of injection volume

It is vital that the correct volume to be injected is calculated. Injection volume is generally calculated as a % of total body weight. This is the clinically relevant value.

Example calculation to obtain 1% of the total weight of a 20 gram mouse

0.01 x 20 = 0.2 mls or 200 µl

The table below provides some examples

Table 1. Recommended maximum injection volumes and needle gauge (G) with length for rodents (NHMRC 2008)

Values	Mouse	Rat
Needle Gauge	25-30 G	23-26 G
Needle Length	13 mm	13 mm
Needle Depth at Injection	0.5cm	1cm
Volume to be injected	Volume approved by the AEC	
Max Injection Volume	1% of bodyweight in a bolus injection	1% of bodyweight in a bolus injection
Attempt allowance	No more than 3 attempts per tail vein is approved. If unsuccessful in 3 attempts, allow another trained and competent person on the project to complete. A second person may try for another 3 attempts.	
Procedure number per animal and frequency	A maximum of 3 total injections per mouse at a maximum frequency of one injection per week. Or as approved by the AEC in your project.	

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 animal injections must be performed by appropriately trained personnel who have been deemed to be competent in the procedure.
- Training for injections collection must be undertaken on models or cadaver animals initially.
- Note for UQBR Training purposes, 1% of total body weight may be injected in a bolus injection.
- 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.
- Face visor or safety goggles
- Heat source with ongoing monitoring *
- Disinfectant*
- Sharps Container
- Appropriate restraint device
- Adhesive tape or sticky matt
- Needles (Mouse 25-30g x 13-25mm) (Rat 23-26g x 25mm)
- Syringes
- Substance for Injection**
- Empty cage base

VIII. PREPARATION

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*
   Ensure equipment is operating as required.

4. Wipe surfaces with disinfectant
   There should be no contamination of needles or substance for injection during this process.

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 volume is limited to 1% of total body weight.
  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.

IV 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 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.

2. Identify animal to be injected – *check animal's identification marks*
3. Use heat to dilate the tail veins which run laterally along each side of the tail
   The dilation of blood vessels may help with visualising the veins and improve injection success rates. In dark pigmented rodents a light source may help to highlight the vein. Pigmentation and strain differences can impact visibility.

4. Restrain the animal in an appropriate restraint of suitable size if the animal is not anesthetised
   Ensure that you do not hold or pull the tail too hard, this may occlude the vein, the animal's movement should be restricted but the animal should not be showing signs of stress.

5. Clean the tail with 70% alcohol using a swab
   This is a quick light wipe, using cold fluid on the vein may cause it to restrict.

6. Stabilise the tail between the thumb and forefinger of your non-dominate hand. Hold the tail above (proximal to) the injection site.

Ensure you do not hold or pull the tail too hard (the tail is an extension of the spinal cord), this may occlude the vein or bruise the tail.

7. Ensure the tail is straight
   This will allow you to assess the angle of your injection site.

8. Locate the vein on one side of the tail at mid-length or slightly distal (further down the tail)
   It is best to begin injections at the end of the tail or mid-length. This will mean any further attempts above this site do not leak from previous puncture sites.

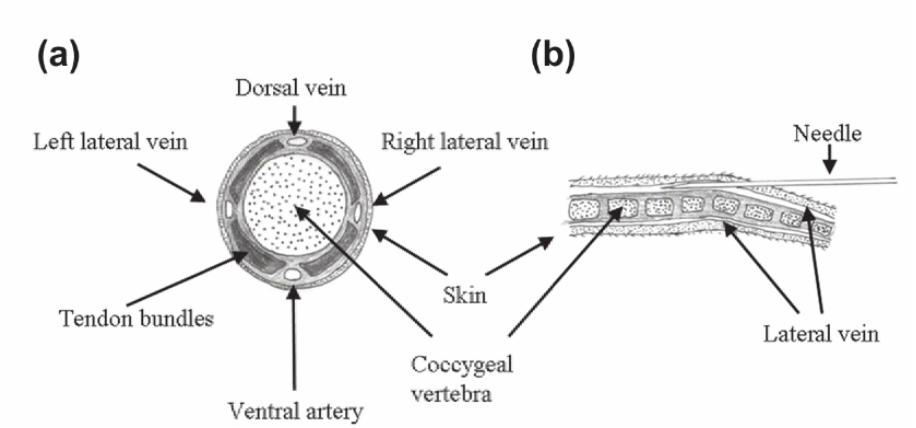

Figure 1 (a) Transverse section view of the mouse tail
(b) Sagittal view of the mouse tail (the tail is turned 90°) (Hedrich 2012)

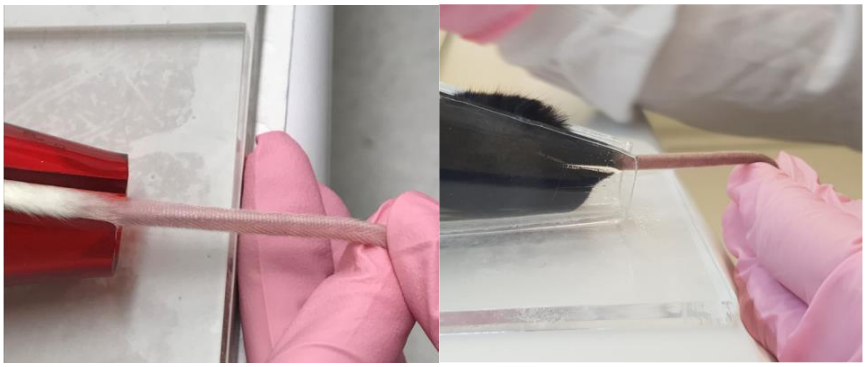

Figure 2 Restraint of the tail after heating

9. Hold the syringe with the dominant hand near the bottom so that the remaining fingers are near the plunger and can easily push the substance plunger without moving the needle in the vein. Insert needle, bevel up, toward animal's head and approximately parallel to the vein.
   The needle placement is shallow and you should be able to see your needle going in, the needle should slide in easily with very little pressure, if you feel resistance in the needle you are not inside the vein. Do not aspirate (pull back on the plunger) as this may cause the vein to collapse. Correct placement may not be verifiable until injection occurs although you may see a flash of blood in hub of needle when it is first placed in the vein.

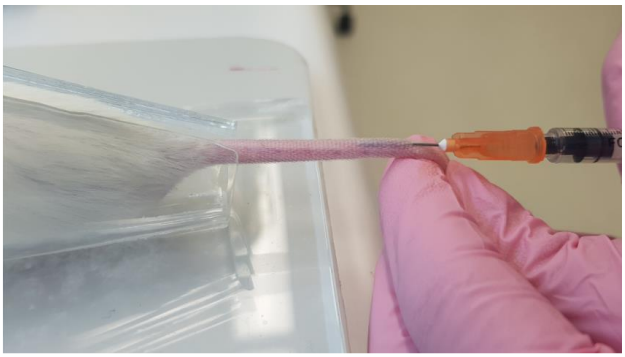

Figure 3 Successful needle placement (UQBR 2020)

10. Inject pre-determined volume slowly. If there is any swelling (blister or bubble) or resistance to injection, stop injecting immediately and remove the needle. Reinsert the needle, choose a site higher up on the tail closer to the animal's body than the site of the previous attempt.
    Refer to approved ethics protocol for volumes, inject the solution at a consistent, steady pace. If the vein blanches all the way to the top of the tail with ease, injection is successful. You may wish to discard the needle and replace with a new one, the sharper the needle the easier it is to successfully complete this technique.

11. Keep the needle in the vein for 5 seconds then remove needle slowly after injection and apply pressure to the injection site to cease bleeding.
    This will also avoid any leakage of the substance. Use clean gauze or tissue to remove any blood.

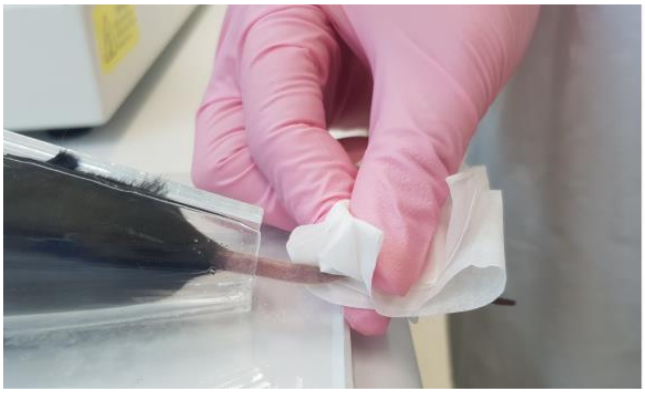

Figure 4. Applying a small amount of pressure to stop bleeding (UQBR 2020)

12. 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.

13. 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.

14. Complete record keeping requirements – a record should be kept of the procedure, date, side of tail, substance, who performed it, and any complications. This record can be kept anywhere appropriate that conforms with the animal code and UQ research policies. A health alert cage card (or similar) needs to be placed indicating which procedure was performed to ensure the animal is monitored carefully for a minimum of at least 2 days. If the study is blinded, the cage card does not need to include substances etc. Record keeping may include UQBR records, lab book, 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.

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

Post-Injection Monitoring

- Animals should be monitored for at least 1 hour following injection
- Animals should be clinically monitored using a score sheet for at least 3 days after the day of injection

Refer to LAB_022 if any abnormal signs are observed. Some indications for veterinary advice following this technique include:

- Sloughing of the tail (skin is shedding or able to be removed from the tail)
- Bruising
- Necrosis (tissue death)
- Skin irritation

These indicators may take 1-3 days to develop.

X. REFERENCE INFORMATION

Injection Considerations

Accuracy - Dependent on many factors, such as the type of substance being administered, small volumes of the injected compound may extravasate from the vein in as many as 20-50% of mouse lateral tail vein injections (Sands & Baker 1999; Groman & Reinhardt 2004).

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.

XI. BIBLIOGRAPHY

1. Groman, Ernest & Reinhardt, Christopher. Method to Quantify Tail Vein Injection Technique in Small Animals. *Lab Anim Sci*. 2004; 43: 35-38.
2. Sands MS, Barker JE. Percutaneous intravenous injection in neonatal mice. *Lab Anim Sci*. 1999; 49(3): 328-330.
3. Hedrich, H. J. (2012). *The Laboratory Mouse* (2nd ed., pp. 1–845). Elsevier Science. https://doi.org/10.1016/C2009-0-60982-X
4. 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
[#]		[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).