 <p>THE UNIVERSITY OF QUEENSLAND AUSTRALIA CREATE CHANGE</p>	<p>UQ Animal Ethics Committee - Standard Operating Procedure</p> <p>LAB_030 Injections - Intravenous (IV) Tail Vein Injection in Mice and Rats</p> <p>Institutional author: UQ Biological Resources</p> <p>AEC Reviewed & Approved: 13th Feb 2020</p>	Version 3
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LAB_030 Injections - Intravenous (IV) Tail Vein Injection in Mice and Rats

I. OBJECTIVE

To describe the IV 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. 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.
- Face visor or safety goggles
- Heat source with ongoing monitoring **
 - E.g. Heat Mat
 - E.g. Infra-red heat lamp
 - E.g. Warmed water (take care with scalding Disinfectant *
- Disinfectant*
- Sharps Container
- Appropriate restraint device
- Adhesive tape or sticky matt
- Needles **
- Syringes
- Substance for Injection**
- Empty cage base

IV. PREPARATION OF EQUIPMENT

1. Check AEC approvals to ensure that the correct procedure and personnel are approved for the planned work

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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. Source the most appropriately sized restraint device for your animals
3. Attach the restraint device to the bench using adhesive tape if required or rubber matting
4. Setup preferred heating source, if you are heating with a heat mat set your empty cage bases on this source
5. Turn on Change station or Biosafety Cabinet *
6. Wipe surfaces with disinfectant *Ensure equipment is operating as required.*

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.

1. Confirm the concentration and volume with the approved AEC protocol
The NHMRC Guidelines for a bolus IV injection volume is 1% of total body weight, any volume larger than this should be clearly cited and justified in the AEC application. For example, a 25g mouse may have up to 250ul injected IV.
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.
2. Unless specific directions are provided in the AEC approved project, refer to NHMRC Guidelines for recommended needle gauge.
Refer to Reference information below for guidance. Changing your needle for each injection is recommended.
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.

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Heating Procedure

Using thermostatically controlled infra-red heat lamp

Rodents must be monitored at all times throughout the heating procedure

A rodent's core temperature is affected by several variables, including:

- Ambient temperature and air flow
- Duration of exposure to heating source
- Intensity of the heat (altered by the heat source distance from the cage and number of mice in the cage)
- The procedure being carried out on them (shaving, opening skin layers can cause a rapid decrease)



Figure 1 Example of temperature controlled heat lamp (UQBR 2020)

Continuous monitoring for signs of over-heating is required. Immediate removal from the heat source is necessary if the below signs of hyperthermia are observed.

- Panting (visible increased in respiration)
- Running from the heat source
- Jumping
- Flattening bodies towards the base of the cage
- General signs of distress
- Over grooming of ears and muzzle if a heat source from above is used

1. Position the heat lamp at an appropriate distance from the animal cage to provide warmth and vasodilation.

A minimum of 15 cm is a recommended distance from the cage

2. Consider any metal components in the cage i.e. wire lids and food hoppers as they may heat up and cause injury such as burns

Remove food hoppers and wires so the animals can be carefully monitored during the heating process

3. Turn the heat lamp on to reach appropriate temperature, set a timer.

35 °C is sufficient heat to dilate the vein. If you cannot leave your hand under the lamp at the level of the cage for approx. 30 seconds, then reduce temperature by increasing distance from the lamp or reducing temperature via control switch.

4. Place the rodent under heating device

Each animal may only be placed under the heat lamp for a maximum of 5 minutes, using a timer is highly recommended to assist in monitoring exposure times.

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Heating Procedure

Using thermostatically controlled heat mat

Rodents must be monitored at all times throughout the heating procedure

1. Position the heat mat underneath a clean empty cage
2. Turn the heat mat on and set the timer for 2-5 minutes aiming for 30°C cage environment

Using a timer is highly recommended to assist in monitoring exposure times.

3. Check the vein for vasodilation

Check that the vein on either side of the tail is easily visualised, in black or darker animals this can be more difficult. Check at the top or bottom of the tail where the pigmentation is usually lighter.

4. Once vasodilation is adequate remove the rodent

The heat mat may remain turned on for any remaining animals however continuing to closely monitor these animals.

Considerations:

- Heat mats with no insulation should not be placed directly onto stainless steel surfaces as the heat may be lost.

Place a rubber or foam mat or bench coat under the heat mat.

- If animals are to be held for extended periods of time, freedom from the heat source should be provided.

This can be achieved by placing the cage half on/off a heat mat.



Figure 2 Examples of temperature controlled heat mats (UQBR 2020)

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Heating Procedure

Using Water Immersion

Rodents must be monitored at all times throughout the heating procedure

Note this is an alternate method used when approved by the AEC and when other equipment is not available.

1. Use running water to fill an appropriate sized container with water at no more than 45°C. Water temperature must be measured using a thermometer.

Ensure both the tube/container and water are clean and free from contamination. If you are using an immunocompromised strain sterile water can be placed in the tube, then allow hot water to run over the tube until it is warmed.

2. Measure the water temperature and if at the correct temperature place the rodent tail into the tube

It is easiest to already have the rodent in the restraint for this step

3. Monitor for vasodilation.

As a guide 15 -30 seconds should be adequate.

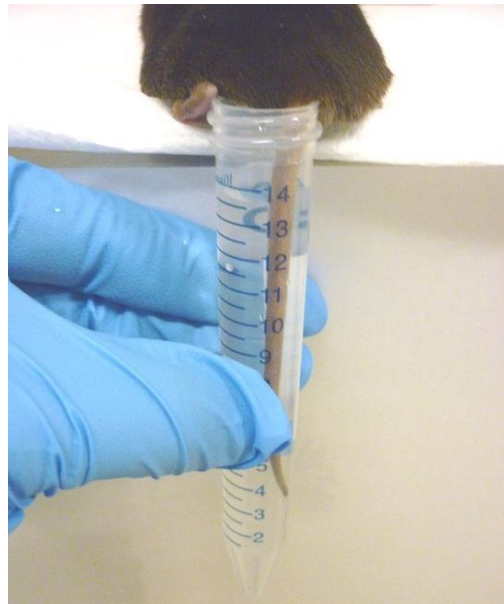


Figure 3 Demonstrating placement of tail into a tube with warmed water (UQBR 2020)

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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. Anaesthetise the animal **

4. Use heat to dilate the tail veins which run laterally along each side of the tail

The increased blood flow will help with visualising the veins and improve injection success rates

5. Restrain the animal in a restraint device if animal is not anaesthetised

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.

6. 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.

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

Ensure that you do not hold or pull the tail too hard occluding the vein or injuring the animal Digital pressure will act as a tourniquet.

8. Put slight tension on the tail ensuring the tail is straight

This will allow you to assess the angle of your injection site.

9. Locate the vein on one side of the tail at mid-length or slightly distal (further down the tail)

In dark pigmented rodents a light source may help to highlight the vein. 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.

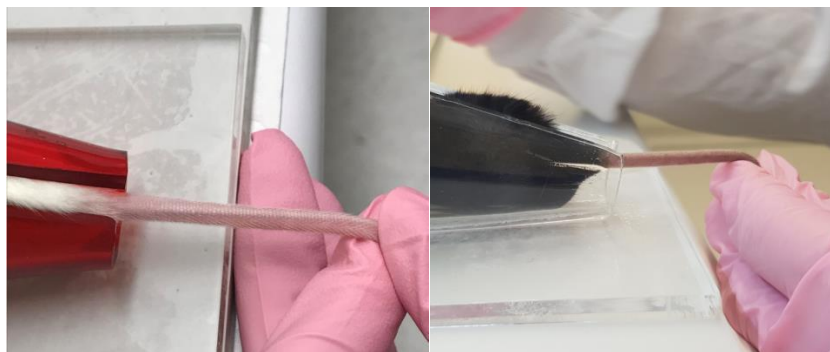


Figure 4 Restraint of the tail after heating (UQBR 2020)

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10. 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 5 Successful needle placement (UQBR 2020)

11. 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. No more than 3 attempts per tail can be done.

12. 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.

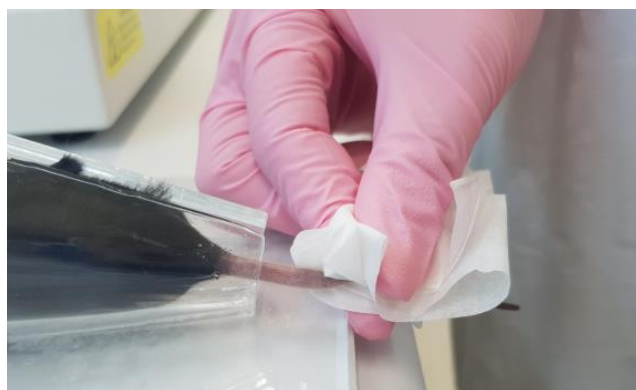


Figure 6. Applying a small amount of pressure to stop bleeding (UQBR 2020)

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13. 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.

14. 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.

15. 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.

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

Post-Injection Monitoring

Factors contributing to a reaction at the tail injection site or necrosis (tissue death) include:

- Heat burns e.g. on ears, along the back or tail
- Formulation of substance
- Not using an aseptic technique
- Too many attempts/punctures to the tail vein

These factors may result in reactions post-injection; necrosis may be observed 1-3 days following injection. If this occurs, follow UQBR Veterinary Care Program.

If injecting cells, it is important to monitor for instances of embolism in capillaries after injection.


VI. REFERENCE INFORMATION

Table 1. Recommended values for Mice and Rat IV Injections (NHMRC 2008)

Values	Mouse	Rat
Needle Gauge	25-30G	23-26G
Needle Length	13-25mm	25mm
Max Injection Volume	1% of bodyweight in a bolus injection	1% of bodyweight in a bolus injection

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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.

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 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.

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VII. REFERENCES

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8. UQ Biological Resources, 2020 *IV Injections*.

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