LAB_019 Blood Collection – Tail Bleed in Rats and Mice (Expiry: March 2026)

I. OBJECTIVE

To describe the standard techniques of blood collection from the tail of rodents, to standardise practice for UQBR staff and researchers within UQBR facilities. This blood collection SOP describes:

- "Tail prick" [venepuncture of the lateral tail vein with a free-held needle] and,
- Needle & syringe [venepuncture of the lateral tail vein with a needle connected to a syringe].

This blood collection SOP **does not describe**:

- "Tail tip" [soft tissue amputation of a small portion of the distal tail]. For this technique, please refer to LAB_013 Blood Collection – Tail Tip (Amputation) Bleed in Rats and Mice

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." (as per, Australian code for the care and use of animals for scientific purposes, 2013)

"Minor" vs "moderate" vs "major" bleed – please refer to table 1 (within VII. Reference Information).

III. COMMENTS/RECOMMENDATIONS

- Relative to animal ethics applications, when using this SOP, the following must be described in the individual ethics application:
  - the frequency and total number of blood collection events,
  - if multiple "minor bleeds" are planned, how will you monitor or support the animal to ensure significant adverse effects do not occur (for veterinary advise: br.vetservices@uq.edu.au),
  - if anaesthesia is being performed, what are the anaesthetic details (including drug selection and doses),
  - any variations intended to this SOP.
- Aseptic technique is necessary when performing this technique to minimise the risk of inadvertent inoculation of research animals (with incidental pathogens) and contamination of biological samples
- A new sterile sharp instrument should be used for each animal
- Rodents should be held in the restraint tube/device for the shortest period required to obtain a viable blood sample. Continuous restraint should never exceed 5 minutes per animal.
- Tail vein sampling is generally suitable for small volumes of blood (e.g. <20 ul)
- A maximum of 3 venepuncture attempts may be made per vein (during any one session)
- Please note: UQ Biological Resources offers training courses to all staff and researchers (including anaesthetic training). For more information email uqbrtraincomp@uq.edu.au

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
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Safety

- Users should further read and understand all relevant risk assessments prior to operation: e.g. 3657 UQBR Handling and restraint of laboratory animals; 3940 Handling rats and mice; 4020 Rodent anaesthesia using isoflurane (available on the UQSafe website).
- All incidents/injuries should be reported to your supervisor, the animal facility manager and via UQSafe online. Potential incidents/injuries include, but are not limited to, needle stick, rodent bite, and musculoskeletal repetitive-strain injury (if performed regularly)

IV. EQUIPMENT

- PPE *
- Change station/Bio-safety cabinet *
- Disinfectant*; including 70% ethanol
- Heat source – heat plate, heat lamp, warm water
- Restraint tube (or equivalent species and age specific restraint device)
- Lubricant for tail massage (if required)
- Needle (25-30G)
- Syringe (0.3 – 1mL) (if performing needle and syringe blood collection)
- Blood collection receptacle – "sample tube" (e.g., Eppendorf tube, capillary tube)
- Tissue / gauze
- Clinical waste bin and sharp's container

V. PREPARATION

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. Turn on Change station or Biosafety Cabinet* and clean with disinfectant
3. Ensure all required equipment is set up and ready for use
   *This includes anaesthetics, if relevant to your protocol**; sample collection tubes (e.g. are they labelled, and do they require anti-coagulant); any relevant monitoring records*

VI. PROCEDURE

"TAIL PRICK" and NEEDLE & SYRINGE BLOOD COLLECTION TECHNIQUES

1. Ensure you have the correct rodent for this procedure
   Check identification marks and ensure this matches the labelling on the collection tube.
2. Apply gentle warming heat to the animal(s) for 2 to 10 minutes. This will cause peripheral vasodilation, making venous access much easier, and less invasive for the rodents.
   a. If using a heat plate, remove the animal from the home cage and place it into a cage without bedding placed on top of the heat mat (this method may take several minutes to cause vasodilation).
   b. If using a heat lamp, CAUTION: lamps can cause overheating and can burn rodents if used inappropriately. Rodents must be monitored throughout. Also, relative to the type of heat lamp being used, a safe distance and duration of exposure must be established. As a guide, a 100W red bulb can be used with rodents in an opaque cage 20-25cm away from the lamp, with a maximum exposure time of 15 minutes.

c. If using a warm water bath, maintain the water temperature between 37-40°C. Gently restraining the animal submerge the tail in the water for 1-2 minutes, then dry the tail with gauze.

Rodents that are experiencing mild-moderate "overheating" may demonstrate redness of the ears and muzzle, and agitated behaviour, including unusual jumping, cleaning of the face and ears, and head shaking.

Figure 1 – Mice on a heating plate

3. Ensure the sample tube is labelled and open, ready for blood collection.
4. Place the rodent into a restraint tube allowing its tail to hang freely. Clean the venepuncture site by gently rubbing with an ethanol-soaked cotton tip or gauze swab. Allow the ethanol to dry.
   Spraying or splashing the venepuncture site with ethanol should be avoided as it often causes excessive wetting of the fur, may splash into the eyes of the animal, and does not actually clean the skin appropriately.
5. The tail may be "massaged" from its base to promote vasodilation. To do this a sufficient volume of lubricant must first be applied to the base of the tail. The thumb and forefinger then grasp the tail from its base and using the lubricant, "glide" along the tail towards the tip without causing friction with the skin. Friction with the tail skin can result in significant tail-pull or degloving injuries to rodents.
   The application of lubricant must be controlled as insufficient volumes may enable friction with the skin and excessive volumes can occlude the venepuncture site and contaminate the sample. [NB: some personnel will use a non-polar lubricant, such as Vasoline®, and allow the blood to bead on top of the lubricant. Always ensure the lubricant used is non-toxic and will not contaminate samples collected].

Figure 2 - Applying lubricant to the tail

6. To perform venepuncture, hold the tip of the tail between your thumb and forefinger of your non dominant hand, gently straightening out the tail but without causing strain to the tissue. The left and right lateral veins should be visible (running down either side of the tail).
    a. For the **"tail prick" technique** - using a 25G needle gently puncture the lateral tail vein. The needle should be advanced through the skin at an angle anywhere between 25-90° with the skin surface.

PLEASE NOTE: using a lancet or #11 scalpel blade for this technique is not standard UQBR procedure. If this technique is to be used training and competency assessment within your lab group must carefully consider how venepuncture is performed; the lancet should be advanced through the skin at ~90° angle in a "poking" action, as compared to a "slicing" action. "Slicing" will unnecessarily transect soft tissue surrounding the vein, causing avoidable and unnecessary harm to the rodent. NC3Rs provides a videographic example of this technique being performed on their webpage: https://www.nc3rs.org.uk/mouse-tail-vein-non-surgical

    b. For the **needle/syringe technique** - using a 25-30G needle connected to a syringe, gently advance the needle tip through the skin and into the lateral tail vein. The needle's bevel should be "facing up" and the needle advanced at ~25° angle to the skin.

The initial site of venepuncture should be made in the distal portion of the tail (i.e. close to the tip of the tail). This is because if repeat blood collection attempts are required, they should be made proximal to the previous venepuncture site.

Figure 3 – Puncture of the lateral tail vein, via the **"tail prick" technique.**

7. To collect blood, securely maintain the position of the tail within your non dominant hand and take the following action with your dominant hand;
    a. For the **"tail prick" technique** – As beads of blood form at the surface of the skin use a sample tube to collect the droplets as they form. If the blood is not readily flowing a pipette may be used to collect the droplets from the incision site.
    b. For the **needle/syringe technique** - Once the needle's tip is within the lumen of the vein gently pull on the syringe's plunger withdrawing only a few microlitres into the syringe. Very slowly continue to withdrawal the syringe's plunger until the desired sample volume is obtained. Rapid withdrawal of the syringe's plunger will cause the vein to "collapse" and may result in a failed blood collection.

Note for UQBR Training purposes, 0.05% of body weight in blood volume will be collected, e.g. 10µl for a 20g rodent).

8. If required, the tail can be gently "massaged" to assist with blood flow – as per step 5.
Ensure sufficient lubricate is present to enable the thumb and fore finger to "glide" without friction.

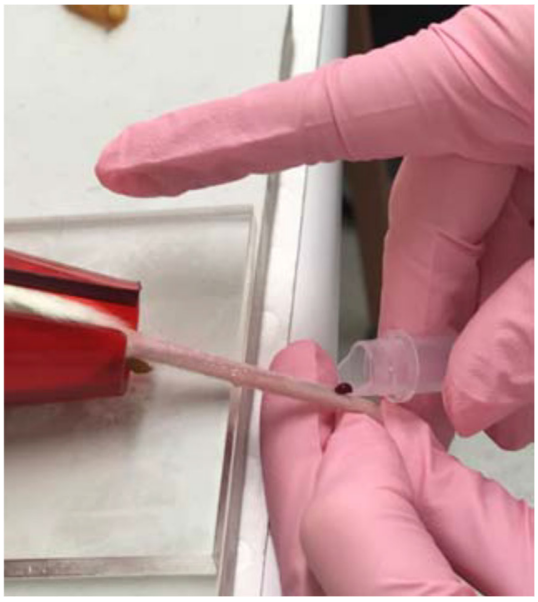

Figure 4 – Blood collection from the lateral tail vein, via the **"tail prick" technique.**

9. Place sharps into the sharp's container and close the sample tube. (If the **needle/syringe technique** was used, the blood will first need to be transferred from the syringe into the appropriate sample tube).
   Ensure the sample tube is not contaminated with fur or dander upon closing.
10. Using a tissue or gauze apply gentle pressure venepuncture site until bleeding has ceased.
    Bleeding should cease easily, usually within 30 seconds.
11. Release the rodent into the holding cage and continue to monitor for recovery and health
    Following venepuncture, the animal should return to normal movement and behaviour. If continued bleeding is observed, repeat step 10. Despite this if bleeding continues seek veterinary advice, refer to UQBR SOP 22 Veterinary Care Protocol.
12. Complete record keeping requirements:
    a. on the cage card: procedure name, date, and initials;
    b. research log books or monitoring records may require: procedure name, date, and initials, animal ID, approximate volume of blood collected.

    Records need to be clear and legible, especially on the cage cards.
13. Store the sample as required (e.g. refrigeration).
14. Repeat from step 1 for the next animal or if finished, pack and clean up equipment and space.

VII. REFERENCE INFORMATION

Table 1. Recommended blood collection volumes based on a mouse's live body weight (NHMRC 2008).
The total amount of blood loss from any blood collection procedure must take into account the sample volume collected as well as any circumstantial haemorrhage (e.g. prolonged bleeding post venepuncture). This total amount of blood lost must be used in relation to this table, not just the desired sample volume.

Mouse Weight	**TOTAL BLOOD VOLUME (TBV)** *[equates to 5-7% of body weight]*	**Minor bleed** *(<7.5% of TBV)*	**Moderate bleed** *(7.5-10% of TBV)*	**Major Bleed** *(10-15% of TBV)*
Recovery period required between bleeds, relative to volume collected:		1 week recovery	2 weeks recovery	3 weeks recovery
18g	1.2mL	<90uL	90-120uL	120-180uL
22g	1.5mL	<115uL	115-150uL	150-225uL
26g	1.8mL	<140uL	140-180uL	180-270uL

Signs of acute blood loss

Animal appears to be weak/cold/pale after blood collection.

Treatment

Seek Veterinary advice. Commonly treatment may include providing warmth and delivering a single dose of up to 5% of body weight in warmed (to ~37°C) sterile normal saline fluids via subcutaneous injection. If the animal is able to eat, then high energy "wet booster" foods may also be of assistance.

Post Procedure Monitoring

If discomfort is observed refer to the UQBR SOP 22 Veterinary Care Protocol.

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.

VIII. BIBLIOGRAPHY

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8. UQ Biological Resources, 2019 *IP Injections.*

Version #	**Reviewing AEC** (note: all other relevant AECs ratify the approval)	**AEC Review Date**	**Approved Until**
5	ABS	09/03/2022	09/03/2025

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