LAB_013 Blood Collection – Tail Tip (Amputation) Bleed in Rats and Mice

I. OBJECTIVE

To describe the UQBR standard technique of blood collection via

- "tail tip" [soft tissue amputation of a small portion of the distal tail] in rodents.

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

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

for these techniques, please refer to LAB_019 Blood Collection – Tail 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).

Pre-weaned pups – rodents not yet separated from their mothers. This separation, termed "weaning" routinely occurs at approximately 21 days of age.

III. COMMENTS/RECOMMENDATIONS

Position statement on the use of this technique (including conditions for general anaesthesia):

There is a use for this technique, however, its use must be justified.

Tail tip (amputation) is broadly considered a standard procedure in laboratory animal research, as it is quick, reliable, and facilitates minimally invasive repeat sampling (when a scab can be removed, without having to re-cut newly formed skin with a scalpel). Under such circumstances **repeat blood collection via this method may be considered to present less of an impact to animal wellbeing than blood collection via repeat venepuncture**, such as that described in LAB_019 Blood Collection – Tail Bleed in Rats and Mice. A common example of this application being glucose tolerance testing, whereby frequent repeat blood collection is required. On the other hand, the initial cutting of the tail in this technique is a form of soft tissue amputation which should be **considered a crude method of blood collection**. If performed inappropriately this technique will cause unnecessary pain and suffering and carries potential for complications such as prolonged pain in the animal (e.g. neuroma formation), thus highlighting the **critical importance of appropriate training and competency**. When using this SOP staff must genuinely reflect on their animal use procedures and have confidence that their use is as "refined" (3Rs) as possible - we all carry this ethical obligation when using animals for scientific purposes. Given the expected tissue damage and associated discomfort, this technique may cause in animals over the age

of 21 days, **this SOP prescribes the use of general anaesthesia when performing tail tip (amputation) in rodents >21 days old.** Following initial amputation, a scab will form at the end of the tail which will be present for ~48hrs. For repeat blood collection, if this scab only needs to be removed (without a scalpel) to collect blood, then general anaesthesia is not considered to be required. If, however, new sensitive tissue has begun to grow over the wound, and repeat cutting of innervated skin (with a scalpel) is required to collect blood, repeat general anaesthesia is considered to be required (e.g. if repeat blood collection is occurring 7 days following initial amputation). **Prospective research projects that wish to use this SOP in rodents over 21 days of age without general anaesthesia must specifically identify this proposed variation to the AEC and provide ethical justification** – i.e. why they believe this method to be the most refined option for application in their model.

- 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,
  - the measures that will be taken to monitor and support the animals (e.g. fluid support) if collecting volumes that would constitute a "major bleed" (see references, table 1); either due to a singular large bleed or many, frequent "minor bleeds",
  - any relevant anaesthetic details (e.g. LAB_060 Rodent Anaesthesia - Isoflurane),
  - any variation 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.
- Please note: UQ Biological Resources offers training courses to all staff and researchers (including anaesthetic training). For more information email uqbrtraincomp@uq.edu.au

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)
- Lancet or Number 11 scalpel blade
- Blood collection receptacle – "sample tube" (e.g., Eppendorf tube, capillary tube)
- Tissue / gauze
- Clinical waste bin and sharp's container

- If applicable, anaesthesia equipment as per LAB_060 Rodent Anaesthesia - Isoflurane

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 TIP" (AMPUTATION) BLOOD COLLECTION TECHNIQUE

1. Ensure you have the correct rodent for this procedure
   Check identification marks and ensure this matches the labelling on the collection tube
2. Ensure the sample tube is labelled and open, ready for blood collection.
3. If rodent is older than 21 days of age, anaesthetise the animal and ensure sufficient dept of anaesthesia, as per LAB_060 Rodent Anaesthesia – Isoflurane.
4. Place the rodent onto a clean, hard cutting surface. If the rodent is conscious secure restraint is required.
5. Clean the tip of the tail by gently rubbing an ethanol-soaked 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, does not actually clean the skin appropriately.
6. Gently secure the distal end of the tail against the cutting surface, using the thumb and forefinger of your non-dominant hand. In this position the fingers immobilise the tail and act somewhat as a tourniquet.
7. Using a fine scalpel (e.g. #11) transect no greater than 1mm of soft tissue from the tip of the tail. This technique must not involve bone, or bony joints: it is exclusively limited to amputation of the soft tissue tip of the tail.
   The length of tail amputated should be as little as possible to permit blood collection. This length will vary from ~0.2mm up to 1mm.
8. Let the blood flow naturally into the collection tube.
   Note for UQBR training purpose 0.05% of body weight in blood volume will be collected, e.g. 10 µl for a 20g rodent.
9. Place sharps into the sharp's container and close the 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 for at least 30 seconds and until bleeding has ceased.
11. If the rodent was anaesthetised, as per LAB_060 Rodent Anaesthesia – Isoflurane, place the animal into a recovery box and continuously monitor, maintained on a heat mat until fully ambulatory.
    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 bleeding (e.g. prolonged bleeding post venepuncture). The total amount of blood lost must be used in relation to this table, not just the 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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7. UQ Biological Resources, 2019 *IP Injections.*

Version #	Reviewing AEC (note: all other relevant AECs ratify the approval)	AEC Review Date	Approved Until
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