LAB_037 Blood Collection – Pedal Vein in Bleed Mice (Expires Dec 2024)

I. OBJECTIVE

To describe the pedal vein blood collection method 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 (most likely blood or anticoagulant) follow the facility emergency spill procedures.

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.
- Disinfectant *
- Sharps container
- Capillary tube
- Lubricant
- Topical anaesthetic ** [e.g. Emla cream®]
- Clinical waste bin
- Change station or Biosafety Cabinet
- Restraint device
- Needle (26-30G)
- Heat Source – heat mat, heat lamp
- Blood collection tube or slides

IV. 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. Set up equipment items
   There should be no contamination of needles or samples tubes during this process.
3. Turn on Change station or Biosafety Cabinet *

4. Wipe surfaces with disinfectant

   Ensure equipment is operating as required. Disinfect tools that will contact the animals, or sterilisation where relevant.

5. Setup heat source **

Aseptic Technique

Use an aseptic technique when performing procedures, this will minimise contamination from pathogens and subsequently infection in research animals.

IV. PROCEDURE

Pedal Vein Bleed Procedure

1. Check that the sample collection tube type is correct. *E.g. does it require anti-coagulant?* Open the sample collection tube so it is ready for blood to drip into or ready capillary tube.
2. Ensure you have the correct mouse for this procedure – *check identification marks and ensure this matches the labelling on the collection tube.*
3. Restrain the animal – refer to UQBR SOP 6 Handling and Restraint in Mice and Rats.

   It is important that the leg is restrained sufficiently to prevent movement during the procedure.
4. Apply topical anaesthetic ointment ** to the top of the paw on the area you will bleed from if stated in the ethics. This will normally need 15 minutes to take effect, the animal may be placed back into the home cage

   A lignocaine gel e.g. Emla cream is commonly used
5. Warm the mouse, this will assist in making the vein prominent *– if you are using a heat mat, remove the animal from the home cage and place in a cage without bedding, if a heat lamp is in use ensure this is at a distance that the animal is not over heating, watch the ears and muzzle for redness, mice will also jump or clean their ears, shake their heads if overheating.*
6. Restrain the mouse using a restraint tube or cone, ensure the tube or cone has a large enough gap to easily slide the hind limb out. Avoid prolonged restraint.

   The opening should be large enough that the mouse can pull its own leg back through, some restraint of the paw will be needed, the pedal vein runs along top of the foot.
7. Clean site with ethanol allowing ethanol to dry. *It is best to use an ethanol wipe or tissue soaked in ethanol to avoid splashing the animal's eyes or over use wetting the fur.*
8. With your thumb and first finger hold the paw around the ankle, *your thumb should be on top of the foot, the medial dorsal pedal vessel is found on the top of the foot. It is important to ensure you do not over extend the limb as this can lead to injury or further discomfort, the leg should hang at a natural angle.*

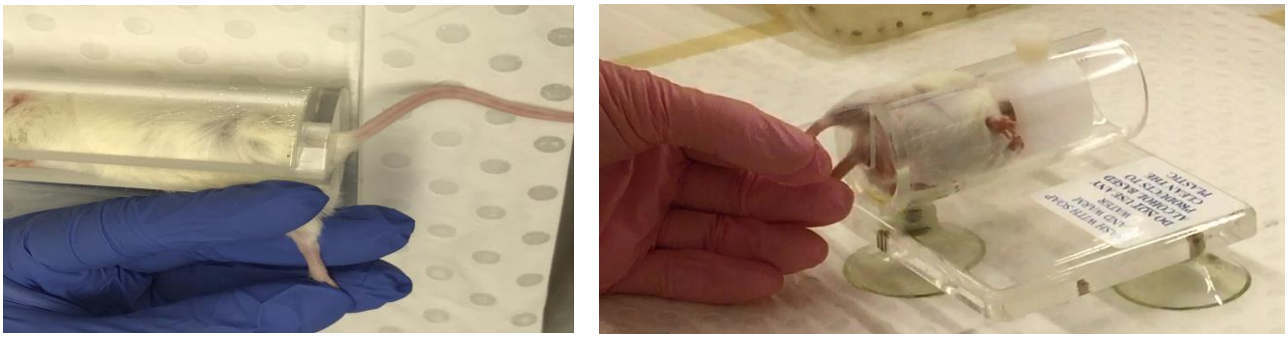

Figure 1 Restraint examples to complete Pedal vein blood collection (UQBR 2019)

9. Apply petroleum jelly or eye lubricant to the foot.

   This may assist the blood to bead and in turn enhance total blood volumes collected. Ensure you only use a small amount of lubricant as large amounts can prevent the blood from flowing or mix with the sample.

10. Gently puncture the pedal vein using your needle.

    The needle can be held like a pencil and angled almost level to the surface of the skin. Please note, the NHMRC guidelines prescribe a 26-30G needle for blood collection, however, many UQBR staff prefer to use a ½ inch (length), 30G needle.

    You may need to release your fingers from around the ankle to ensure blood flows, if this is necessary very gently hold the paw. Blood should drip from the site of puncture. If this does not occur this is commonly due to the puncture being too shallow or the site being incorrect – No more than 3 attempts should be performed per paw.

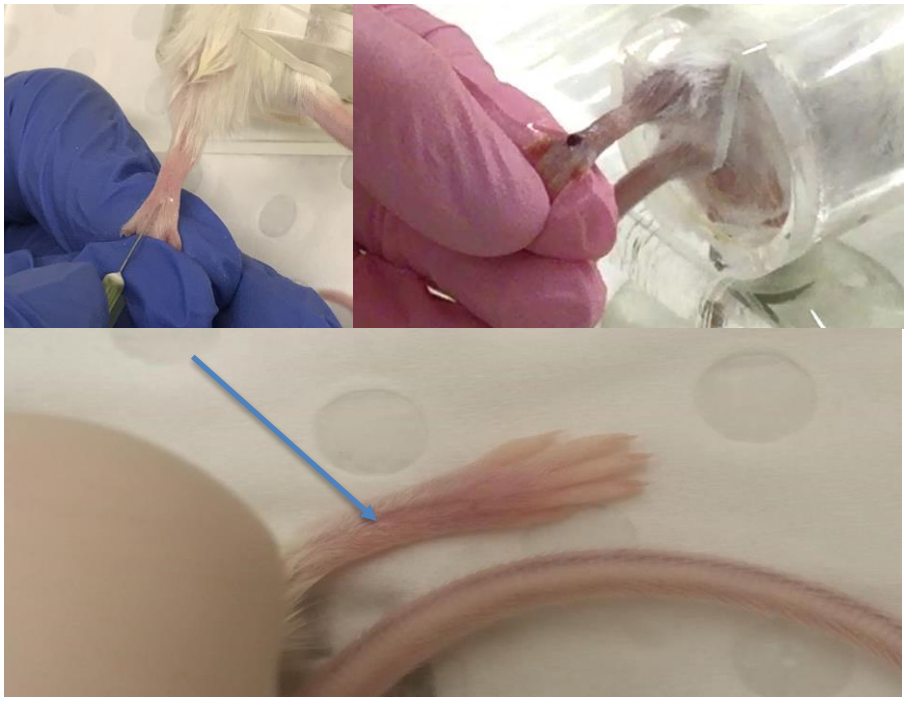

Figure 2 Location of Pedal Vein and puncture site (UQBR 2019).

11. Collect blood into a capillary tube, pipette, or paper test kit to collect the droplets as they form. Note for UQBR Training purposes, 0.05% of body weight in blood volume will be collected, (e.g. 10µl for a 20g mouse)

    Take care not to contaminate the tube with urine/faeces etc. Blood is collected by capillary action into a haematocrit tube. This method is ideal for collection of approximately 50uL (from the adult mouse). If the blood is not flowing very gently massage the area above the puncture site in a downward direction, avoid touching the puncture site.

12. Place sharps into sharps container
13. Apply pressure to the puncture site with a tissue/gauze before releasing into the cage.

    This will reduce the amount of blood loss and decrease stress with re-handling. Do not squeeze the paw as they are fragile and you can cause injury, simply apply a clean tissue or gauze and hold the area.

14. Release rodent into holding cage and continue to monitor for recovery and health

    Following the procedure the animal should return to normal movement and behaviour. If you observe the animal not bearing weight on the foot or excessive bleeding seek veterinary advice.

    In the rare case that an animal appears weak refer to treatment section below. The volume of blood collected should be reviewed as per SOP reference information prior to sampling the next animal. Refer to UQBR SOP 22 Veterinary Care Protocol.

15. Place needle into sharps container and close the sample collection tube.

    The sample collection tube should be closed without contamination and stored appropriately (e.g. refrigerated if required by the research), a new needle should be used for each animal.

16. 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. *Records need to be clear and legible on each record to allow others to read and understand.*
17. Store the sample as required (e.g. refrigeration).
18. Repeat from step 1 for the next animal or if finished, pack and clean up equipment and space.

V. REFERENCE INFORMATION

Table 1. Recommended blood collection volumes based on a mouse's live body weight (NHMRC 2008).

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/col

d/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 degrees) saline fluids via subcutaneous or intraperitoneal injection. If the animal is able to eat then nectar/wet boost food 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.

VI. REFERENCES

1. 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-usedscientific-purposes
2. Office of the Gene Technology Regulator (OGTR) n.d., viewed 11 April 2019, http://www.ogtr.gov.au/

3. University of Queensland n.d., *Health, safety and wellbeing,* viewed 11 April 2019, https://staff.uq.edu.au/information-and-services/health-safety-wellbeing
4. University of Queensland n.d., *Incidents, injuries and hazard,* viewed 11 April 2019, https://staff.uq.edu.au/information-and-services/health-safety-wellbeing/health-safetyworkplace/incidents-injuries-hazards
5. UQ Biological Resources n.d., *UQBR SOP's, viewed 11 April 2019, https://biologicalresources.uq.edu.au/secure/reference-information#SOP's*
6. UQ Biological Resources, 2019 *Restraint for Sub-Mandibular Bleed.*