LAB_036 Blood Collection – Saphenous Vein in Mice

I. OBJECTIVE

To describe the saphenous 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
- Clinical waste bin
- Change station/Bio-safety cabinet *
- Equipment for hair removal **
- Restraint device*
- Needle (26-30G) **
- Blood collection tube or capillary tube
- Petroleum jelly

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

Saphenous 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.
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. It can be useful to use a restraint device to assist in preventing movement of the mouse. Ensure the restraint is clean to prevent pheromones induced stress or potential cross –infection.

   The hind leg can be extended by applying light pressure above the knee joint, this will also stretch skin over the ankle, improving access to remove hair from the ankle and immobilising the ankle.

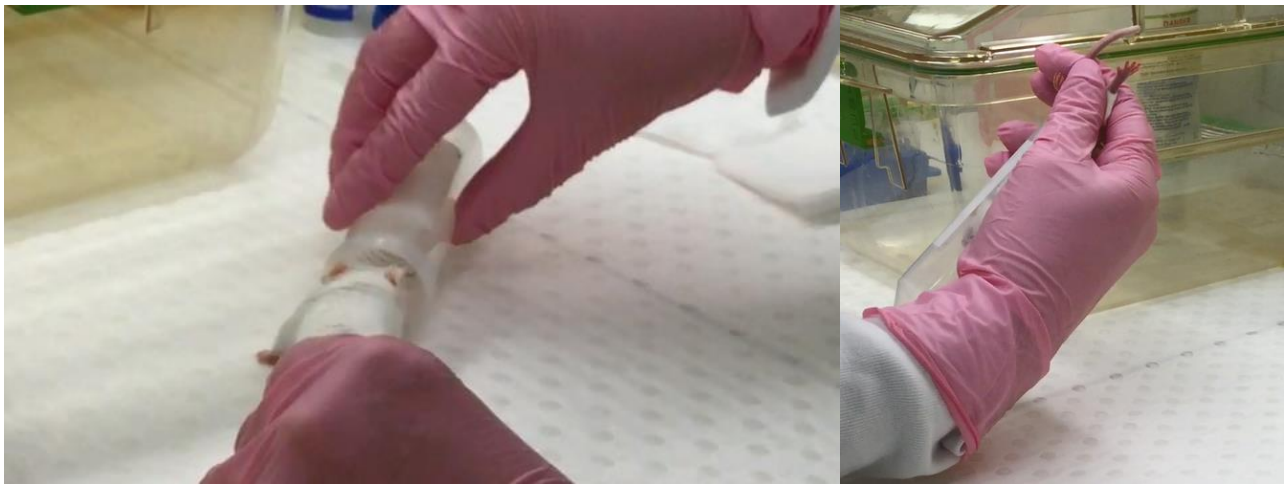

Figure 1 Appropriate restraint for this technique (UQBR 2019).

4. Remove hair from the ankle area – Refer to UQBR Guideline 3 Rodent Hair Removal
    a. The method of hair removal that may be used for training purposes in this technique include hair plucking, clipping, and use of depilatory cream.

    The lateral saphenous vein runs dorsally and then laterally over the tarsal joint.

    The area of hair removed should be minimal while still allowing for effective outcomes of the procedure. Using clippers or depilatory cream are the preferred methods for this procedure. Avoid hair removal methods that remove epidermal layers of the skin such as shaving.

5. Prepare skin using a clean technique to remove excess hair

    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.

6. Consider applying a small amount of petroleum Jelly to the site with a clean tissue or similar.

    Application of petroleum jelly to the site may assist the blood to bead and in turn increase the total blood volume collected.

7. Puncture the vein swiftly (<1s) to a depth of 1-2mm.

    Only use the appropriate sized needle (26-30G). A needle that is too large can cause more trauma and higher blood volume resulting in haemorrhage and decreased circulating blood volume.

    The needle should be held like a pencil while resting your wrists together to provide stability during puncture. 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 incorrect site. No more than 3 attempts should be performed per side. Sometimes the puncture wound in the vein and skin may become mal-aligned if the animal moves it leg. In this case, a bruise will develop, do not attempt another puncture on this leg.

8. Collect blood sample into capillary tube, holding the capillary tube over a blood collection tube to capture the sample.

    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 ~50uL (adult mice) and maximum once per fortnight.

    This method should not be used for more frequent sampling. The mice will often bleed a few more drops after collection and thus equalling up to the maximum volume. This must be included when assessing the volume of blood collected per mouse. Excessive turbulence of the blood during collection will rupture RBC and the serum or plasma sample will be contaminated with haemoglobin.

9. Apply pressure to the site using gauze or a tissue to slow blood flow.
10. Release rodent into holding cage and continue to monitor its health.

    Animal is returned to cage to recover and monitored for normal movement and behaviour. Minor bleeding should have ceased within ~15s after release and this should be the case for ~99% of animals.

    In the rare case that bleeding continues, animal should be restrained again and a piece of gauze/tissue can be firmly pressed to the site for 30-60s to encourage clotting at the site. Do not apply and remove pressure frequently (e.g. every 5s) as this disrupts the clotting process.

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.

11. Place needle and capillary tube 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 and capillary tube should be used for each animal.
    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.
12. Store the sample as required (e.g. refrigeration).
13. 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.*