LAB_020 Blood Collection – Facial Bleed (Sub-Mandibular) in Mice (Expiry: March 2026)

I. OBJECTIVE

To describe the facial 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 *
- Blood collection tube or capillary tube
- Lancet 3-5mm, depending on size and age of the animal

IV. PREPARATION

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 rodent 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 head is restrained sufficiently to prevent movement during the procedure. It can be useful to scruff the animal with a 'pinch' grip with thumb and forefinger, placing the third finger on the opposing cheek to prevent the mouse from turning its head away. Consider if you are left or right handed.

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

The inferior facial vein can be located by using the 'hair whorl' that is distinguishable on most rodents. Move away from the nose and above this landmark. Puncture can be performed on either side of the head.

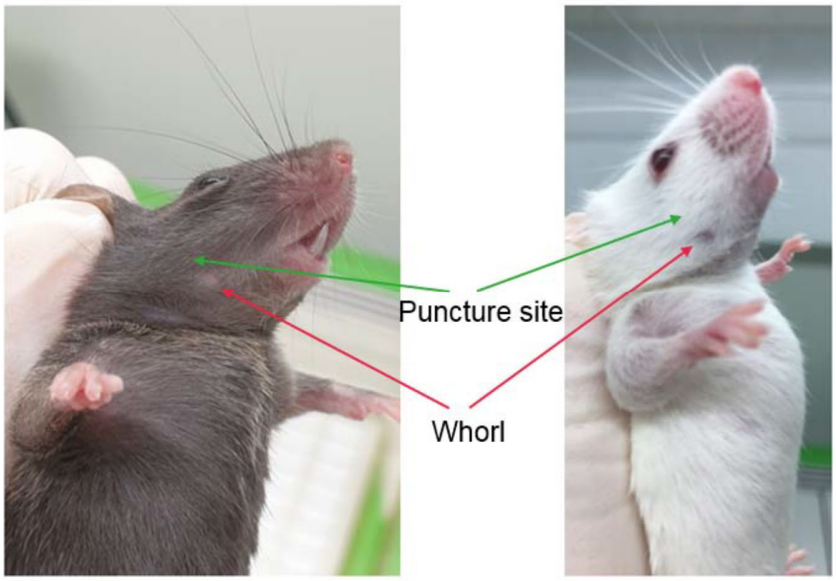

Figure 2 Location of the whorl as an anatomical landmark for the puncture site in white and black mice (UQBR2019).

4. Position the animal above the sample collection tube in preparation for puncture.

   Taking care not to contaminate the tube with urine/faeces etc. The lancet should be held like a pencil and it can be beneficial to rest the wrists together to provide stability during puncture. The lancet should be angled to puncture perpendicular (90 degrees) to the surface of the skin.

5. Puncture the vein swiftly (<1s) to a depth of 2-3mm.

The angle of the lancet should be perpendicular to surface of the skin/cheek. 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 side.

Consider the appropriate sized lancet, a lancet that is too large can cause more trauma and higher blood volume resulting in haemorrhage and decreased circulating blood volume.

During the training stage, individuals may be trained in anaesthetised animals, progressing to conscious animals depending on the trainee's skills and confidence. Sufficient blood should be drawn on the first or occasionally (<10%) the second attempt.

6. Collect blood sample into blood collection tube.

   The exact quantity of blood that drips from the site can be difficult to control – this method is ideal for collection of up to 200uL in the adult mouse, observing the recovery periods specified in table 1. This method should not be used for more frequent sampling or methods that only require much smaller volumes of blood. 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. If insufficient blood is collected recheck the site and depth of puncture.

7. Release rodent into holding cage and continue to monitor its health.

   If a reasonable or high flow of blood was collected apply pressure to the site with a tissue/gauze for 20-30 seconds before releasing into the cage, this will reduce the amount of blood loss and decrease stress with re-handling the animal.

   Animal is returned to cage to recover and monitored for normal movement and behaviour. Animals should clean their faces and bleeding should have ceased within 15 seconds after release.

   If blood is seen coming from the mouth or ears then puncture has been too deep – most animals will recover from this without further adverse effects if left undisturbed. In the rare case that bleeding continues, the animal should be restrained again and a piece of gauze/tissue securely held to the site for 30-60s to encourage clotting. Ensure pressure is consistent and firm, but not hard.

   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. The trainee should demonstrate knowledge of common first aid measures for acute blood loss. Refer to UQBR SOP 22 Veterinary Care Protocol.

8. Place lancet 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 lancet should be used for each animal.

   Sharps must be handled safely and placed in clear view when preparing for use. The sharps bin needs to be placed away from the immediate working area to prevent accidental injury but close enough to access.

9. 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.

10. Store the sample as required (e.g. refrigeration).

11. 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

Table 2. Recommended lancet tip size.

Lancet Tip Size	
4mm	Maximum body weight of 28g
4.5mm	Best for use in mice 6 weeks to 2 months old
5mm	Best for use in mice 2 months to 6 months

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 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

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
- If this SOP has not been reviewed and approved by a UQ AEC within the last three years it is no longer valid and cannot be used in animal ethics applications until reapproved (see "AEC Reviewed/Approved" date in this document's header).

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.*