LAB_018 Blood Collection – Retro-orbital bleed in mice under general anaesthesia (Expires May 2027)

I. OBJECTIVE

To describe the standard retro orbital blood collection procedure used in mice across UQ research projects, also reflecting the procedure used to train workers across UQ by UQBR.

II. CONDITIONS FOR USING THIS SOP

It must be performed under anaesthesia. When citing this SOP you must also describe your chosen anaesthetic technique (or quote the relevant SOP you will be following)

You must log all complications of this technique. Because of the potential complications of this technique, you are required to keep a log of any complications associated with retro-orbital blood collection in this project. Severe complications should be reported to the AEC as an unexpected adverse event

This can only be performed a maximum of 2 times per eye (a total of 4 blood collections per mouse)

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

III. 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."[1]
Retro-Orbital – situated or occurring behind the orbit of the eye

IV. COMMENTS / RECOMMENDATIONS

- Retro-orbital bleeding under anaesthesia must be performed by appropriately trained people who have been deemed to be competent in the procedures.
- Retro-orbital blood collection is only allowed as a terminal bleed in some international jurisdictions. This is because of the potential adverse effects of this procedure if not performed correctly. It is therefore important that people undertaking this procedure, as a recovery technique, ensure they are competent and perform the procedure with great care.

Monitoring

- Mice should be monitored for a minimum of 2 days following this procedure. Signs to check for include corneal opacity (cloudy eye), swelling or bulging near the eye, less active mouse.

[1] NHMRC, 2013, *Australian code for the care and use of animals for scientific purposes*, National Health and Medical Research Council (NHMRC).

Possible adverse effects

- Retro-orbital haemorrhage resulting in haematoma and excessive pressure on the eye
- Corneal ulceration, keratitis, pannus formation, rupture of the globe and micro-ophthalmia caused by proptosis of the globe.
- Damage to the optic nerve and other intra-orbital structures which can lead to deficits in vision and blindness
- Fracture of the fragile bones of the orbit and neural damage by the micro-pipette
- Penetration of the eye globe itself with a loss of vitreous humour
- These will show various signs including cloudiness, swelling, shutting the eye, and facial grimace

Calculation of blood volume to collect

It is vital that the correct volume of blood to be collected is calculated. Blood collection volume is generally calculated as a % of total blood volume. This is the clinically relevant value. The average blood volume of a mouse is 7% of body weight where 1 ml of blood is assumed to be equivalent to 1 g of body weight.

Example calculation to obtain 10% of the total blood volume of a 20 gram mouse

total blood volume is 7% of 20 g

0.07 x 20 = 1.4 mls

10% of total blood volume is 0.10 x 1.4 = 0.14 ml (or 140 µl)

The table below provides some examples

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)*
Minimum Recovery period required between bleeds:		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
Attempt allowances	Two attempts per eye on any mouse.			
Procedure frequency	Dependant on bleed volume as advised in this table. Maximum of 4 collections per mouse (two times per eye)			

V. SAFETY AND COMPLIANCE

1. The person undertaking this task must ensure all relevant approvals are in place, training has been undertaken and risk assessments have been performed. If unsure, consult your supervisor.
2. Facility protocols should be followed.
3. Possible risks include mouse bite injury, needle stick injury, spills, exposure to infectious agents, repetitive task musculoskeletal injury and psychosocial harm.

VI. TRAINING CONSIDERATIONS

- All unsupervised animal blood collection must be performed by appropriately trained personnel who have been deemed to be competent in the procedure.
- Training for retro-orbital blood collection must be undertaken on models or cadaver animals initially.
- Further training should be undertaken on animals under general anaesthesia.
- For UQBR training purposes animals, 0.05% of body weight (about 7% of blood volume) in blood volume will be collected, e.g. 10µl for a 20g mouse). 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.

VII. 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 tubes – heparinised or non-heparinised
- Clinical waste bin
- Change station or Biosafety Cabinet
- Anaesthesia equipment**
- Blood collection tube or slides *Check that you have the appropriate tube for your project sample. I.E heparinised or no anticoagulant etc*

VIII. 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 Prepare for anaesthesia**

IX. PROCEDURE

Retro-orbital Bleed Procedure

1. Check that the capillary collection tube size and type is correct. Open the sample collection tube so it is ready for blood to drip into.
   Does it require anti-coagulant? Anticoagulant in the capillary tube can be helpful when collecting plasma as it prevents the blood from clotting during the collection process. Serum will be obtained if no anti coagulant is used. Use a new capillary tube for each animal, using either the heparinised (red tip) or non-heparinised (blue tip) version of the capillary tube as needed.

2. Ensure you have the correct animal for this procedure
   Check identification marks and ensure this matches the labelling on the collection tube.

3. **Anaesthetise the animal as per approved AEC protocol****.

4. Restrain the animal – Refer to LAB_006 Handling and Restraint in Mice and Neonates
   Hold the anaesthetised mouse with your hand closer to the head ensuring the head is unable to move but is not restricting breathing around the chest. If the mouse moves, the depth of anaesthesia may not be sufficient. It is important that there is no movement during the procedure. Avoid prolonged restraint. The eye should protrude sufficiently to continue the procedure.
   *For refinement it is ideal to apply a small drip of ocular local anaesthetic to the eye. The details of this (drug, dose etc) should be added to the application ***

Figure 1 Appropriate restraint and location of capillary tube placement before tilting the mouse (UQBR 2019).

Use clean technique when using the collection tube when performing procedures. For example, use a clean collection tube that has not touched the mouse, surfaces etc. This will minimise contamination from pathogens and subsequently infection in research animals.

5. Position the animal above the sample collection tube, the tube is placed into the correct location and then the mouse is held tilted so that the eye faces the floor.

Taking care not to contaminate the tube with urine or faeces etc. The capillary tube can be held between the tip of the thumb and index finger and it can be beneficial to rest the elbows to provide stability during the technique. The capillary tube should be angled perpendicular (90 degrees) to the surface of the skin.

6. Insert the capillary tube coloured end first between the eyeball and inner eye lid (3rd eyelid) then begin to gently twist the capillary tube to create blood flow.
   Ensure the capillary tube is placed in the correct location to avoid scratching the eye globe and result in damage to the eye. Blood should flow from the top of the capillary tube and down into the collection tube. If this does not occur this is commonly due to the needing slightly more force when twisting the tube or the site being incorrect – no more than 2 attempts to collect blood should be performed each time.

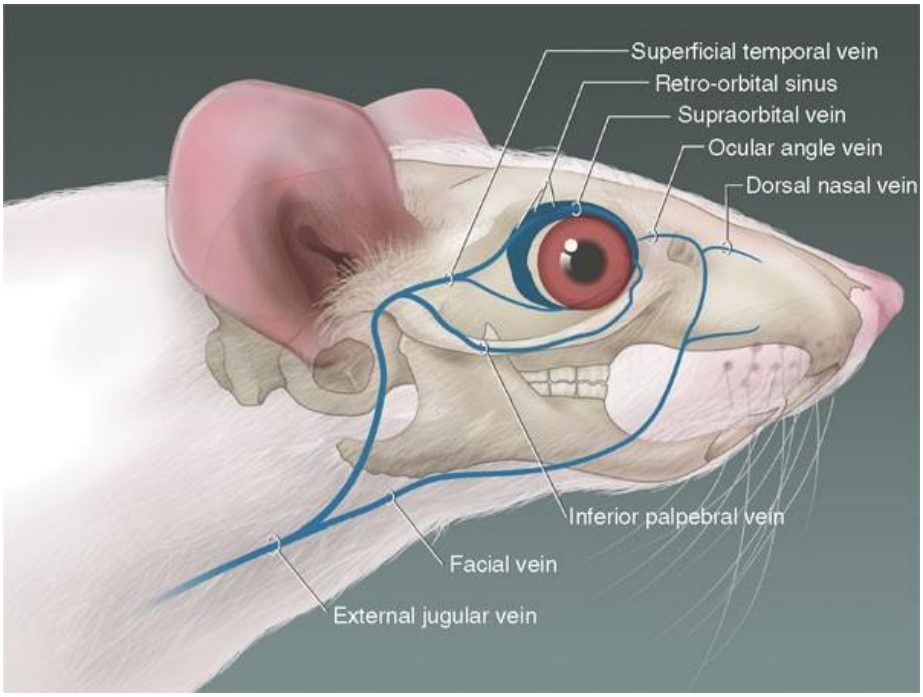

Figure 2. The blood vessels contributing to the retro-orbital sinus of the mouse (Yardeni *et al.* 2011)

7. Allow the required amount of blood to fill the capillary tube.
   Consider blood remaining in the capillary tube and additional drops of blood that may occur post procedure when calculating blood volume collected for each mouse. A standard capillary tube will hold 75µL of blood. The total quantity of blood collected from each animal must observe the volume limitations and recovery periods as specified in table 1.

8. Remove capillary tube and remove any excess blood from the eye by using a clean tissue. Apply gentle pressure onto the closed eyelid with a tissue if there is any remaining blood dripping.
9. Apply a small drop of sterile eye lubrication, a mist spray or eye antibiotic gel to the affected eye to support recovery
10. Release rodent into holding cage and continue to monitor for recovery and health

Care of mouse after blood collection

- Monitor until the mouse is fully recovered from anaesthesia
- Mice should be monitored for a minimum of 2 days following this procedure. Record must be kept of this monitoring. Signs to check for include corneal opacity (cloudy eye), swelling or bulging near the eye, less active mouse.
- Following the procedure, the mouse should return to normal movement and behaviour. If you observe small amounts of pooling blood gently encourage the eye lids to close by pinching for a short period of time while the broken vessels begin to clot, avoid squeezing.
- 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.
- 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.
- The most common problems found following this procedure are damage to the structures around the eye, eye muscles, and Hardaerian gland, corneal ulceration, inflammatory reactions and haemorrhages (NHMRC 2008).
- In the rare case that an animal appears weak or unexpected symptoms refer to Refer to LAB_022.

11. Blood remaining in the capillary tube can be placed into the blood collection tube by using an empty pipette.
    A standard capillary tube when full will can contain 75µL of blood.
12. Place 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 capillary tube should be used for each animal.
13. Complete record keeping requirements – a record should be kept of the procedure, date, side of eye, volume collected, who performed it, and any complications. This record can be kept anywhere appropriate that conforms with the animal code and UQ research policies. A health alert cage card (or similar) needs to be placed to ensure the animal is monitored carefully for a minimum of at least 2 days. Record keeping may include UQBR records, lab book, AEC animal monitoring paperwork and the relevant research sample collection labelling/records.
14. Store the sample as required (e.g. refrigeration).
15. Repeat from step 1 for the next animal or if finished, pack and clean up equipment and space.

X. 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-used-scientific-purposes
2. Huizing, M., Morris, H. D., Yardeni, T., Eckhaus, M., & Hoogstraten-Miller, S. (2011). Retro-orbital injections in mice. *Lab Animal*, *40*(5), 155–160. https://doi.org/10.1038/laban0511-155

Version #	Reviewing AEC (note: all other relevant AECs ratify the approval)	AEC Review Date	Approval To Date
[#]		[DD/MM/YYYY]	

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).