LAB_018 Blood Collection – Retro-orbital bleed in Mice

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
- Capillary tubes – heparinised or non-heparinised
- Clinical waste bin
- Change station or Biosafety Cabinet
- Anaesthesia equipment**
- 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. Prepare for anaesthesia**

Anaesthesia Procedure

UQ Biological Resources offers anaesthetic training courses to all staff and researchers. For more information email uqbrtraincomp@uq.edu.au

Aseptic Technique

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

V. PROCEDURE

Retro-orbital Bleed Procedure

1. Check that the capillary collection tube 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. 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 if this is approved.
4. Restrain the animal – refer to UQBR SOP 6 Handling and Restraint in Mice and Rats.

   Restrain the mouse with your hand closer to the head ensuring the head is unable to move but is not restricting breathing around the chest. It is important that the head is restrained sufficiently to prevent movement during the procedure. Avoid prolonged restraint. The eye should protrude sufficiently to continue the procedure.

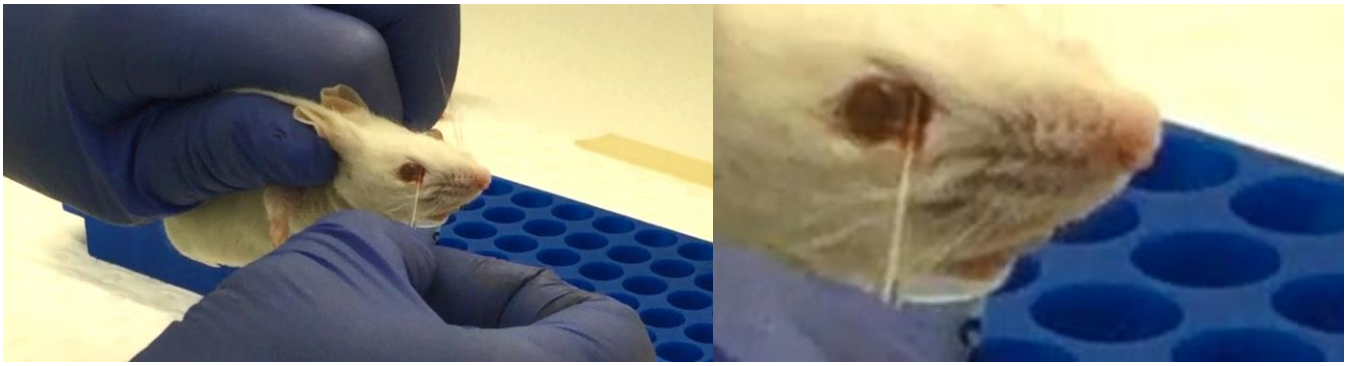

Figure 1 Appropriate restraint for this technique and location of capillary tube placement (UQBR 2019).

5. Position the animal above the sample collection tube and tilt the mouse 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 eye and inner eye lid then begin to gently twist the capillary tube to create blood flow.

   Ensure the capillary tube is placed in the exact 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 3 attempts should be performed per side.

7. Allow the required amount of blood to fill the capillary tube.

   Note for UQBR Training purposes, 0.05% of body weight in blood volume will be collected, e.g. 10µl for a 20g mouse).

   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

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

   Following the procedure the mouse should return to normal movement and behavior. 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 procedures 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 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.

10. 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.*

11. 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.

    Complete record keeping requirements – note procedure, date, side of eye, 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.

VI. 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/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

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.

VII. 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 Retro-orbital Bleed.*