LAB_019 Blood Collection – Tail Bleed in Rats and Mice

I. OBJECTIVE

To describe the procedure for blood collection in rodents, to standardise practice for all UQBR staff and researchers 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
- Pipette or collection tube
- Lubricant for tail massage
- Tissue / gauze
- Clinical waste bin
- Change station/Bio-safety cabinet *
- Appropriate restraint device
- Needle (25G) or scalpel blade/surgical scissors*
- Heat source – heat mat, heat lamp, warmed water

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 substance for injection 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 **
6. Prepare for anaesthesia ** [anaesthetic details and procedure must be provided within AEC application]

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

1. Ensure you have the correct rodent for this procedure *– check identification marks and ensure this matches the labelling on the collection tube.*
2. Use heat to dilate the tail vein, for this procedure the lateral vein is used. Warming the rodent 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, warmed water may also be used. Watch the ears and muzzle for redness, mice will also jump or clean their ears, shake their heads if overheating. Heating the animal for tail vein blood collection will significantly reduce collection time and therefore stress to the animal.

Figure 1 – Mice on a heating plate

3. 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 your pipette.
4. Place the rodent in a restraint cone or tube allowing its tail to hang freely. Clean site with ethanol allowing ethanol to dry. Apply lubricant to the base of the tail.

   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. Ensure you only use a small amount of lubricant at the base of the tail as large amounts can prevent the blood from flowing or mix with the sample.

Figure 2 - Applying lubricant to the tail

5. Hold the tip of the tail between your thumb and forefinger of your non dominant hand, pulling the tail straight but not causing stress to the animal, the lateral vein should be visible on either side. Using a 25 G needle gently puncture the lateral tail vein, this puncture is to be made as close to the tip of the tail as possible to allow for further attempts if necessary.

   The needle should be removed after puncture. A maximum of 3 attempts per animal should be performed on either side of the tail moving towards the base each time.

Figure 3 – Puncture the lateral tail vein

6. To assist with blood flow gently massage the tail from the base using the pre-applied lubricate.

Use your thumb and forefinger to hold the tail and gently pull down from base to tip, be mindful of the pressure applied as this may restrict blood flow or harm the animal.

7. Collect blood into blood collection tube or use a capillary tube 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 rodent). If the blood is not flowing easily a pipette may be used to collect the droplets

   Tail vein sampling is suitable for a small volumes of blood (less than 20 ul).

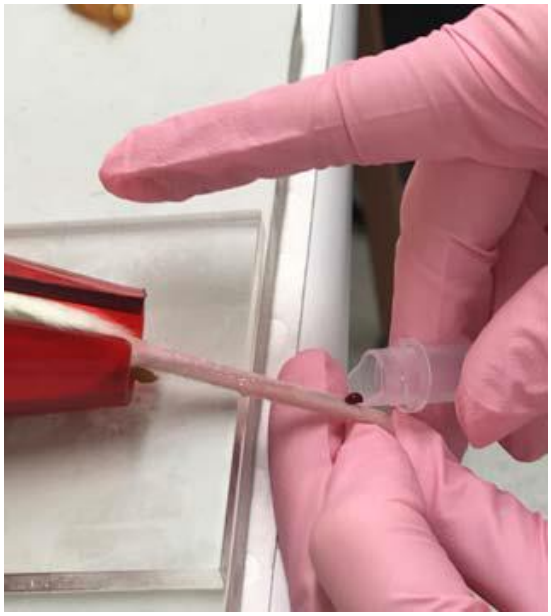

Figure 4 – Blood Collection from the Tail Vein

8. Place sharps into sharps container and close the sample collection tube.
9. Apply pressure until bleeding has ceased.

   Bleeding should cease easily, the aim should be not to have the animal restrained for long periods of time.

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

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

11. 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.

Tail Tip Blood Collection Procedure- For use in Juvenile or Anaesthetised adult rodents

Snipping the tail surgically is a crude method of sampling and should be avoided as it involves the removal of soft tissue from the tip of the tail resulting in permanent damage to the tail and pain to the rodent (NC3Rs).

If this method is to be used in an adult rodents for blood collection anaesthesia such as isoflurane must be used [as described within the approved AEC application].

1. Ensure you have the correct rodent for this procedure *– check identification marks and ensure this matches the labelling on the collection tube*
2. Anaesthetise the rodent (If the rodent is over 3 weeks of age anaesthesia is mandatory)
3. Pinch tail of the rodent between thumb and forefinger; fingers act as a tourniquet.
4. Wipe distal portion of the tail with 70% alcohol
5. Snip appropriate amount of tail using scissors or scalpel blade

   Remove 0.2 - 1 mm only, this should be soft tissue with no bone exposed. It is best practice to use a new blade or clean scissors for each animal.
6. Let the blood flow naturally into the collection tube. Note for UQBR training purpose 0.05% of body weight in blood volume will be collected, e.g. 10 µl for a 20g rodent.

   Tail vein sampling is suitable for a small volume of blood.
7. Place sharps into sharps container and close the sample collection tube.
8. Apply pressure until bleeding has ceased, at least for 30 seconds.

   Bleeding should cease easily, allowing the animal to be restrained for only a short period of time.
9. Release rodent into holding cage and continue to monitor for recovery and health

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

   Following the procedure the animal should return to normal movement and behavior. If you observe excessive bleeding seek veterinary advice. 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. 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.
11. Store the sample as required (e.g. refrigeration).
12. 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 Center for the Replacement Refinement and Reduction of Animals in Research (NC3Rs) n.d., viewed 12 December 2019, https://www.nc3rs.org.uk/rodent-tail-vein-non-surgical*
2. 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
3. Office of the Gene Technology Regulator (OGTR) n.d., viewed 11 April 2019, http://www.ogtr.gov.au/

4. University of Queensland n.d., *Health, safety and wellbeing,* viewed 11 April 2019, https://staff.uq.edu.au/information-and-services/health-safety-wellbeing
5. 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
6. UQ Biological Resources n.d., *UQBR SOP's, viewed 11 April 2019, https://biologicalresources.uq.edu.au/secure/reference-information#SOP's*
7. UQ Biological Resources, 2019 *IP Injections.*