LAB_014 Cardiac blood collection (terminal bleed)(Expiry: August 2026)

INFORMATION REQUIRED FOR ANIMAL ETHICS APPLICATIONS

****You will need to state if the cardiac bleed is being undertaken under heavy anaesthesia or just following death. The method of anaesthesia or humane killing needs to be described in the application (or a relevant current SOP referenced).**

I. OBJECTIVE

To describe the procedure for blood collection within UQBR facilities.

II. 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]

III. COMMENTS / RECOMMENDATIONS

- Cardiac blood collection is used as a terminal procedure to collect a maximum volume of blood directly from the heart.
- Cardiac puncture is a suitable technique to obtain a single, large, good quality sample from a mouse. It is appropriate for all strains of mouse.
- Blood collection by cardiac puncture is a terminal procedure and cannot be carried out on conscious animals.
- The mouse must be anaesthetised and once the blood sample is collected the mouse must be humanely killed.
- Alternatively, blood collection can be successfully performed after humane killing. This needs to be performed immediately after death has been confirmed. Sample collection will be less successful as the time since death increases.
- UQ Biological Resources offers anaesthetic training courses to all staff and researchers. It is highly recommended that anaesthesia training is completed before anesthetising rodents. For more information email uqbrtraincomp@uq.edu.au.

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.

IV. 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

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

- Clinical waste bin
- Change station/Bio-safety cabinet *
- Blood sample tube
- CO2 or anaesthesia equipment** [details and procedure must be provided within AEC application]
- Needle and syringe size will depend on the age and species of the animal

"Add anticoagulant to the syringe, if required. Anticoagulant in the syringe can be helpful when collecting plasma as it prevents the blood from clotting during the collection process."

V. 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

Ensure you do not contaminate 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.

VI. 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.

   Add anticoagulant to the syringe if required. Anticoagulant in the syringe can be helpful when collecting plasma as it prevents the blood from clotting during the collection process.

2. Ensure you have the correct rodent for this procedure *– check identification marks and ensure this matches the labelling on the collection tube.*
3. Either heavily anaesthetise the animal as per approved AEC protocol **, or euthanise and ensure death before beginning the procedure**.

   Blood collection by cardiac puncture is a terminal procedure and cannot be carried out on conscious animals. Blood collection can be successfully performed immediately after death has been confirmed. Sample collection will be less successful as the time since death increases. Cervical dislocation as a euthanasia technique can result in the loss of blood from the circulatory system so this should be considered in choosing a euthanasia technique.

Cardiac Puncture Blood Collection Procedure Closed Technique

1. Lay the animal in dorsal recumbence (on its back) with the head pointing either away from you or towards your non-dominate side.

   It can be helpful to lay the needle over the animal's chest before starting to get an estimate of how far to insert the needle. Needle gauge and length and syringe volume will vary with the age and species of rodent.

2. Pinch the finger and thumb of your non-dominate hand over the apex of the chest and press the chest wall gently.

   The pressure of your finger and thumb helps to slightly compress the chest wall and reduces the likelihood of movement of the heart in the chest when the needle is entering the heart.

3. With the bevel up insert the needle either
   a) Just below the ribs either on the midline or slightly to the left of the midline. The needle can be angled between 0-20° relative to the bench top.
   b) In line with the armpit at 90° to the chest wall

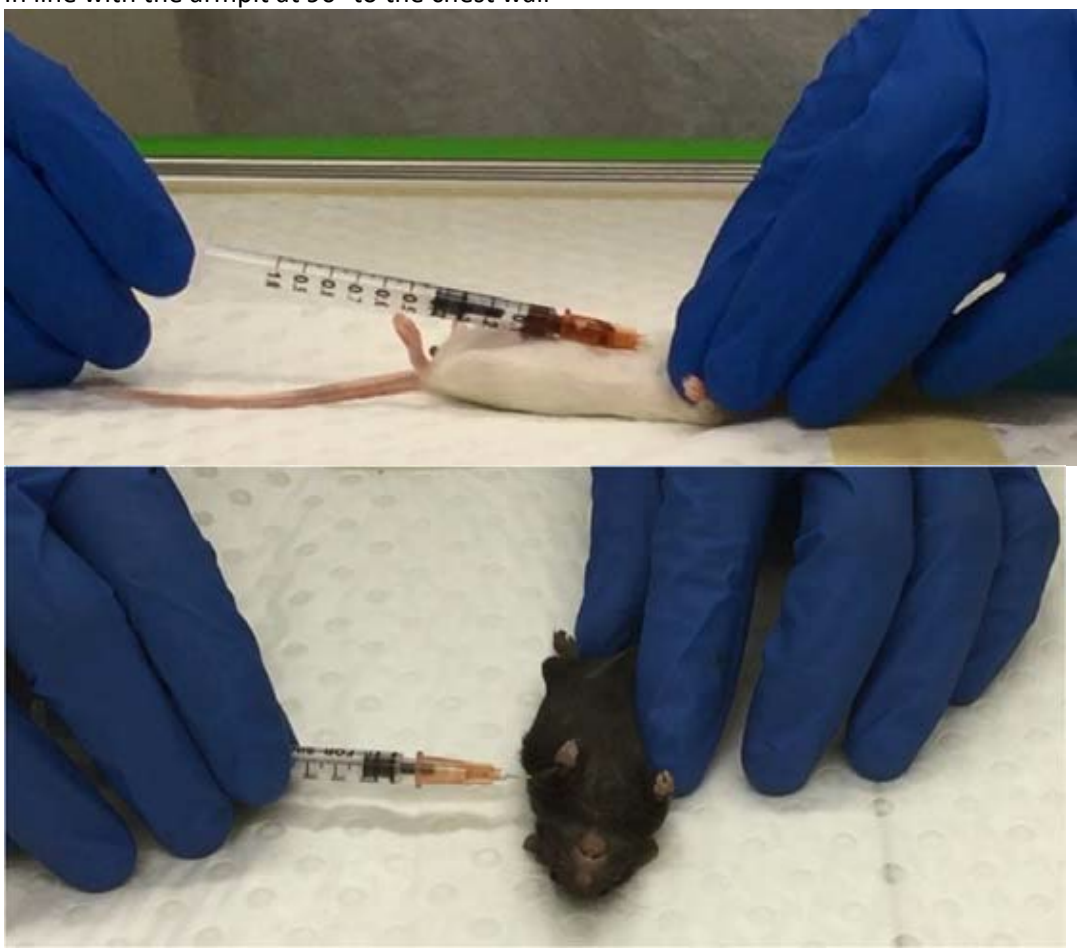

Figure 1 Appropriate position of the needle for two different alternative approaches when performing cardiac bleed

4. Insert the needle until a small flash of blood appears in the hub or you have exceeded the anatomical likelihood of entering the heart.

   It may be beneficial to draw back on the syringe to create a vacuum once the needle has entered the thoracic cavity to assist placement of the needle as blood will flow immediately into the syringe.

5. Holding the needle steady release your pinch over the chest and gently withdraw the plunger on the syringe.

   If blood enters the syringe continue to withdraw blood.

   If the flow slows rotate or advance or retract the needle slightly or try briefly compressing the chest using the pinch technique.

   If no blood appears in the syringe retract the needle very slowly while continuing to slightly withdraw the plunger until you have passed the level of the diaphragm.

If you have retracted the needle past the anatomical likelihood of being in the heart with the needle still in the rodent advance the needle again at a slightly different angle and repeat the attempt.

6. If only a small sample has been collected despite the techniques above release the plunger and withdraw the needle. Try again with a fresh needle and syringe.

   As this is a terminal procedure on an unconscious animal there is no limit to the number of attempts however the likelihood of success decreases with each attempt. A fresh needle and syringe is required because repeated attempts at blood collection will often result in a clot inside either the needle or the hub of the syringe which will hinder further collection attempts.

7. Once a sufficient blood sample has been collected withdraw the needle from the rodent.
8. Remove the needle from the syringe and gently depress the plunger to place blood sample in the collection tube.

   It is important to remove the syringe so when transferring blood from the syringe to the collection tube the red blood cells (RBC) to not break from this force.

9. If the collection tube contains anticoagulant dribble the sample down the side of the tube and then gently invert the tube several times.

   Excessive turbulence of the blood during collection will rupture RBC and the serum or plasma sample will be contaminated with haemoglobin.

10. Place the needle in the sharps bin.
11. Ensure the animal is deceased. If in doubt a second method of euthanasia may be used.

    Signs of death include no respiration rate, no pulse rate, and no toe pinch reflex.

12. Follow facility specific methods for body disposal.
13. Complete record keeping requirements – note procedure, date and initials on cage card and the relevant research sample collection labelling/records.
14. Store or process the sample as required (e.g. refrigeration).
15. Repeat for the next animal or if finished, pack up and clean up equipment and space.

    Use a new needle and syringe for each animal.

Cardiac Puncture Blood Collection Procedure Open Technique

**Gaseous anaesthesia may not be a suitable anaesthetic because animal breathing is affected by opening the thoracic cavity. Injectable anaesthesia or humane killing immediately before cardiac blood collection is more suitable. The method must be approved by AEC.

1. Following on from 'Preparation of the animal' section above
2. Lay the animal in dorsal recumbence (on its back) with the head pointing either away from you or towards your non-dominant side.
3. Spray the abdomen and chest with ethanol or any wetting agent.

   As this is a terminal procedure aseptic technique is not required however wetting the hair helps to prevent it from contaminating the body cavities.
4. Open the abdominal cavity using scissors and cut the diaphragm where is meets the chest wall on both sides. Cut the ribs 1/3 of the distance between the sternum and the spine up to the level of the 2nd or 3rd rib. Reflect the free section of chest wall backwards and secure with a solid pin or a clamp.

Figure 2 Open method and incision line through the ribs

Figure 3 Open method and insertion of needle (Morris & Levy 1964).

5. With the bevel up insert the needle into the apex of the left ventricle and advance until a flash of blood appears in the hub of the needle or you have exceeded the anatomical likelihood of entering the left ventricle.

The left ventricle is on the left side of the heart, it extends to the apex of the heart and is a slightly different colour to the right ventricle.

6. Holding the needle steady, gently withdraw the plunger on the syringe. If blood enters the syringe continue to withdraw blood. If the flow slows rotate the needle slightly or try slightly retracting or advancing the needle.
7. If only a small sample has been collected despite the techniques above release the plunger and withdraw the needle. Try again with a fresh needle and syringe.
   As this is a terminal procedure on an unconscious animal there is no limit to the number of attempts however the likelihood of success decreases with each attempt. A fresh needle and syringe is required because repeated attempts at blood collection will often result in a clot inside either the needle or the hub of the syringe which will hinder further collection attempts.
8. Once a sufficient sample has been collected withdraw the needle from the heart
9. Remove the needle from the syringe and gently depress the plunger to place blood sample in the collection tube.
   It is important to remove the syringe so when transferring blood from the syringe to the collection tube the red blood cells to not break from this force.
10. If the collection tube contains anticoagulant dribble the sample down the side of the tube and then gently invert the tube several times.
    Excessive turbulence of the blood during collection will rupture RBC and the serum or plasma sample will be contaminated with haemoglobin.
11. Place the needle in the sharps bin.
12. Ensure the animal is deceased if in doubt use a second method of euthanasia.
    Opening the chest cavity prevents respiration so the animal is very likely to be deceased.
13. Follow facility specific methods for body disposal.
14. Complete record keeping requirements – note procedure, date and initials on cage card 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.
15. Store or process the sample as required.
16. Repeat for the next animal or if finished pack up and clean up equipment and space.
    Use a new needle and syringe for each animal.

VII. REFERENCE INFORMATION

Table 1. Recommended values for needle size, gauge, volume and expected total blood collection volumes.

	Blood Volume Collected	Needle Gauge (G)	Needle Length	Syringe Size
Adult Mice	0.5 to 1mL	25G	¾ inches	1mL
Adult Rats	4 to 8mL	22G	1 ¼ inches	5 to 10mL

VIII. 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. Morris, J & Levy, M 1964, 'Cardiac Puncture Catheterization: Experience in 80 Patients*', Diseases of the Chest, vol. 45, no. 5, pp. 449-483.
3. NeuroScience Associates (NSA) n.d., *Perfusion Protocol/Transcardial,* viewed 11 July 2019, https://www.neuroscienceassociates.com/instructions/perfusion-protocol/
4. Office of the Gene Technology Regulator (OGTR) n.d., viewed 11 April 2019, http://www.ogtr.gov.au/
5. University of Queensland n.d., *Health, safety and wellbeing,* viewed 11 April 2019, https://staff.uq.edu.au/information-and-services/health-safety-wellbeing
6. 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
7. UQ Biological Resources n.d., *UQBR SOP's, viewed 11 April 2019, https://biologicalresources.uq.edu.au/secure/reference-information#SOP's*
8. UQ Biological Resources, 2019 *IP Injections.*

Version #	Reviewing AEC (note: all other relevant AECs ratify the approval)	AEC Review Date	Approval To Date
[#]		03/08/2023	

Please leave this table blank. The Veterinary Officer will fill in this section.

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).