LAB_014 Cardiac blood collection (terminal bleed) (Expiry: Sept 2029)

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.
- The mouse must be in surgical or deep depth of anaesthesia, or alternatively, blood collection can be successfully performed after humane killing- immediately after death has been confirmed. Sample collection will be less successful as the time since death increases.
- Blood collection by cardiac puncture is a terminal procedure (i.e. the animal's death must be confirmed post collection) and cannot be carried out on conscious or lightly anaesthetised animals.
- For ethics approval it should be made clear to the animal ethics committee (AEC) if the procedure is to be performed under anaesthesia as compared to after humane killing. Note that if this procedure is to be performed after humane killing it does not technically need to be included in the ethics application provided that collection occurs after confirmation of death.

Table 1. Recommended blood collection values

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

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

IV. 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.

V. TRAINING CONSIDERATIONS

All animal procedures MUST be performed by appropriately trained personnel who:

- have been deemed to be competent in the procedure.
- are confident in completing the procedure.
- OR are under the direct supervision of a person who is competent.

VI. 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 sample tube
- CO2 or anaesthesia equipment** [details and procedure must be provided within AEC application]
- Needle and syringe size will depend on the age/size 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."

VII. 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.

VIII. 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. Check that the sample collection tube type is correct. *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.*
3. Either heavily anaesthetise the animal as per approved AEC protocol ** or euthanise and confirm death before beginning the procedure.

   Blood collection by cardiac puncture is a terminal procedure and cannot be carried out on conscious or lightly anaesthetised animals. Blood collection can be performed after confirming deep anaesthetic depth - lack of withdrawal reflex and slow breathing rate (Less than 1 breath per second) OR immediately after death

has been confirmed (absence of heartbeat and respiration, fixed, dilated pupils and pale, grey mucous membranes). Cervical dislocation as a euthanasia technique can result in the loss of blood from the circulatory system so this should be a consideration when choosing the 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-dominant 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. Spray ethanol over the animal: this can assist in visualising the landmarks more clearly as well as following a clean technique.

3. Stabilise the animal using your non-dominant hand.
   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.

4. Proceed with the chosen method/technique. There are 3 different methods to choose from, based on the location of needle insertion. This will be based on personal preferences.
   a. Vertical adjacent to Sternum: At the point of intersection of the lines between the points of the elbows and midline. The syringe and needle should be kept vertical.

      Note: the point of the needle may need to be walked 1-2mm laterally either to the left or right to avoid the bony sternum.

   b. Horizontal and lateral point of the elbow on the left, keeping the syringe and needle horizontal. The needle should have the bevel pointing upwards.

   c. Obliquely next to the Xiphoid and under the Sternum, under or adjacent to the Xiphoid Cartilage with the syringe held at 20-30° and the needle advanced toward the head. The needle will need to have the bevel pointing upwards and be longer to allow further advancement compared to the other 2 methods.



Figure 1A Closed method, horizontal approach



Figure 1B Closed method, oblique and under the xiphoid/sternum approach

5. Insert the needle until a small flash of blood appears in the hub.

   It may be beneficial to draw back slowly 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.

6. Holding the needle steady, gently and slowly 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.

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 a deeply anaesthetised, unconscious animal, there is no limit to the number of attempts. However, the likelihood of success decreases drastically 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 blood sample has been collected, slowly withdraw the needle from the rodent.
9. Remove the needle from the syringe, open the blood collection tube and gently depress the plunger to place blood sample in the collection tube.

   Removing the needle is so that the red blood cells (RBC) are not damaged from the force/pressure of transferring the blood to the collection tube.

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. Confirm the animal is deceased. A second method of euthanasia must be used to confirm.

    Signs of death include absence of heart beats/respiration, no pulse detected, pale extremities, glazed: fixed, dilated eyes, cold to touch.

13. Follow facility specific methods for body disposal. This usually involves disposing the cadaver in a biohazard bag and placing it in the cadaver freezer
14. Complete record keeping requirements – note procedure, date and initials on cage card and the relevant research sample collection labelling/records. Ensure the cage card reflects the change of animal number and that Mosaic is updated
15. Store or process the sample as required (e.g. refrigeration).
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.

Cardiac Puncture Blood Collection Procedure Open Technique

Injectable anaesthesia (resulting in deep surgical anaesthetic depth) or humane killing must be performed immediately before open cardiac blood collection.

Gaseous anaesthesia is not recommended as the animal can no longer breathe in the anaesthesia. When the diaphragm is opened, the lungs will collapse due to equalising pressure in the chest cavity. Whilst the animal will die, it is not instant.

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. .

   As this is a terminal procedure aseptic technique is not required however wetting the hair helps to minimise hair contamination and smoother incision process.
4. Incise the skin at the mid-abdomen. 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, slowly 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. Once a sufficient sample has been collected withdraw the needle from the heart.
8. Remove the needle from the syringe and gently depress the plunger to place blood sample in the collection tube.
   Removing the needle is so that the red blood cells (RBC) are not damaged from the force/pressure of transferring the blood to the collection tube.
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 use a second method of euthanasia.
    Opening the chest cavity prevents respiration so the animal is very likely to be deceased.
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.
    Records need to be clear and legible on each record to allow others to read and understand.
14. Store or process the sample as required.
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.

IX. 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/

Version #	Reviewing AEC	AEC Review Date	Outcome
4.0	Anatomical Biosciences AEC, Health Sciences AEC, Molecular Biosciences AEC and Laboratory Biomedicine AEC	03/08/2023	Approved to 03/08/2026
5.0	Anatomical Biosciences AEC, Health Sciences AEC, Molecular Biosciences AEC and Laboratory Biomedicine AEC	August 2026	Deferred approval
5.1	Molecular Biosciences AEC	02/09/2026	Approved to 02/09/2029