LAB_012 Euthanasia - Transcardial Perfusion in Mice and Rats

I. OBJECTIVE

To effect safe and humane killing of laboratory mice and rats under general anaesthesia via transcardial perfusion for the purpose of tissue fixation and associated sample collection.

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. COMMENTS / RECOMMENDATIONS

- Consideration must be taken as to the potential for stressful auditory, visual or olfactory stimuli that may be perceived by other animals. Efforts must be made to isolate these potential stressors:
  - Euthanasia or laboratory rodents should only occur in "terminal procedure rooms"(*),
  - Biosafety cabinets or fume hoods should be used for the procedure wherever possible,
  - Ensure the area is cleaned prior to use, and between animals,
  - Different species (i.e. rats and mice) should not be euthanised in the same area at the same time.
- This procedure must be performed in a fume cabinet, or similar device, that provides protection of users from formaldehyde fumes

III. EQUIPMENT

- PPE (*)
  Although PPE is facility dependent, minimum expectations include: disposable gloves, clean log-sleeved laboratory gown, hair bonnet, eye protection, face mask, closed in shoes.
- Home cage - enclosure
- Needle and syringe prepared, containing general anaesthetic
  Needle gauge (mice: 25-27G; rats: 23-26G), and maximum volume (<1% bodyweight equivalent) as per LAB_028 Injections - Intra-peritoneal (IP) in Mice, Rats and Neonates
- General anaesthetic:
  - As per LAB_025 Rodent Anaesthesia - Injectable Agents (e.g. 10mg/kg xylazine, 100mg/kg ketamine);
  - alternatively, pentobarbitone (Lethabarb) at >50mg/kg live bodyweight.

  Note: pentobarbitone will result in "recoverable" general anaesthesia at doses ~50mg/kg (i.e. the rodent should "wake up" after 1-2 hours). Lethal (>200mg/kg) or sub-lethal doses (i.e. 200>50mg/kg) of pentobarbitone may be used to effect euthanasia via transcardial perfusion, because exsanguination and perfusion will result in death while the animal is anesthetised. Sub-lethal doses of pentobarbitone are however not appropriate for lethal Injection in mice and rats on its own. As detailed in the relevant SOP, LAB_011 Euthanasia - Lethal Injection in Mice and Rats, without exsanguination and perfusion, >200mg/kg is required to reliably effect euthanasia.
  - or, if the animal is already deeply anaesthetised (as part of AEC approved project activities (**)), a general anaesthetic injection is not required [deeply anaesthetised = unconscious (loss of righting reflex), non-responsive (no "toe-pinch" reflex, no "blinking" reflexes), rhythmic breathing].
- Dissection instruments: large and fine scissors, bone cutters, haemostats

- Perfusion needle: a blunt-ended or olive-tipped needle is recommended, as large as 15G for rats
- Perfusion device
    - Pump (e.g. Medical peristaltic pump)
    - Luer lock syringe (20-50mL)
- Phosphate Buffered Saline (PBS) (~0.1M, approximately 20mL for mice, and 150mL for rats)
- Fixing agent: e.g. 4% formaldehyde (approximately 150mL for mice, and 450mL for rats)

SUPPLEMENTARY EQUIPMENT

- Spatula
- Falcon tubes
- Work tray (e.g. polystyrene or cork board within a plastic collection tray)
- Cryostat cutting compound (e.g. OTC Tissue Tek)
- -80°C freezer

IV. PROCEDURE

Procedure using a perfusion pump

1. Retrieve the animal from its home cage, restrain the animal and inject the general anaesthetic into the intraperitoneal (IP) space using the needle and syringe (as per LAB_028 Injections - Intra-peritoneal (IP) in Mice, Rats and Neonates).

2. Return the animal to the home cage and monitor for loss of righting reflex and withdrawal reflexes (e.g. toe pinch).
   The righting reflex is the animal's ability to maintain an upright dorso-ventral position (when standing, sitting or lying down) - loss of this reflex is correlated with a loss of consciousness. The withdrawal reflex is an involuntary recoil from painful stimuli, which is often most accurately assessed by a firm pinch of the hind limb footpad - loss of this reflex is correlated with a loss of pain sensation.

3. Once loss of the righting and withdrawal reflexes is achieved remove the rodent from the home cage and place it within dorsal recumbence (i.e. on its back) within your work station (e.g. fume cabinet).
   If loss of the righting and withdrawal reflexes is not achieved within 10 minutes repeat steps 1-2.

4. Using dissection instruments an incision is made through the skin and underlying sternum. The ribcage is manipulated to visualise the heart. The ribcage may be retracted or resected. Further dissected is then required to release the heart from its surrounding tissue.

5. The blunt-ended 29 gauge needle, connected via tubing to a perfusion pump, is pierced through the left ventricle, advancing the needle tip diagonally into the ascending aorta. The needle is then secured in position using haemostats. A secondary incision is often required in the right atrium or ventricle to aid exsanguination and perfusion.
   The tubing and pump should be primed with PBS.

6. PBS is then pumped through at a rate of approximately 60mL/min to "clear" the blood from the mouse's circulatory system.

7. Immediately following, 4% formaldehyde is pumped through the system using the same parameters.

8. Upon completion of perfusion, using the dissection instruments, the animal is decapitated, the brain and any other tissue removed, as required.

Manual perfusion procedure (UQBR training procedure)

For UQBR Training purposes animals will be euthanised and the procedure performed on fresh cadavers. Animals should not be cervical dislocated this procedure tears many significant blood vessels, prohibiting successful perfusion.

1. Correctly identify rodent and complete steps 1-3 of "Procedure using a perfusion pump" (above)
2. Place the rodent in the dorsal recumbancy (i.e. on its back) within the work station (e.g. fume cabinet), then restrain each limb to the work surface.
   Pin each limb through the skin of the axilla (armpits) and through the skin behind the stifle/knee. Ensure the limbs are immobile but not taught.

Figure 1. The rodent is pinned to a board or foam surface (UQBR 2021).

3. Using the forceps lift the skin up to create tenting of the skin, then use scissors to make a small incision up to 5 mm in line with the bottom of the xyphoid process of the sternum (ribs).

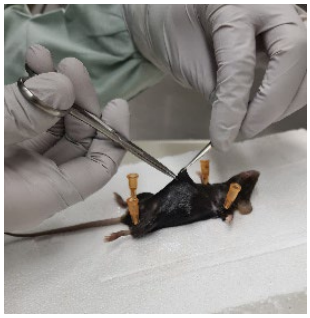

Figure 2. Small incision (UQBR 2021).

4. Use a blunt or sharp dissection technique to expose the external thoracic cavity.

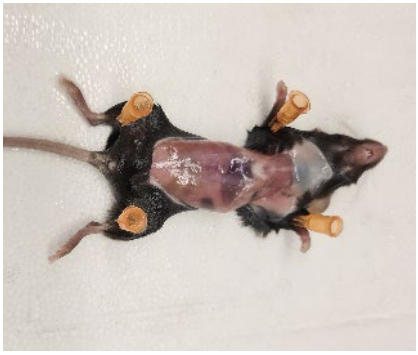

Figure 3. The external thoracic cavity is exposed (UQBR 2021).

5. Using forceps lift the xyphoid process and cut into the peritoneal cavity

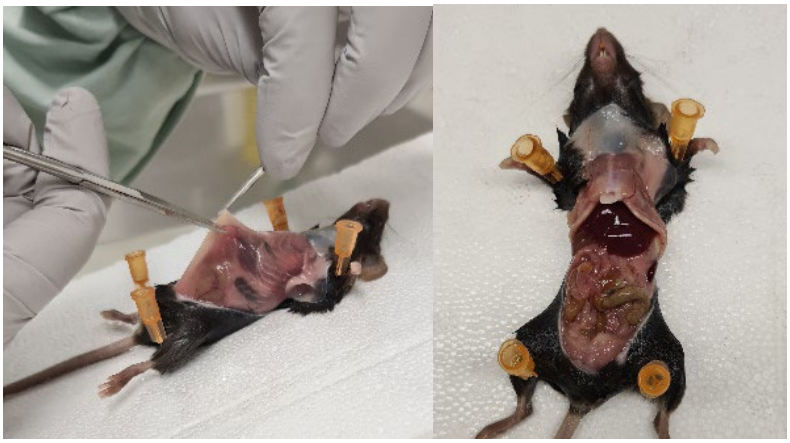

Figure 4. Opening the peritoneal cavity (UQBR 2021).

6. Whilst still holding the xyphoid process and being careful not to damage the organs within the abdomen incise through the diaphragm to enter the thoracic cavity.

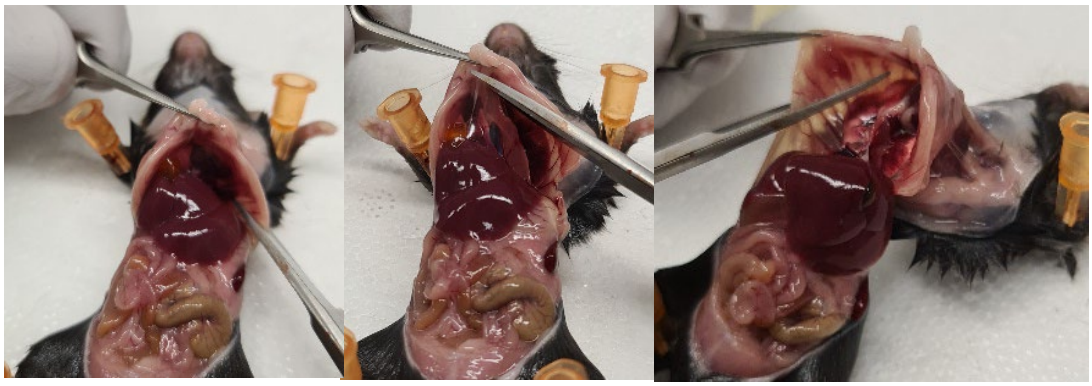

Figure 5. Accessing the heart (UQBR 2021).

7. Cut along the outer edges of the rib cage meeting above the heart, then removing the sternum

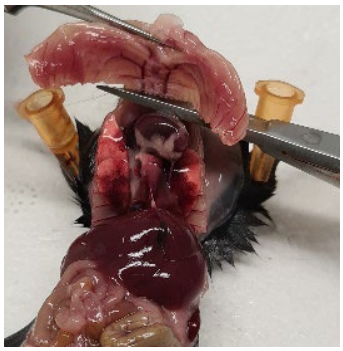

Figure 6. Removing the sternum (UQBR 2021).

8. Grip the heart with forceps to stabilise, then insert the needle at a shallow depth so the bevel is just below the surface into the left ventricle of the mouse

Ensure the heart is stabilised but still able to beat. Ensure your hand is steady to avoid movement of the needle within the heart to avoid piercing through the heart or moving into the atrium.

Figure 7. Stabilising the heart (UQBR 2021).

9. Then once the needle is in the left ventricle, using scissors cut the right atrium of the rodent
   This is where fluid will exit the rodent during the procedure

10. Depress the plunger to inject the substance, using either

    10a. Phosphate Buffered Solution (PBS) - *Generally PBS is used first to flush blood cells out of the body. This will avoid blood cells from fixing in the tissue. This flush may continue until the liver turns pale and blood is no longer exiting the atrium. Use the colour of the tissue to gauge the volume of solution to use.*

    10b. Paraformaldehyde (PFA) – *When the PFA is used the animal may convulse as the tissues become fixed, this is normal.*

    If using a smaller 20mL syringe avoid applying excess pressure, this may burst blood vessels within the body. If using a 50mL a significant amount of pressure may be required. It can be helpful to brace your hand against a stable object to apply force to the plunger without moving the needle, this is why a 'luer lock syringe' is advised, injection of PFA may be completed. If more than one syringe of solution is required the needle can stay within the heart and the syringe switched over. Use the colour of the tissue to gauge the volume of solution to use.

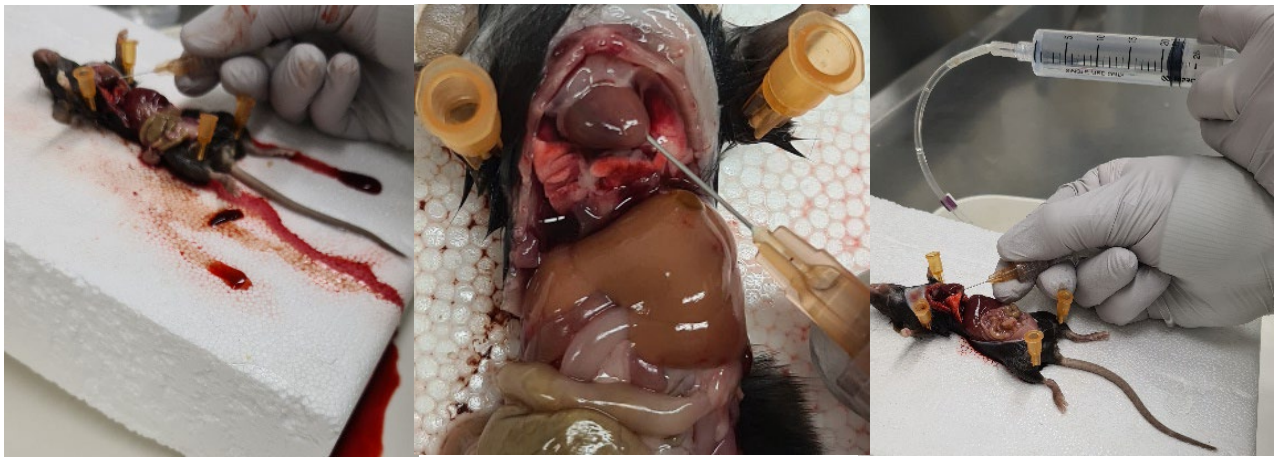

Figure 8. Using a syringe to inject the substance (UQBR 2021).

11. Upon completion of perfusion, the animal is decapitated, the brain and any other tissue is removed as required.

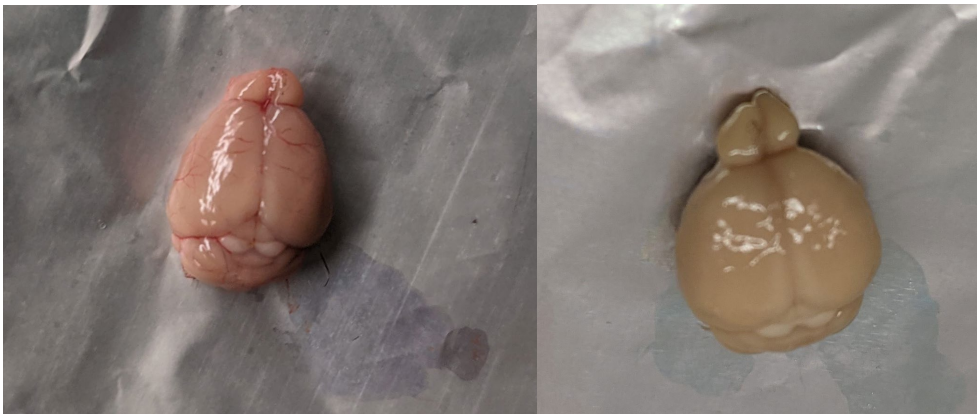

Figure 9. Example of the brain, the left is normal in colour, the right has been perfused (UQBR 2021).

12. Refer to your individual project to handle and store tissue as necessary, this will vary amongst projects.
13. Dispose of the cadaver once all tissue is collected
14. Place needles into sharps container
15. Complete any project specific paperwork

V. BIBLIOGRAPHY

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5. Lamberts, R., & Goldsmith, P. C. (1986). Fixation, fine structure, and immunostaining for neuropeptides: perfusion versus immersion of the neuroendocrine hypothalamus. The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society, 34(3), 389–398. https://doi.org/10.1177/34.3.2419392
6. UQ Biological Resources, 2021 Perfusion in rodents.

Version #	Reviewing AEC (note: all other relevant AECs ratify the approval)	AEC Review Date	Approval To Date
~~#1~~	~~HS~~	~~18/02/2021~~	~~18/02/2024~~ superseded
#2	HS	21/04/2022	21/04/2025

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).