Standard Operating Procedure	Title: Heart Dissection	
	Doc. Number: ESLIM_020_001 Rev No.	Date Issued: 01/06/04

1.0 Purpose:

1.1 Relaxation, dissection, weighing and fixation of heart for histological analysis. Changes in heart weight and wall thickness are linked to cardiovascular phenotypes. This protocol describes the relaxation, dissection, weighing and fixation of the hearts to prepare the tissue for sectioning.

2.0 Scope:

2.1 Individuals who have been trained, and are competent in performing the procedures described herein must follow this procedure.

2.2 Any queries, comments or suggestions, either relating to this SOP in general or to a specific problem encountered during a procedure, should be addressed to the leader of cardiovascular investigations.

2.3 Any deviances from this protocol must be reported to the leader of cardiovascular investigations.

3.0 Safety Requirements:

3.1 General laboratory procedures should be followed, which include: no eating, no chewing gum, no drinking, and no applying of cosmetics in the work area. Laboratory coats and gloves must be worn at all times in the work area, unless the protocol specifically describes the appropriate attire for the procedure.

4.0 Associated Documents:

- Tissue fixation with 4% buffered paraformaldehyde
- Trimming fixed tissues from necropsy
- Tissue processing and embedding in paraffin
- Sectioning from paraffin embedded tissues
- Haematoxylin and eosin staining of histological sections

5.0 Notes

5.1 Procedure is to be carried out on 3 males and 3 females at the end of pipeline 1.

5.2 The validity of results obtained from cardiovascular phenotyping is largely dependent on methods of animal husbandry. It is of vital importance that individuals following this procedure are experienced and aware of the animal's welfare, and is familiar with the animal being tested, in order to reduce the anxiety levels of the animal prior to testing.

5.3 The majority of mouse cardiovascular studies are age/sex/strain dependent. It is important to keep these parameters comparable throughout a single experiment.

6.0 Quality Control:

6.1

7.0 Equipment:

Fine forceps
Surgical scissors
Fine surgical scissors
"1 ml" syringes
"1.0 mm" needles
Centrifuge
Tips
Precision pipettes
"1.5 ml" tubes
Down draft table/fume hood certified for paraformadehyde use
Lab balance
~~Dissection tools~~

8.0 Supplies:

Ketamine (Imalgene®, Mérial)
Xylazine (Rompun® 2%, Bayer)
Sterile saline (0.9 % NaCl)
4% Paraformaldehyde diluted in PBS (4% PFA-PBS)
C-fold towel or surgical compress

~~8.1 250mM KCl~~
~~4% buffered paraformaldehyde~~
~~10ml syringe~~
~~26G butterfly needle connected to luer valve (e.g. vacutainer blood collection kit)~~
~~7.5ml Bijou containers with lids~~
~~Labelling for bijous~~
~~C-fold or equivalent paper towels~~

9.0 Procedure:

Summary of protocol steps:

- Anaesthetize the mouse
- Intracardiac blood collection
- Heart collection ~~Collection of the heart~~and weight
- Heart ~~weight and~~ storage for histology

9.1 ~~Anaesthe~~Anaesthetize the mouse~~sia~~

9.1.1 Dilute ketamine and xylazine in sterile saline (NaCl 0.9%) in order to inject 150 mg/kg ketamine and 10 mg/kg xylazine to mice in 100 µL volume (for a 25 g-weight mouse).

9.1.2 Hold the mouse in your hand by the dorsal skin so that its head is up and its rear legs are down. Hold its tail with fingers.

9.1.3 Use "1mL" syringes and "0.5 mm" needles to inject anaesthesia solution and administrate 100 µL of solution per mouse (for a 25 g-weight mouse) by an intraperitoneal injection.

9.1.4 Check that mouse is deeply anaesthetized.

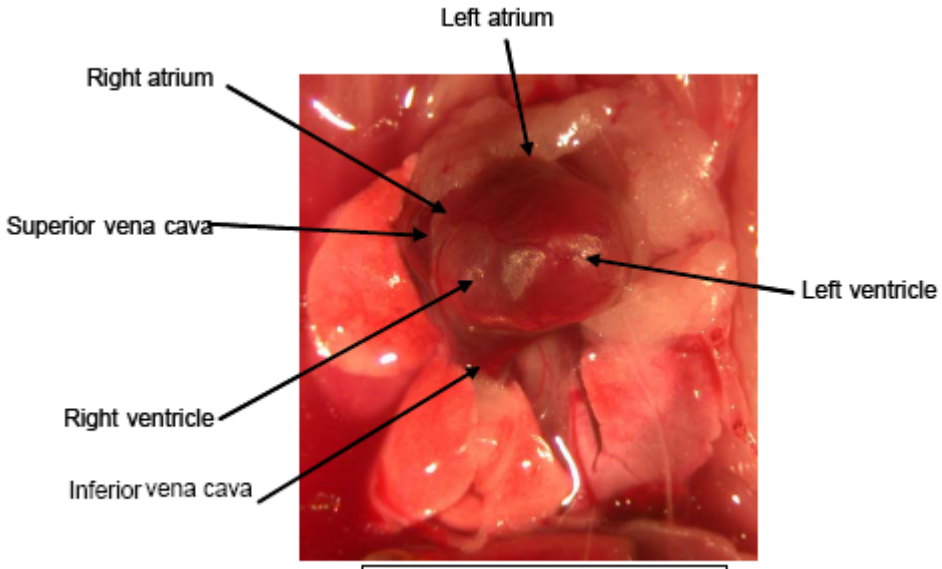
9.2 Intracardiac blood collection

9.2.1 Lay the anesthetized mouse on its back.

9.2.2 Cut abdominal skin and wall to open peritoneal space.

9.2.3 Cut diaphragm and ribs on both sides of the thoracic cage.

9.2.4 Pull up the thoracic cage.



In situ view of the heart

9.2.5 Carefully insert a "1.0 mm" needle connected to a "1 mL" syringe into the left ventricle and draw blood slowly in order that the wall does not collapse.

9.2.6 Transfer the blood into a "1.5 ml" tube coated with heparin.

9.2.7 Centrifuge blood (5000 rpm for 15 min at 4°C).

9.2.8 Collect the resulting supernatant (plasma) and store at –80°C until use.

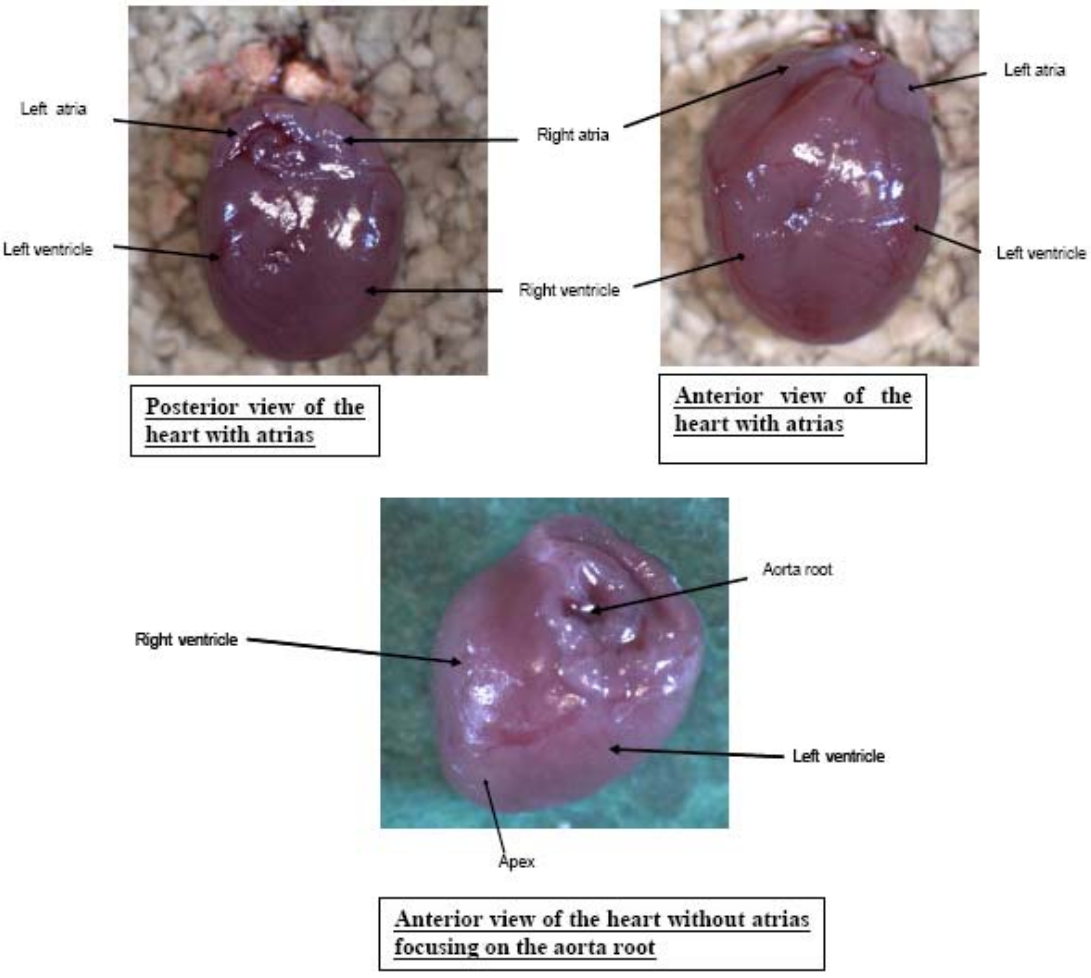
9.3 *Heart collection ~~Collection of the heart~~ and weight*

9.3.1 Note if there is excessive fat surrounding the heart.

9.3.2 Remove the heart from the pericardiac membrane

9.3.3 Note (text and photograph) if there are any abnormalities

9.3.4 Cut the major vessels at the point they enter/exit the atria and excise the heart.



~~9.3.5~~ 9.3.5

~~Label the bijous with the mouse ID~~
~~Load the KCl into the syringe, attach the butterfly needle and ensure all air bubbles are cleared.~~
~~Record the weight of the mouse~~
~~Give mouse terminal anaesthetic~~

~~Record the anaesthesic type and dose~~
~~Once pedal reflex has ceased, but before the heart stops, open up the ventral abdominal cavity and expose the inferior vena cava below the liver and the ascending aorta above the heart.~~
~~Introduce KCl into the inferior vena cava. Once 0.5ml has been introduced, sever the carotid and perfuse another 5ml of KCl through the heart (or until the solution runs clear).~~
~~Note if there is excessive fat surrounding the heart.~~
~~Remove the heart from the pericardiac membrane~~
~~Note (text and photograph) if there are any abnormalities~~
~~Cut the major vessels at the point they enter/exit the atria.~~
Gently squeeze the heart empty and tap it on~~on~~ the C-fold towels/surgical compress. D and ~~d~~ab it dry to remove all remaining liquid in the heart.

9.3.6 Record the weight of the heart

9.4 *Heart storage for histology (wax embedding)*
9.4.1 Place the heart into 5ml of freshly made paraformaldehyde in a bijou and fix overnight at room temperature.

9.4.2 Embed the hearts in wax as per *Tissue processing and embedding in paraffin*

9.4.3 Trim the block from the atrial end until no more atria tissue is visible

9.4.4 Collect a single 5µm section and stain in H&E as described in *Sectioning from paraffin embedded tissues* and *Haematoxylin and eosin staining of histological sections*

9.4.5 Collect image of the whole section at 20x (minimum resolution of 0.5µm per pixel)

9.4.6 Note if there are abnormalities visible in the section

9.5 Alternative heart storage for histology (removal of atrias and cryopreservation and storage at -80°C)
9.5.1 Remove both atria. Then cut a slice of the ventricles. Fix tissues in 4% formalin for 24 hours. Then store in 70% ethanol for further histological investigation.

9.5.2 Quickly freeze the apex in liquid nitrogen and store at -80°C for further investigation.

Parameters to record~~ed~~ for each mouse

- Animal weight (g)
- Method of anaesthesia
- Anaesthetic dose
- Presence of excessive fat surrounding the heart (yes/no)
- Abnormal morphology of heart (yes/no) if yes, free text description
- Option of images taken to record abnormalities
- Heart weight (mg)
- Heart weight (mg)/tibia length (mm) from X-ray
- Other comments relating to the tissue collection
- Comments relating to the tissue and section processing
- Image of H&E stained section
- Comments on image

10.0 Data Records and Reports:

10.1

11.0 History Review:

11.1 Not applicable

12.0 Emergency Procedures:

12.1 Ensure that appropriate steps are taken to eliminate the possibility of exposure to mouse allergens, needle sticks and paraformaldehyde poisoning.