WHOLE ANIMAL PERFUSION

Perfusion removes blood from organs of interest and rapidly and uniformly preserves tissues for further assessment. While this technique can be used for most species including mice, rats, cats, and non-human primates, it is important to note that physiological pressures are dependent on the species used. Techniques for perfusion fixation vary depending on the tissue to be fixed and how the tissue will be processed following fixation.

**Description of the procedure:**

1) The animal is deeply anesthetized as described in the IACUC protocol. Adequate anesthetic depth is confirmed using a firm toe pinch. If the animal reacts, additional time or anesthetic is given.

2) Once the animal has reached a surgical plane of anesthesia, a ventral midline incision is made through the skin and abdomen from the xiphoid process to approximately the level of the umbilicus.

3) The liver is carefully separated from the diaphragm.

4) Using curved, blunt scissors, an incision is made in the diaphragm along the entire length of the rib cage to expose the pleural cavity.

5) The ribs are then cut on both sides of the animal to the level of the collar bone.

6) The sternum is lifted and any tissue connecting the rib cage to the heart is carefully trimmed.

7) The tip of the sternum is then clamped with a hemostat and the hemostat placed over the head to retract the rib cage. When done properly, the thymus lifts away from the heart along with the sternum, providing a clear view of the major vessels.

8) A large gauge (size dependent on species) perfusion needle is passed through the left ventricle into the ascending aorta, but not up to the aortic arch where the brachial and carotid arteries diverge. Depth to which the needle is inserted into the aorta is determined by being able to see the tip of the needle through the wall of the aorta.

9) A hemostat is used to clamp the heart to secure the needle and prevent leakage.

10) A small incision is made in the animal's right atrium using delicate scissors.

11) After verification that there are no bubbles in the infusion fluid lines, perfusion is started with normal or phosphate-buffered saline followed by a fixative such as formalin as indicated by the research needs.
12) If the fluid does not flow adequately, the needle may be abutted against the heart wall. In such cases, the angle of the needle is adjusted to achieve a maximum flow rate.

**Detailed reference including an informative video:**

http://www.jove.com/video/3564/whole-animal-perfusion-fixation-for-rodents