

COMPARATIVE MEDICINE
LABORATORY ANIMAL FACILITIES

STANDARD OPERATING PROCEDURES
FOR
WHOLE BODY PERFUSION FIXATION OF MICE

1.0 Purpose:

The goal of perfusion fixation is to use the vascular system of a deeply anesthetized animal to deliver fixatives to the tissues of interest. This is the optimal method of tissue preservation because the tissues are fixed before autolysis begins. Perfused tissues are less susceptible to artifacts caused by handling. Techniques for fixation vary depending on the organ and the desired processing. Appropriate literature should be consulted to determine the ideal technique for the organ of concern. The following technique is appropriate for harvesting brain and organs with circulation supplied by the left side of the heart. This method combines tissue fixation with euthanasia and can only be performed as a terminal procedure.

2.0 Scope:

This procedure applies to all CMLAF veterinary staff and principal investigators and their associated staff performing tissue fixation via the vascular system.

3.0 Procedure:

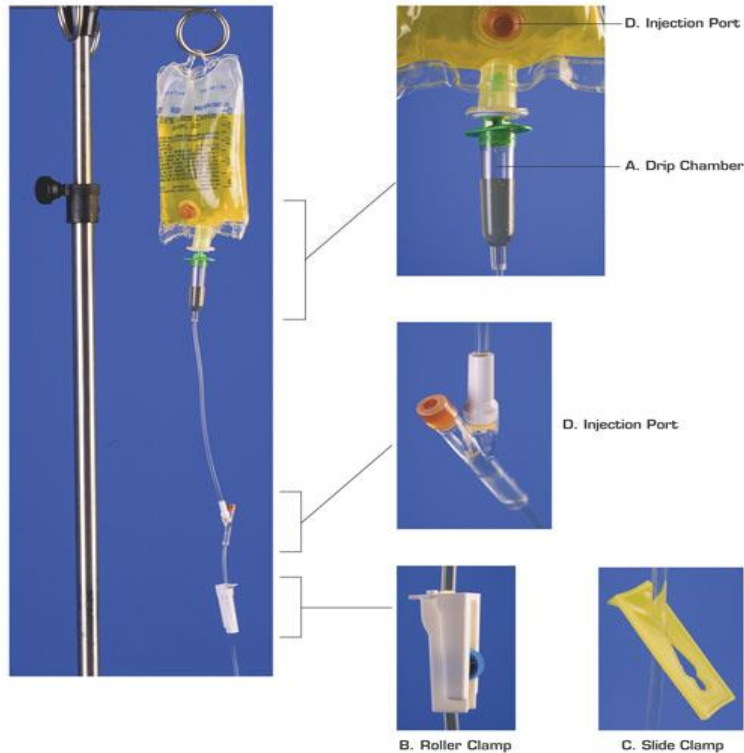
3.1 Supplies:

- 3.1.1 2 empty 250ml fluid bags with fluid lines attached (see diagram)
- 3.1.2 60 ml syringe and large bore needle
- 3.1.3 IV stand
- 3.1.4 Shallow, disinfectable pan to catch waste fluids
- 3.1.5 15 gauge blunt- or olive-tipped perfusion needle
- 3.1.6 2-3 Mosquito hemostats
- 3.1.7 Iris scissors and Metzenbaum scissors
- 3.1.8 200 mL freshly prepared 4% paraformaldehyde (PFA)
- 3.1.9 200 mL phosphate buffered saline (PBS) or preferred buffer
- 3.1.10 Anesthetic (as per approved IACUC protocol)
- 3.1.11 Chemical fume hood

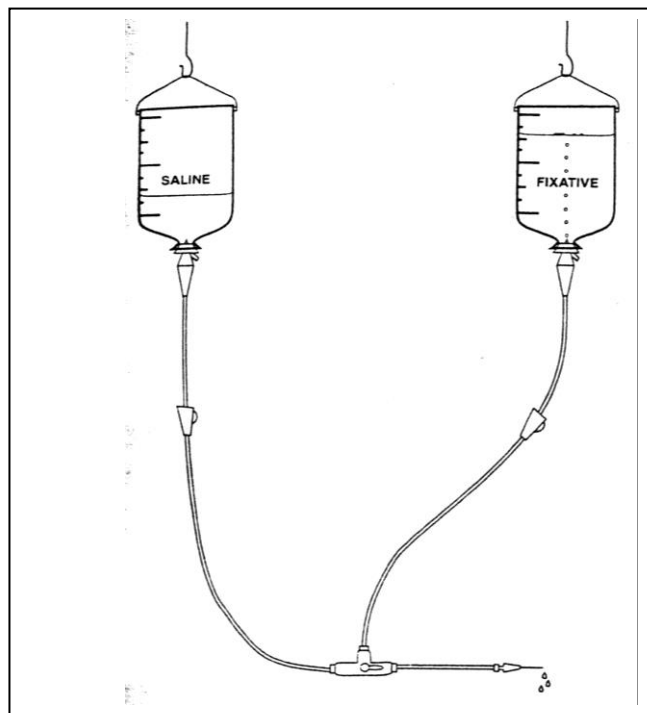
3.2 Set Up

- 3.2.1 4% PFA must be made fresh on the day of the procedure inside a chemical fume hood.
- 3.2.2 The perfusion process should be performed in a chemical fume hood for the best personal protection. Perfusion can be performed in a well-ventilated area if a chemical fume hood is not available.
- 3.2.3 Fill one empty fluid bag with fresh 4% PFA using a 60 ml syringe and a large bore needle. Fill the second fluid bag with phosphate buffered saline (PBS).

- 3.2.4 Set up the fluid lines in a “piggy back” fashion (see picture) with a perfusion needle attached to the end.
- 3.2.5 Hang the bags from the IV pole at least 30 cm, but not more than 120 cm, above the animal
- 3.2.6 Flush the fluid lines thoroughly with PBS to remove all fixative and air bubbles before beginning. Use caution throughout the procedure to avoid introducing air bubbles, as they will impede complete perfusion of tissues.



Example fluid bag and line set-up



Example of a commercially available “piggy back” set up for perfusion. A “piggy back” set up can also be made out of two regular IV lines by attaching a large bore needle to the end of one of the IV lines and inserting the needle into the injection port of the other line.

3.3 Anesthesia

3.3.1 Animals must be anesthetized prior to starting perfusion fixation.

3.3.2 The method of anesthesia specified in the approved IACUC protocol must be used. Pharmaceutical grade anesthetic agents must be used.

3.3.3 Anesthetic options:

- a. Sodium pentobarbital (Fatal-Plus® or Nembutal®): 100 mg/kg IP
- b. Ketamine/Xylazine: Rat: 80-100 mg/kg/5-10 mg/kg (respectively) IP or IM Rat :80-100 mg/kg/5-10 mg/kg, Mouse: 100-200 mg/kg/5-10 mg/kg (respectively) IP or IM
- c. Isoflurane via nose cone

3.3.4 A deep surgical plane of anesthesia must be ensured prior to beginning the procedure.

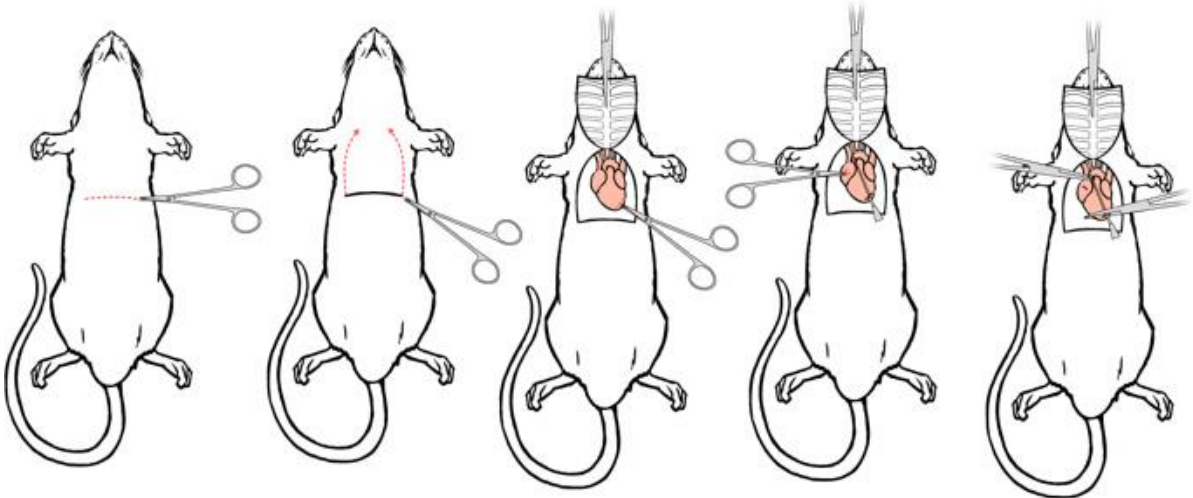
3.4 Perfusion

3.4.1 Place the mouse in dorsal recumbency (on its back) in the shallow pan, and secure the limbs to the pan.

3.4.2 Check the withdrawal reflex again to assure adequate depth of anesthesia. Administer additional anesthetics as needed.

3.4.3 Make a 5-6 cm incision through the skin and body wall just below the ribs. Carefully separate the liver from the diaphragm.

3.4.4 Using the Metzenbaum scissors (blunt scissors) carefully incise along the length of the diaphragm, then continue through the ribs on each side of the thorax until the sternum can be lifted away.

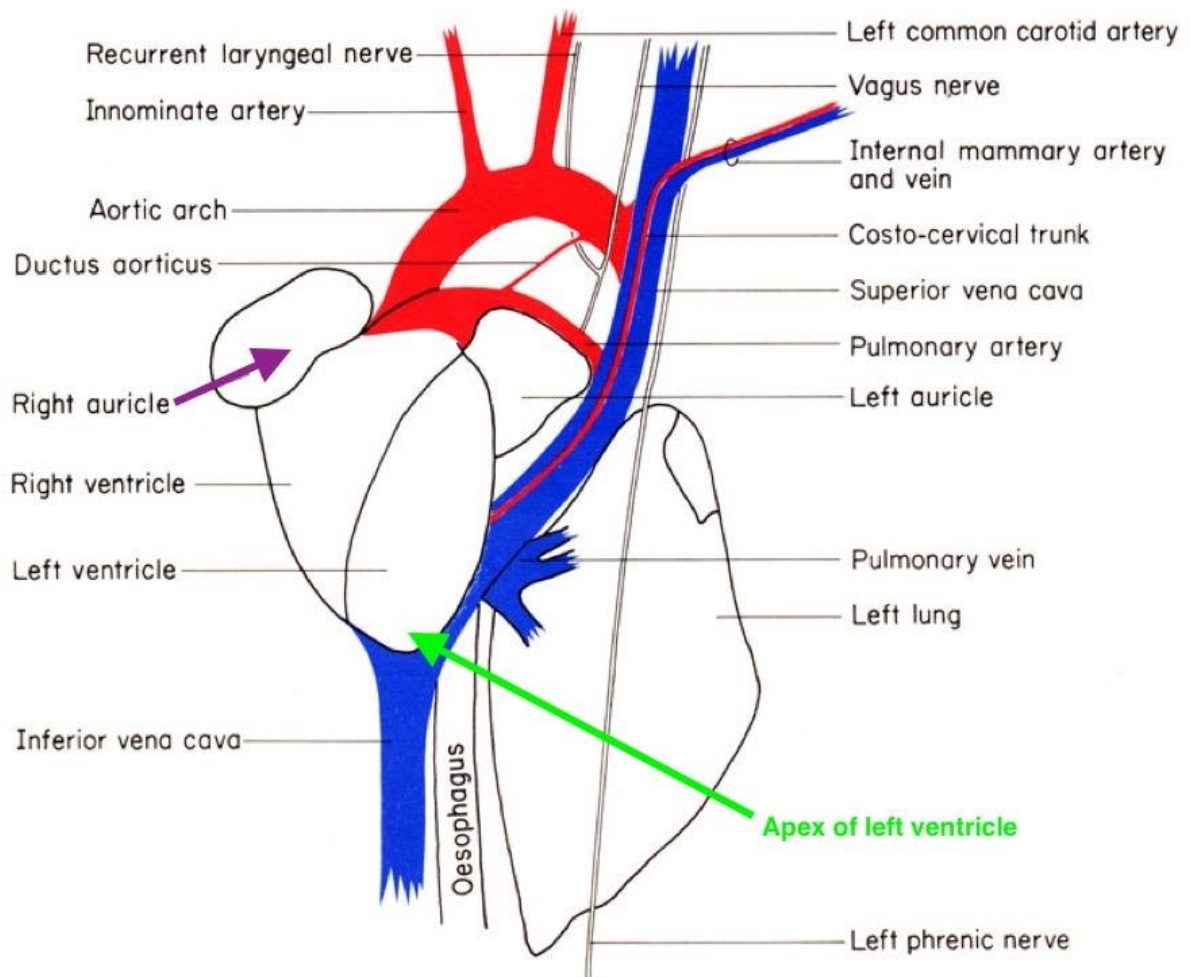


Gage, Gregory J., et. al. "Whole Animal Perfusion Fixation for Rodents." *Journal of Visualized Experiments*, no. 65, 2012, doi: 10.3791/3564.

3.5 Clamp the tip of the sternum at the level of the xiphoid process with hemostats, then lift the sternum away and lay the hemostats above the mouse's head.

3.6 Carefully remove the thymus and any connective tissue over the heart to provide a clear view of the major vessels.

- 3.7 Use iris scissors to make a small nick in the apex of the left ventricle. The wall of the left ventricle is thicker than the wall of the right ventricle. If a blood sample is needed it should be collected at this time.
- 3.8 Pass the 15 gauge blunt- or olive-tipped perfusion needle through the incision in the left ventricle until it reaches the ascending aorta. The needle should be visible through the wall of the vessel. Use care to not pass the needle all the way to aortic arch, as the brachial and carotid arteries diverge here.
- 3.9 Clamp the heart with hemostats to secure the needle and prevent leakage.
- 3.10 Using iris scissors, make an incision in the right auricle to provide an outlet for the perfusate.



Anatomy of the mouse heart. Green arrow is identifying the apex of the left ventricle where the perfusion needle is inserted. Purple arrow indicates the position of the right auricle that will be incised to allow drainage of the perfusate.

- 3.11 Start the flow of the PBS. Check the drip chamber on the IV line to assure adequate flow. The position of the needle in the aorta may need to be adjusted to optimize flow.
- 3.12 When the fluid exiting the mouse is clear of blood, close the buffer line and open the 4% PFA line.
- 3.13 Muscle contractions and blanching of the liver and mesenteric blood vessels are signs of good perfusion.

- 3.14 Perfusion is complete when all muscle contractions have stopped, the liver and mesenteric vessels are blanched, and the desired amount of preservative has passed through the circulatory system. The mouse should be stiff.
 - 3.15 PFA and other fixatives must be collected after the perfusion is complete and stored appropriately as hazardous chemical waste. Contact Environmental Health and Safety at 829-3301 for disposal procedures.
- 4.0 Troubleshooting
- 4.1 Fluid dripping from the mouse's nose
 - 4.1.1 Indicates too much fluid pressure.
 - 4.1.2 Lower the height of the IV bag until the fluid stops dripping from the nose.
 - 4.2 Fluid is not seen dripping in the IV chamber
 - 4.2.1 Indicates that the fluid is not flowing into the mouse's circulatory system.
 - 4.2.2 Close the fluid line, reposition the perfusion needle, reclamp the heart, and reopen the fluid line.