 <p>University at Buffalo The State University of New York Lab Animal Facilities</p>	<p align="center">STANDARD OPERATING PROCEDURE</p> <p align="center">Whole Body Perfusion of Small Rodents</p>	<p>Quality Form SOP # 2.A.12 Revision: 01 Last Reviewed: 5/23/2025</p>
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1.0 PURPOSE:

To describe a standardized procedure for terminal whole body perfusion in small rodents such as mice, rats, and hamsters.

2.0 SCOPE:

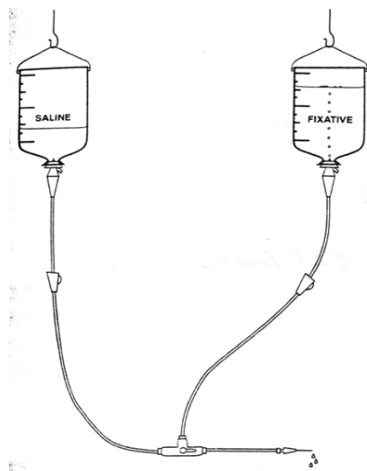
This applies to personnel performing terminal perfusion in small rodents prior to tissue collection.

3.0 DEFINITIONS: NA

4.0 PROCEDURES:

4.1 Set Up

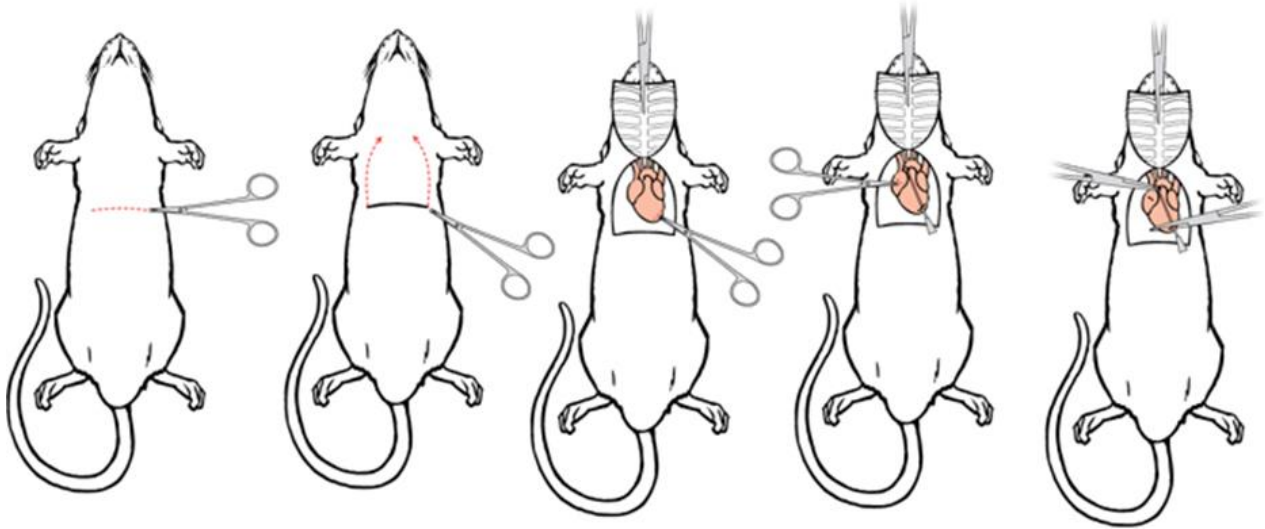
- 4.1.1 Any fixatives to be used should be prepared fresh and handled inside a chemical fume hood.
- 4.1.2 If fixatives are to be used, the perfusion process should be performed in a chemical fume hood for the best personal protection. Perfusion can be performed in a well-ventilated area, such as on a down draft necropsy table, if a chemical fume hood is not available.
- 4.1.3 Either a peristaltic pump or gravity feed system can be used to flush saline and fixatives through the animal's circulatory system.
 - a. If using a pump system, place the fixative and saline in the appropriate containers (glass) for the system, and set the pump rate for the species to be perfused.
 - b. If using a gravity feed system, fill an empty fluid bag with fixative and suspend both the fixative and saline bags 30-120 cm above the work surface (an IV pole works well). Set up the fluid lines attached to the bags in a piggyback fashion.
- 4.1.4 Ensure all lines are flushed and fluid filled prior to beginning perfusion. Air can impede proper perfusion of tissues.



Example of a commercially available "piggyback" set up for perfusion. A "piggyback" 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.

4.2 Perfusion Procedure

- 4.2.1 The animal must be deeply anesthetized using the method of anesthesia described in the approved IACUC protocol.
- 4.2.2 Place the animal in dorsal recumbency (on its back) in a shallow pan or on an absorbent pad and secure the limbs.
- 4.2.3 Make an incision through the skin and body wall just below the ribs. Carefully separate the liver from the diaphragm.
- 4.2.4 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.

- 4.2.5 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 animal's head.
- 4.2.6 Carefully remove the thymus and any connective tissue over the heart to provide a clear view of the major vessels.
- 4.2.7 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.
- 4.2.8 Pass 15-20G 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 into the aortic arch, as the brachial and carotid arteries diverge here.
- 4.2.9 Clamp the heart with hemostats to secure the needle and prevent leakage.
- 4.2.10 Make an incision in the right auricle to provide an outlet for the perfusate.
- 4.2.11 Start the flow of saline to flush the circulatory system. The position of the needle in the aorta may need to be adjusted to optimize flow.
- 4.2.12 When the fluid exiting the right auricle is clear of blood, stop saline perfusion. If fixative perfusion is required, begin the flow of fixative.

- 4.2.13 Muscle contractions and blanching of the liver and mesenteric blood vessels are signs of good perfusion. When fixative perfusion is complete, the animal will be stiff.
- 4.2.14 Fixatives and any materials that have been in contact with fixatives (absorbent pads) should be disposed of as hazardous waste. Contact EH&S for disposal procedures.
- 4.3 Troubleshooting
 - 4.3.1 Fluid dripping from the animal's nose indicates too much fluid pressure. Reduce the fluid rate on the pump or lower the height of the fluid bag until the dripping ceases.
 - 4.3.2 If fluid is not entering the heart/circulation, as evidenced by backing up of fluid in the fluid bag, no flow from the pump, or now fluid exiting the right auricle, reposition the needle in the heart as it has likely become obstructed in the wall of the aorta.

References:

1. Hedrich, Hans J. "Perfusion Methods." The Laboratory Mouse, Elsevier. Academic Press, 2004, p. 501.
2. Gage, Gregory J., et. al. "Whole Animal Perfusion Fixation for Rodents." Journal of Visualized Experiments, no. 65, 2012, doi: 10.3791/3564.
3. Slamdot, Inc. "Perfusion Protocol, Pump Calibration." Neuroscience Associates, 26 June 2017, www.neuroscienceassociates.com/instructions/perfusion-protocol/.

Approvals:

Name	Title	Date of Approval
Jennifer Peirick, DVM	LAF Director/Attending Veterinarian	5/23/25
Jolie McCutcheon, DVM	LAF Clinical Veterinarian	5/23/25

Change History:

Revision #	Description of Change	Effective Date
01	Adapted into new SOP format. Changed title from "Whole Body Perfusion Fixation of Mice" to include the perfusion of rats and hamsters. Minor edits to content	5/23/25