

University Animal Care Committee Standard Operating Procedure		
Document No: 10.15	Subject: Whole Body Perfusion for Small Rodents (Rats)	
Date Issued: April 11 th , 2012	Revision: 2	Page No: 1

Location: Queen's University

Responsibility: Principal Investigators, Research Staff, Veterinary Staff

Purpose: The purpose of this Standard Operating Procedure (SOP) is to describe perfusion methods used in rats.

1. Introduction and Definitions:

In deeply anesthetized animals, the vascular system is utilized to perfuse or deliver fixatives and other compounds (such as PBS) to the tissues of choice. This is the optimal method for tissue preservation as the tissues are fixed prior to autolysis occurring. For the collection of immunohistochemistry data, care must be especially taken when administering fixatives; a perfusion pump may be used as an alternative method. Appropriate Personal Protective Equipment (PPE) must still be worn and the pump manufacturer's instructions should be followed.

The following technique is appropriate for harvesting brain and organs with circulation supplied by the left side of the heart. This method combine's tissue fixation or flushing with euthanasia and can only be performed as a terminal procedure.

Abbreviations: Animal Care Services **ACS**, Principal Investigator **PI**, subcutaneous **SC**, intravenous **IV**, intraperitoneal **IP**, intramuscular **IM**, per os **PO**, per rectum **PR**

2. Materials:

- Two 250ml fluid bags with fluid lines attached
 - Various large syringes (10 – 60 ml dependent on species, perfusion compound and technique)
 - Large bore needles
 - IV stand
 - 18-23g butterfly catheter (species and size dependent)with blunt and sharp needles
 - Bandage tape
 - Mosquito hemostats
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- Iris scissors
- Small forceps
- 0.9% sodium chloride (20-200 ml species, size and technique dependent)
- Freshly prepared paraformaldehyde (PFA) or 10% buffered formalin (60-200 ml species dependent)
- 70% alcohol
- Gauze
- Anesthetic
- Chemical fume hood or down draft table
- Test tube rack
- Instrument or dissection pan
- Personal Protective Equipment (PPE); gloves, goggles, lab coat, mask
- Perfusion pump

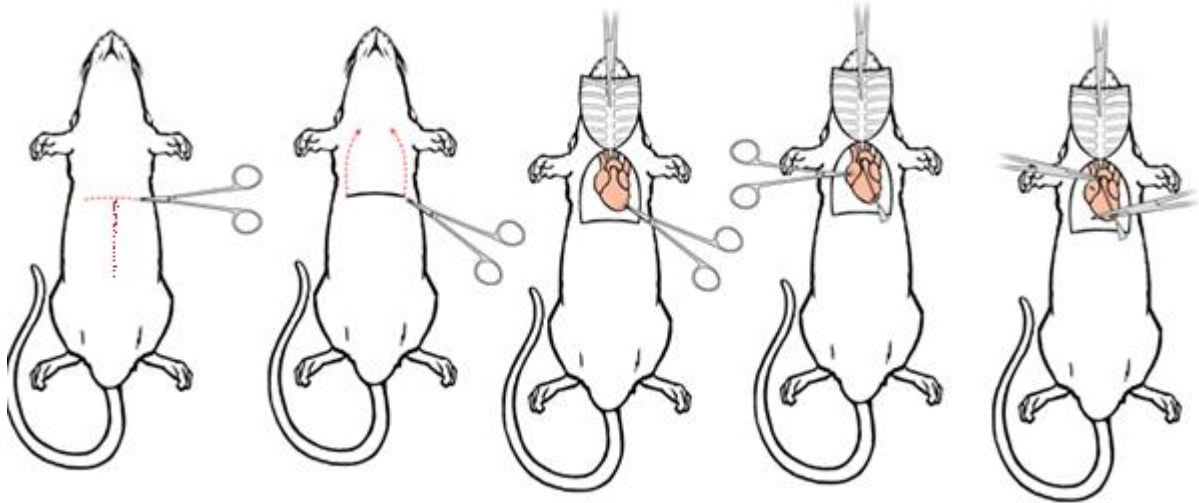
3. Procedures:

- The perfusion process should be conducted within a chemical fume hood or on a necropsy down draft table.
 - Place test tube rack over instrument or dissection pan if a down draft table is not available.
 - Fill one IV bag with 4% PFA or 10% buffered formalin using a 60ml syringe and a large bore needle.
 - Fill the other IV bag with 0.9% NaCl.
 - Hook the IV bags on the IV stand side by side. Attach the blunt butterfly needle to the end of the saline line. The bags should be at least 30cm above the animal but no more than 120cm high.
 - Flush any air bubbles out of the lines.
 - Following the respective anesthetic protocols, administer the anesthetic and allow the animal to reach a deep surgical plane of anesthesia (loss of palpebral, corneal and pedal reflexes, slow respiration rate).
 - Once the animal is in a deep surgical plane of anesthesia, place in dorsal recumbency and secure each limb to the test tube rack or necropsy table.
 - Wet the fur of the ventral skin surface with alcohol.
 - Make a midline incision from the thoracic inlet to the pelvis. This will help to observe blanching of organs with a successful perfusion.
 - Make a lateral incision through the integument and abdominal wall just beneath the rib cage.
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- Carefully separate the liver from the diaphragm.
 - Grasp the xyphoid process and lift up, exposing the diaphragm muscle. Using a combination of blunt dissection and scissor-assisted dissection techniques, open the thoracic cavity by cutting the diaphragm from one lateral aspect to the other lateral aspect while avoiding cutting any visceral organs.
 - With scissors, cut along one lateral side of the ribs, carefully displacing the lungs, through the rib cage up to the collarbone. Make a similar cut on the contralateral side.
 - Lifting the sternum away, carefully trim any tissue connecting it to the heart. Clamp the tip of the sternum with the hemostat and place the hemostat over the head. When done properly, the thymus lifts away from the heart along with the sternum, providing a clear view of the major vessels. In mice, the ribcage may be completely removed. This is not recommended with larger rodents due to the larger vasculature.
 - With forceps grasp the heart gently and lift it slightly out of the chest.
 - Make a small incision to the posterior end (apex) of the left ventricle using iris scissors.
 - Pass the butterfly needle through the cut ventricle into the ascending aorta. The tip may be visible through the wall of the aorta, and should not reach the aortic arch where the brachial and carotid arteries diverge.
 - Use a hemostat to clamp the butterfly and heart, this secures the needle and prevents leakage.
 - Make an incision to the animal's right atrium using iris scissors to create as large an outlet as possible without damaging the descending aorta.
 - Start the flow of the saline flush and watch the chamber of the IV line to make sure the fluids are flowing.
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<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3476408/>

- When the fluid exiting the animal is running clear (free of blood), close the flush line and open the PFA/formalin line. Fixation tremors when using a fixative should be observed within seconds. These muscle contractions and blanching of the liver and mesenteric blood vessels are signs of a successful perfusion. 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 animal should feel rigid.
- Depending on the quality of tissue needed, an approved option is to use a large syringe with a needle or butterfly needle attached. Manual perfusion is performed. It is important to recognize that the perfusate is not pushed through the heart at a constant flow rate using this method. This method is often used with PBS or Hank's Buffer.
- As a general rule, you can use a pump in cases where syringes would be sufficient, but you cannot use syringes when the procedure requires a pump such as IHC.

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References:

- Gage, G. J., Kipke, D. R., Shain, W. Whole Animal Perfusion Fixation for Rodents. *J. Vis. Exp.* (65), e3564, doi:10.3791/3564 (2012).
- <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3476408/>

SOP Revision History:

Date	New Version
April 11 th , 2012	SOP Created
February 28 th , 2019	Triennial Review
December 6 th , 2021	Triennial Review
June 10 th , 2024	Triennial Review, updated format.