

TRANSCARDIAL PERFUSION FIXATION (SOP-29)

INTRODUCTION

Although immersion fixation can allow for the adequate cytological preservation of small pieces of tissue for routine histological stains, optimal preservation of large blocks of body tissues ordinarily requires perfusion fixation at the time of euthanasia. This is especially true for central neural tissues, particularly if they are to be used in immunohistochemical or other specialized neurohistological procedures.

The following is a model protocol for perfusion fixation that is suitable for all rodent species and animals of any age. The "rinse" solution is generally a heparinized saline, while the "fixative" typically comprises a buffered solution of paraformaldehyde or mixed aldehydes.

PERFUSION EQUIPMENT

The perfusion equipment is quite simple, comprising a peristaltic pump attached to an IV line fitted with a cannula (a metal spinal needle or hypodermic needle cut flush and ground to a smooth finish). The perfusion equipment should be completely set up before the animal is anesthetized, and the line(s) should be completely cleared of air and filled with the first solution to be perfused, typically a heparinized normal saline solution.

The size of the cannula should be suited to the size of the animal. It is best to select the largest gauge cannula that can be inserted atraumatically into the aorta; a 16 gauge cannula is appropriate for adult rats. The rate of flow of perfused solutions should be calibrated so as not to exceed the normal rate of blood flow.

As fumes from the fixative solutions are potentially hazardous to the health of humans and animals, the perfusion of animals and dissection of fixed tissue should be performed in a fume hood.

TRANSCARDIAL PERFUSION

1. The animal is injected with 2x the anesthetic dose of chloral hydrate-pentobarbital or other appropriate anesthetic agent .
2. Closely monitor the animal's breathing; ideally the procedure should be initiated immediately prior to respiratory arrest induced by the anesthetic overdose. That is, respirations should be slow and shallow, but the animal should not be cyanotic. *Before proceeding with the thoracotomy, it is absolutely essential to confirm that the animal has reached a deep plane of anesthesia.* Pedal and corneal reflexes should be completely absent.
3. Using a large, sharp scalpel blade quickly cut through the skin and muscle of the thorax on each side of the body along the mid-axillary line, extending from the axilla to the inferior margin of the rib cage. Make a third

- incision across the ventral aspect of the trunk at the level of the xiphisternum.
4. Grasp the xiphoid process with a large hemostat and elevate the anterior body wall. Using a large scissors, cut through the abdominal muscles along the inferior margin of the ribs.
 5. Cut through the diaphragm along its attachment to the ventral and lateral margins of the rib cage. Note: Cutting the diaphragm collapses the lungs. Therefore the following steps up to the point of perfusion of the saline rinse (Step 10) should proceed as rapidly as possible to minimize anoxic damage to the tissue. Alternatively, the animal can be placed on a respirator.
 6. Complete the thoracotomy by cutting through the lateral part of the rib cage on both sides. Use the hemostat attached to the xiphoid process to retract the anterior part of the rib cage superiorly and hold it out of the field.
 7. Open the pericardial sac using a small pair of sharp scissors. Cut open the right atrium.
 8. With the scissors, make a transverse cut through the posterior left aspect of the heart to open the left ventricle.
 9. Stabilize the heart with forceps and insert the perfusion cannula through the opening in the left ventricle into the ascending aorta. Fix the cannula in place by clamping a straight hemostat across the superior part of the ventricles. Note: It is essential that before the cannula is inserted the perfusion lines be completely cleared of air and filled with the saline/rinse solution. At this point, pump should be either turned off or set at a slow drip.
 10. Confirm the placement of the cannula tip within the aorta ~1-3 mm above the top of the heart. Turn up the pump to perfuse the saline rinse solution (a pump rate setting of 2-3 is usually optimal in the rat). The descending aorta may be clamped at this point in the procedure.
 11. As soon as the fluid escaping from the atrium clears (i.e. the saline solution has replaced the blood within the animal's vascular system), turn off the pump, quickly transfer the pump input line from the saline solution to the fixative solution and resume pumping at the selected rate. As the fixative solution enters the body and the muscles begin to fix, the body should rapidly begin to stiffen in full extension.
 12. As the procedure continues it is generally advisable to gradually reduce the rate of infusion of the fixative solution to compensate for the constriction of the vasculature (for rats, the setting on the pump may gradually be reduced to 1). Emergence of perfusion fluid from the nostrils is a sign that the perfusion pressure is too high.
 13. After perfusing the desired volume of fixative, turn off the pump and remove the cannula from the aorta
 14. Before perfusing the next animal, be sure to clear the line of fixative and air, and refill it with the saline/rinse solution.