

TRANSCARDIAL PERFUSION FIXATION OF ANIMALS WITH REPLICATION COMPETENT VIRAL INJECTIONS (SOP-71)

PERFUSION EQUIPMENT AND MATERIAL

The perfusion equipment comprises 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 is completely in place before the animal is anesthetized, and the line(s) are completely cleared of air and filled with the first solution to be perfused, *i.e.*, 1X saline solution.

The size of the cannula that is inserted atraumatically into the aorta is a 16 gauge cannula for adult rats and a 20 gauge cannula for adult mice.

The fixative is 4 % (w/v) paraformaldehyde in phosphate buffered saline. 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 must be performed in a ducted biosafety cabinet.

If such a ducted cabinet is not available, we will use a ductless biosafety cabinet and all personnel involved with perfusions will wear a formaldehyde dosimeter for three consecutive perfusions. These will be returned to the vendor for analysis. If analysis results are over the exposure limit per OSHA requirements this procedure will not be continued and an alternate process determined by the lab and EH&S will be used and this SOP will be updated.

TRANSCARDIAL PERFUSION

All procedures are to be performed with double gloves.

1. The animal is injected with Fatal Plus, as per IACUC protocol.
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 large rounded scissors 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. Using large rounded scissors, quickly cut through the skin and muscle across the ventral aspect of the trunk at the level of the xiphisternum.
5. 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.

6. 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.
7. 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.
8. Open the pericardial sac using a small pair of rounded scissors. Cut open the right atrium and rounded-end scissors.
9. Stabilize the heart with a small straight hemostat and insert the perfusion cannula into the left ventricle and optionally advance it into the ascending aorta. Fix the cannula in place by clamping a straight hemostat across the superior part of the ventricles. 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 left ventricle or in the aorta ~1-3 mm above the top of the heart. Turn up the pump to perfuse the saline rinse solution at normal cardiac flow rates. 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. After perfusing the desired volume of fixative, typically 1 ml per gram weight of the animals, turn off the pump and remove the cannula from the aorta.
13. Remove the brain and place in fixative, which further acts to destroy residual virus, for 2-12 hours before further processing.
14. All formaldehyde waste will be collected and sent to EH&S for disposal as chemical hazardous waste.