
1. PURPOSE

The intent of this Standard Operating Procedure (SOP) is to describe procedures for transcatheter perfusion in laboratory animals.

2. RESPONSIBILITY

Principal investigators (PI) and their staff, veterinary care staff or any individual performing transcatheter perfusion, or assisting in those procedures.

3. MATERIALS

- 3.1. Anesthetics
- 3.2. 70% Alcohol
- 3.3. Surgical instruments
- 3.4. Winged infusion set (25G for mice, 21-23G for rats)
- 3.5. Needles (16-18G for large animals)
- 3.6. Syringes
- 3.7. Peristaltic pump and tubing
- 3.8. Saline
- 3.9. Fixative
- 3.10. Personal protective equipment, refer to section 6.3
- 3.11. Certified chemical fume hood, Type II B2 or Type II B1 Biological Safety Cabinet (BSC), or ventilated downdraft workstation, where applicable

4. PROCEDURE FOR RODENTS

- 4.1. Anesthetize animal as per Anesthesia SOP.
- 4.2. Prior to starting the procedure, verify that the animal has reached a surgical plane of anesthesia by confirming loss of pedal withdrawal (toe pinch) reflex.
- 4.3. Position animal in dorsal recumbency. The perfusion set up should allow collection of used aldehyde-based fixatives.
- 4.4. If a blood sample is needed, collect blood via intracardiac puncture.
- 4.5. Wet the fur with alcohol over the entire ventral area.
- 4.6. Make an incision on the ventral aspect of the neck, just over the salivary glands. Using blunt dissection, carefully expose the jugular veins, located just under the salivary glands.
- 4.7. Make a second incision in the abdomen, below the xiphoid process, through the skin and abdominal muscle wall. Open the thoracic cavity by carefully cutting the ribs on both sides of the sternum. Cut or reflect the sternum to expose the heart.
- 4.8. Carefully cut the jugular veins. Blood will start to flow from the vessels.
- 4.9. Insert the needle of the winged infusion set into the left ventricle, making sure the needle is not inserted too deep so that it remains in the ventricle and does not advance into the left atrium.

- 4.10. Use a syringe or peristaltic pump to perfuse saline into the heart with gentle but constant pressure. Observing the area where the blood vessels were severed. Saline should flush the blood out of the vascular system. It is important that the pressure exerted on the syringe or from the pump is enough to push the blood through and out of the vascular system, but not so excessive as to cause damage to any of the organs.

Note: Using gravity pressure to drive saline or fixative is not as effective as using a syringe or pump to completely wash out blood from the tissues.

- 4.11. If using inhalant anesthetics, anesthesia can be stopped when the perfusion starts.
- 4.12. If required, continue perfusion with fixative after saline.
- 4.13. Fixation tremors are usually observed within seconds; this should be considered the true time of fixation.
- 4.14. Alternative procedures for rodents:
 - 4.14.1. Rather than isolating and incising the jugular veins, an incision can be made to the right atrium. Care should be taken to avoid damaging the descending aorta. Perfusion can be continued from step 4.9.
 - 4.14.2. If the only organ of interest is the brain, perfusion can be optimized by using a small hemostat to clamp the descending aorta and vena cava between the liver and lungs. This will occlude the vessels of the lower body.

5. PROCEDURE FOR LARGE ANIMALS

- 5.1. Anesthetize animal as per Anesthesia SOP.
- 5.2. Prior to starting the procedure, verify that the animal has reached a surgical plane of anesthesia by confirming loss of pedal withdrawal (toe pinch) reflex.
- 5.3. Position animal in dorsal recumbency. Perfusion of large animals should occur on a ventilated downdraft workstation to allow adequate collection of used aldehyde-based fixatives.
- 5.4. Wet the fur with alcohol over the entire ventral area.
- 5.5. Make an incision in the abdomen, below the xiphoid process, through the skin and abdominal muscle wall. Open the thoracic cavity by carefully cutting the ribs on both sides of the sternum. Reflect the sternum to expose the heart.
- 5.6. Make an incision in the right atrium. Care should be taken to avoid damaging the descending aorta.
- 5.7. Insert the needle into the left ventricle, making sure the needle is not inserted too deep so that it remains in the ventricle and does not advance into the left atrium. The needle may be clamped in place with hemostats.
- 5.8. Use a peristaltic pump to perfuse saline into the heart. Observing the area around the right atrium; saline should flush the blood out of the vascular system.

Note: Using gravity pressure to drive saline or fixative is not as effective as using a pump to completely wash out blood from the tissues.
- 5.9. If using inhalant anesthetics, anesthesia can be stopped when the perfusion starts.
- 5.10. If required, continue perfusion with the fixative after the saline.
- 5.11. If the only organ of interest is the brain, perfusion can be optimized by clamping the descending aorta and vena cava between the liver and lungs. This will occlude the vessels of the lower body.

6. SAFETY

- 6.1. Aldehyde-based fixatives such as formaldehyde, paraformaldehyde, formalin, and glutaraldehyde are corrosive, flammable, poisonous and chronic exposure is toxic. Such fixatives are sensitizers, known carcinogens and upper respiratory tract and eye irritants. Refer to the Safety Data Sheet.
- 6.2. Routes of exposure include inhalation of vapors during use and splashes to the skin, eyes and mucus membranes during manipulations.

- 6.3. The following personal protective equipment must be worn at all times when handling aldehyde-based fixatives:
 - 6.3.1. 2 pairs of gloves
 - 6.3.2. Mask
 - 6.3.3. Gown or labcoat
 - 6.3.4. Disposable protective sleeves
 - 6.3.5. Safety glasses, goggles or face shield
- 6.4. Any handling of aldehyde-based fixatives, including weighing of powder, preparation of dilutions, perfusions and handling of perfused tissue specimens must be conducted in a certified chemical fume hood, Type II B2 or Type II B1 Biological Safety Cabinet (BSC), or ventilated downdraft workstation.
- 6.5. All solutions of aldehyde-based fixatives and tissues preserved in such fixatives must be stored in tightly sealed, labeled containers to prevent leakage, spills and evaporation.
- 6.6. Used aldehyde-based fixatives must be collected and stored for disposal by the Waste Management department. Do not pour formaldehyde or formalin waste into sinks or drains.
- 6.7. In the event of accidental exposure, promptly complete a McGill University Accident, Incident & Occupational Disease Report form: <https://www.mcgill.ca/ehs/forms/forms/accident-and-incident-report>