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**1. PURPOSE**

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

**2. RESPONSIBILITY**

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

**3. MATERIALS**

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- 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. Phosphate Buffered Saline (PBS) or 0.9% Sodium Chloride solution
- 3.9. Heparin sodium (1000 U/ml), if applicable
- 3.10. Fixative
- 3.11. Personal protective equipment
- 3.12. Chemical fume hood, Type II B2 Biological Safety Cabinet (BSC), or ventilated downdraft workstation, if applicable

**4. CONSIDERATIONS**

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- 4.1. Transcatheter perfusion utilizes the vasculature for systemic delivery of fixative to the animal to preserve tissues.
- 4.2. All materials, equipment, and solutions should be prepared before anesthetizing the animal.
- 4.3. A washout solution, Phosphate Buffered Saline (PBS) or 0.9% Sodium Chloride solution, is used before the fixative to remove clots, blood cells, and other intravascular debris.
  - 4.3.1. Heparin may be added to the washout solution to help inhibit blood clot formation and preserve the patency of the vascular system. If applicable, prepare a 10 U/ml heparin solution.

**5. PROCEDURE FOR RODENTS**

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- 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 animal's pedal withdrawal (toe pinch) reflex. Anesthesia should be maintained until death is confirmed.
- 5.3. Position animal in dorsal recumbency over a dissection board.
- 5.4. If a blood sample is needed, collect blood via intracardiac puncture.
- 5.5. Wet the fur with alcohol over the entire ventral area.
- 5.6. 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.

- 5.7. 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.
- 5.8. Carefully cut the jugular veins. Blood will start to flow from the vessels.
- 5.9. 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.
- 5.10. Use a syringe or peristaltic pump to perfuse the washout solution into the heart, with gentle but constant pressure, until the venous outflow is clear of blood, clots, or debris. 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 solutions is not as effective as using a syringe or pump to completely wash out blood from the tissues.

- 5.11. If required, continue perfusion with the fixative.
- 5.12. Fixation tremors are usually observed within seconds; this should be considered the true time of fixation.
- 5.13. Alternative procedures for rodents:
  - 5.13.1. 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.
  - 5.13.2. 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.

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## 6. PROCEDURE FOR LARGE ANIMALS

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- 6.1. Anesthetize animal as per Anesthesia SOP.
- 6.2. Prior to starting the procedure, verify that the animal has reached a surgical plane of anesthesia.
- 6.3. Position animal in dorsal recumbency.
- 6.4. 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.
- 6.5. Make an incision in the right atrium. Care should be taken to avoid damaging the descending aorta.
- 6.6. 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.
- 6.7. Use a peristaltic pump to perfuse washout solution into the heart until the venous outflow is clear of blood, clots, or debris.

Note: Using gravity pressure to drive solutions is not as effective as using a pump to completely wash out blood from the tissues.
- 6.8. If required, continue perfusion with the fixative.
- 6.9. 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.

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## 7. SAFETY

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- 7.1. 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.
- 7.2. Routes of exposure include inhalation of vapors during use and splashes to the skin, eyes and mucus membranes during manipulations.

- 7.3. The following personal protective equipment must be worn at all times when handling aldehyde-based fixatives:
  - 7.3.1. 2 pairs of gloves
  - 7.3.2. Mask
  - 7.3.3. Gown or labcoat
  - 7.3.4. Disposable protective sleeves
  - 7.3.5. Safety glasses or goggles
- 7.4. 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 Biological Safety Cabinet (BSC), or ventilated downdraft workstation.
- 7.5. All solutions of aldehyde-based fixatives and tissues preserved in such fixatives must be stored in tightly sealed, properly labeled, containers to prevent leakage, spills, and evaporation.
- 7.6. 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.
- 7.7. 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>

## 8. REFERENCES

- 8.1. McFadden, W.C., Walsh, H., Richter, F. et al. Perfusion fixation in brain banking: a systematic review. *acta neuropathol commun* 7, 146 (2019). <https://doi.org/10.1186/s40478-019-0799-y>

## SOP REVISION HISTORY

DATE	NEW VERSION
2021.05.14	3.8. Phosphate Buffered Saline (PBS) or 0.9% Sodium Chloride solution
2021.05.14	3.9. Heparin sodium (1000 U/ml), if applicable
2021.05.14	3.13. Chemical fume hood, Type II B2 Biological Safety Cabinet (BSC), or ventilated downdraft workstation, if applicable
2021.05.14	<p><b>4. CONSIDERATIONS</b></p> <p>4.1. Transcardiac perfusion utilizes the vasculature for systemic delivery of fixative to the animal to preserve tissues.</p> <p>4.2. All materials, equipment, and solutions should be prepared before anesthetizing the animal.</p> <p>4.3. A washout solution, Phosphate Buffered Saline (PBS) or 0.9% Sodium Chloride solution, is used before the fixative to remove clots, blood cells, and other intravascular debris. Heparin may be added to the washout solution to help inhibit blood clot formation and preserve the patency of the vascular system. If applicable, prepare a 10 U/ml heparin solution.</p>
2021.05.14	<p>5.10. Use a syringe or peristaltic pump to perfuse <del>saline</del> the washout solution into the heart, with gentle but constant pressure, <del>Observing the area where the blood vessels were severed, until the venous outflow is clear of blood, clots, or debris. Saline should flush the blood out of the vascular system.</del> 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.</p> <p>Note: Using gravity pressure to drive <del>saline or fixative solutions</del> is not as effective as using a syringe or pump to completely wash out blood from the tissues.</p>
2021.05.14	5.11 If required, continue perfusion with the fixative <del>after the saline.</del>
2021.05.14	6.2. Prior to starting the procedure, verify that the animal has reached a surgical plane of anesthesia. <del>by confirming loss of animal's pedal withdrawal (toe pinch) reflex.</del>
2021.05.14	<del>6.4. Wet the fur with alcohol over the entire ventral area.</del>
2021.05.14	<p>6.7. Use a peristaltic pump to perfuse <del>saline</del> washout solution into the heart <del>until the venous outflow is clear of blood, clots, or debris. Heparin (10,000 IU/L mixed with the saline solution to perfuse can be used. Observing the area around the right atrium; saline should flush the blood out of the vascular system.</del></p> <p>Note: Using gravity pressure to drive <del>saline or fixative solutions</del> is not as effective as using a pump to completely wash out blood from the tissues.</p>
2021.05.14	6.9. If required, continue perfusion with the fixative <del>after the saline.</del>
2021.05.14	<p><b>8. REFERENCES</b></p> <p>8.1. McFadden, W.C., Walsh, H., Richter, F. et al. Perfusion fixation in brain banking: a systematic review. <i>acta neuropathol commun</i> 7, 146 (2019). <a href="https://doi.org/10.1186/s40478-019-0799-y">https://doi.org/10.1186/s40478-019-0799-y</a></p>
2022.03.21	5.2. Prior to starting the procedure, verify that the animal has reached a surgical plane of anesthesia by confirming loss of animal's pedal withdrawal (toe pinch) reflex. <b>Anesthesia should be maintained until death is confirmed.</b>