Document Number: ANP046	Title: CARDIAC PERFUSION OF RATS FOR SYSTEMIC BLOOD COLLECTION	Effective Date: 5/17/23
Section: Animal Research		Supersedes Date: 2/12/09
Subsection: Facility	CONFIDENTIAL INFORMATION MOLECULAR MEDICINE RESEARCH INSTITUTE	Page: 1 of 4

1.0 OBJECTIVE

1.1 The objective of this Standard Operating Procedure (SOP) is to describe the surgical procedure used for the cardiac perfusion of rats at the Molecular Medicine Research Institute (MMRI) Animal Facility (AF).

2.0 SCOPE

2.1 This SOP applies to all rats in the AF and covers all animal protocols requiring perfusion via the cardiac route.

3.0 POLICY

3.1 It is the policy of MMRI to establish written and approved procedures to assure that the animals are treated in a humane manner according to the Guide for the Care and Use of Laboratory Animals and educating personnel utilizing animals in their research.

4.0 RESPONSIBILITY

4.1 It is the responsibility of Manager of Animal Research or designated alternate to implement this SOP and revise it when necessary.

5.0 REFERENCES

5.1 A videographic reference outlining the entire procedure. “Isolation of Mononuclear Cells from the Central Nervous System of Rats with with EAE. Christine Beeton, K. George Chandy Department of Physiology and Biophysics, University of California, Irvine Journal of Visualized Experiments http://www.jove.com/index/Details.stp?ID=527

6.0 PROCEDURE

6.1 Anesthesia

6.1.1 Animals are anesthetized with a choice of the following anesthetics: sevofluorane; halofluorane; isofluorane; pentobarbital; or ketamine/xylazne cocktail (see Table 1)

6.1.2 Animals are maintained under deep anesthesia by use of an anesthetic nose cone for the duration of treatment and/or perfusion. Animals are typically anesthetized for a maximum period of 20 minutes.

6.2 Materials:

6.2.1 Perfusion buffer:
Ringer solution, 0.9% saline, 1x phosphate buffered saline (PBS), 10% formalin, or 4% paraformaldehyde (PFA)

4% PFA solution: 100 mL PBS - heat to 65 ^{o}C in a water bath or microwave. Add 4 g PFA, and 100 uL 10N sodium hydroxide. Stir on magnetic stirrer in fume hood until dissolved. Filter sterilize and cool on ice.

Table 1.

Species	Drug	Dose (mg/kg)	Route	Times/day	Duration
Rat	Sevofluorane	5.0 – 6.0%	Inhalation	1 or 2	Max. 20 min
Rat	Haloflorane	3.0 – 4.0%	Inhalation	1 or 2	Max. 20 min
Rat	Isofluorane	3.0 – 4.0%	Inhalation	1 or 2	Max. 20 min
Rat	Pentobarbitol	30-80 mg/kg	IP[1]	1 plus boost	20 min
Rat	Ketamine/Xylazine	Ketamine (40-80 mg/kg)/ Xylazine (5-10 mg/kg)	SC[2] or IM[3]		

IP[1]: intraperitoneal SC[2]:subcutaneous IM[3]: intramuscular

6.2.2 Irrigation tubing

6.2.3 Syringe pump: a peristaltic pump capable of providing a perfusion rate of a minimum of 10 mL per minute and 30 mL per minute. When a pump is not available, gravity may be used.

6.2.4 160 mL syringe tubes (for perfusion buffer reservoirs) and clamp-stand (gravity-assisted perfusion)

6.2.5 10 mL syringe containing gauze/kimwipes - soak with anesthetic for maintaining deep anesthesia

6.2.6 3-way Laur stop-cocks for multiple buffer perfusions

6.3 Perfusion procedure while the animal is under anesthesia

6.3.1 The heart is exposed by first lifting the skin just under the xiphoid process with the Oehker standard forceps and cutting the skin and abdominal muscles with curved standard scissors to expose the bottom of the diaphragm.

6.3.2 The diaphragm is then cut.

6.3.3 Holding the xiphoid process with the Oehler forceps, the curved standard scissors are used to cut vertically up both sides of the sternum, making an incision with a V-shape and exposing the heart.

6.3.4 The V-shaped flap is held back to avoid interference with further surgical procedures by clamping a hemostat onto the xiphoid process and then taping the hemostat up, and out of the way.

6.3.5 The pericardial sac is then removed.

6.3.6 The heart is then held with Singley heart holding forceps, and while held, a probe point blunt needle is inserted through the left ventricle, just into the base of the ascending aorta, and is held in place with a Dieffenbach vessel clip.

6.3.7 The blunt needle is connected to irrigation tubing attached either to a syringe pump, or to a gravity perfusion system.

CARE SHOULD BE TAKEN TO ENSURE THAT NO BUBBLES ARE PRESENT IN THE LINE OR SYRINGE OR IN THE BLUNT NEEDLE.

6.3.7.1 Syringe pump perfusion

a. 160 mL syringe (attached to a syringe pump) filled with ice cold Ringer perfusion buffer

b. Once the blunt needle is in the ascending aorta and is clamped in place, the syringe pump is started and immediately the right auricle is cut with Cartroviejo scissors to drain blood during perfusion.

c. 60 mL of ice-cold perfusion solution is then delivered via the syringe, at a rate of approximately 15 mL per minute

d. Perfusion is complete after approximately 4 min.

6.3.7.2 Gravity assisted perfusion

a. 160 mL syringe (attached to a drip system) filled with ice cold Ringer perfusion buffer

b. Once the blunt needle is in the ascending aorta and is clamped in place, the drip is started and immediately the right auricle is cut with Cartroviejo scissors to drain blood during perfusion.

c. Maintaining the reservoir at an approximate height of 80 cm, 60 mL of ice-cold perfusion solution is then delivered via the syringe, at a rate of approximately 10 mL per minute (flow rate will increase with height of reservoir)

d. Perfusion is complete after approximately 6-7 min.

6.3.7.3 Perfusion using mixed solutions (using either the pump perfusion or gravity-assisted perfusion technique

a. Prepare two reservoirs and fill the first with at least 100 mL of cold sterile saline or 1x PBS. The addition of heparin at 1000 units/L is advised to improve perfusion

b. The second reservoir is filled with at least 100 mL of 10% formalin or freshly prepared 4% PFA (see for PFA preparation procedure)

c. Once the blunt needle is in the ascending aorta and is clamped in place, the drip is started and immediately the right auricle is cut with Cartroviejo scissors to drain blood during perfusion.

d. Using the syringe pump, 60 mL of ice-cold perfusion wash solution is then delivered via the syringe, at a rate of approximately 15 mL per minute. The reservoirflow is then switched using a 3-way Luer Valve, and 60 mL of 4% PFA solution is then delivered, again at a rate of approximately 15 mL per minute. Perfusion will be complete after approximately 8-10 minutes

e. Using gravity-assistance, each reservoir is held at an approximate height of 80 cm, and each connected to a 3-way Luer Valve. 60 mL of ice-cold perfusion wash solution is then delivered via the syringe, at a rate of approximately 10 mL per minute. The reservoir flow is then switched using a 3-way Luer Valve, and 60 mL of 4% PFA solution is delivered, again at a rate of approximately 10 mL per minute. Perfusion will be complete after approximately 14-15 minutes

6.3.8 Animal carcasses are then stored at -20^{o}C until pick-up by Stericycle