



Pathology Core Protocol

Protocol Section: Clinical Biochemistry: General Lab Procedure
Protocol Subject: Retro-orbital blood collection on mouse

Policy No: CC-PPC1-GLP-RETRO_ORB_BLOOD

Effective Date: Apr 28, 2016

Date Reviewed: Sept 6, 2016

Date Revised: Sept 13, 2016

Introduction: Collecting blood from a mouse requires skilled techniques and collectors must follow the CCAC guidelines to minimize stress and pain on the animal when performing these procedures. Improper blood collection techniques and excessive stress on the animal during collection can create artifacts on the sample such as hemolysis, which compromise the accuracy of the research data. Depending on the blood volume required there are different blood collection routes that collectors can consider to avoid damage to certain tissues. This protocol describes the procedures for blood collection from a mouse using the retro-orbital route, which is a terminal procedure that yields a large volume of blood (600-800ul of whole blood) but can damage eye tissues.

Materials:

- Heat pad
- Anesthetic vaporizer (Benson Medical Industries Inc. Model:T3ISO)
- Induction chamber (homemade with plastic jar)
- Isoflurane 99.9% (Pharmaceutical Partners of Canada. Cat no. M60303)
- Ice in styrofoam box
- Centrifuge (Eppendorf. Model:5424R)
- 1.5ml disposable microtubes

Anticoagulant tubes:

- Heparinized capillary tubes (Fisherbrand. Cat no. 22-260-950) or
- K2EDTA tubes (BD Microtainer Cat. #365974)
- BD Microtainer-PST tubes with Lithium Heparin (BD. Cat no. 365985)

Procedures:

1. Place mouse cage on a heat pad to increase blood circulation in the mouse before collection.
2. Turn on vaporizer, together with oxygen supply; fill the induction chamber with 5% isoflurane.
3. Place the mouse in the induction chamber and observe it reaches surgical plane.
4. Take the mouse out of the chamber and bring the mouse's nozzle to the respiration tubing connected to the vaporizer. Check for vital signs to ensure surgical plane.
5. With your index finger and thumb, pull skin away from the eyeball, so that the eyeball is protruding out of the socket as much as possible.
6. Insert the tip of a heparinized capillary tube into the corner of the eye socket underneath the eyeball, directing the tip at a 45-degree angle towards the middle of the eye socket.
7. Rotate the tube between your fingers during forward passage.
8. Apply gentle downward pressure and then release until the vein is broken and blood is visualized entering the pipette.
9. Allow blood to fill the tube until the blood starts dripping on the distal end and collect the dripping blood into the anticoagulant tube. Mix the tube well to allow for the anticoagulant to mix with the blood.
10. Euthanize the mouse to ensure death by cervical dislocation or CO2 chamber after the required blood volume is obtained. See GP-PPC1-GLP-EUCerv-01 "Euthanasia of the Mouse-Cervical Dislocation" and GP-PPC1-GLP-EUCO2-01 "Euthanasia of the mouse-CO2" for details on the procedures.

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11. Gently invert the collection tube to mix with the anticoagulant. DO NOT shake or mix the tube vigorously.
12. Keep whole blood sample on ice until all samples are collected and ready for centrifuging.
13. Turn on centrifuge and do a dry run until it reaches 8°C internally.
14. Set the centrifuge to spin at 5000 rcf for 10 minutes at 8°C.
15. After centrifugation, pipette out the plasma portion of the whole blood and transfer to a 1.5ml microtube.
16. Plasma can be frozen at -80°C for long term storage.



Reference: Methods of Blood Collection in the Mouse. Hoff, Janet. Lab Animal. November 2000 Vol. 29, No. 10. pp 47-53.

Issued by Lab Manager: _____ **Date:** _____

Approved by Facility Management: _____ **Date:** _____