



Standard Operating Procedure: Blood Collection in the Mouse, Retro-orbital Bleed

These SOPs were developed by the Office of the University Veterinarian and reviewed by Virginia Tech IACUC to provide a reference and guidance to investigators during protocol preparation and IACUC reviewers during protocol review. They can be used as referenced descriptions for procedures on IACUC protocols.

However, it is the sole responsibility of the Principal Investigator to ensure that the referenced SOPs adequately cover and accurately represent procedures to be undertaken in any research project. Any modification to procedure as described in the SOP must be outlined in each IACUC protocol application (e.g. if the Principal Investigator plans to use a needle size that is not referenced in the SOP, simply state that alteration in the IACUC protocol itself).

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I. Procedure Summary and Goal

Describes procedures for the collection of small blood samples from the orbital venous sinus as a survival or terminal procedure in the mouse.

Considerations:

Please refer to the Guidelines for Injections in Rodents and Rabbits, Virginia Tech Office of the University Veterinarian for recommended volumes and needles sizes.

Blood volume collection determination (ARAC Guidelines)

- a. The total circulating blood volume of a rodent is estimated to be approximately 8% of body weight.
- b. Of the circulating blood volume, approximate percentages of the total volume which can safely removed are as follows:
 - i. 10% every two to four weeks
 - ii. 7.5% every seven days
 - iii. 1% every 24 hours

II. Personal Protective Equipment (PPE) and Hygiene

- a. Ensure appropriate PPE is used to protect technician from accidental exposure to blood and other body fluids, such as:
 - i. Gloves
 - ii. Eye protection
 - iii. Mask
 - iv. Other PPE as required by protocol/facility
- b. Inspect capillary tubes/Pasteur pipettes for broken or chipped sections to avoid increased risk of breakage while collecting blood samples.
- c. Hands should be washed and/or gloves changed between animals.
- d. Promptly dispose of used sharps in the provided leak-proof, puncture resistant sharps container.

III. Supply List

- a. Heparinized or non-heparinized capillary tubes or Pasteur pipettes
- b. Capillary tube sealant, for example Crito-O-Seal® (optional for procedure)
- c. General anesthetic - either alone or a combination of the following:
 - i. Isoflurane – inhaled to effect, usually approximately 3%
 - ii. 70% CO₂ + 30% O₂ gas – inhaled to effect



- iii. Ketamine (90-120mg/kg) + Xylazine (8-12mg/kg) combination
 - 1. Intramuscular, subcutaneous, or intraperitoneal injection
- d. Topical ophthalmic anesthetic (e.g., Proparacaine Hydrochloride Ophthalmic Solution)
- e. Ophthalmic ointment
- f. Gauze pads

IV. Detailed Procedure

- a. Frequency
 - i. A minimum of seven days should be allowed between sampling of same orbit to allow for tissue repair.
 - ii. Alternate orbits can be sampled based upon skill of technician.
 - iii. Maximum number of bleeds for each animal is two bleeds per eye.
- b. Anesthesia
 - i. General anesthesia is required.
 - ii. Additionally, apply a single drop of topical ophthalmic anesthetic (Proparacaine Hydrochloride) prior to procedure. Allow a minimum of 30 seconds for the medication to take effect before collecting the blood sample.
- c. Procedure
 - i. Anesthetize the animal with the selected anesthetic agent prior to sample collection procedures.
 - ii. Apply topical anesthetic solution to eye to be sampled as described above (Figure 1).
 - iii. Place the animal in ventral recumbency after the animal has reached an appropriate plane of anesthesia.
 - iv. Using one hand, gently scruff the mouse to open the eye, allowing the globe of the eye to slightly protrude.
 - 1. It is acceptable to move the animal to lateral recumbency to obtain a better view of the eye being sampled.
 - v. Insert a new capillary tube (use the end of capillary tube that is not color banded) or Pasteur pipette tip into the medial canthus of the eye under the nictating membrane at a 30-45° angle. The tube should be positioned between the globe of the eye and bony orbit of the eye (Figure 2).

CAUTION: Avoid letting the tip of the capillary tube or Pasteur pipette touch the surface of the eye as this can create trauma.
 - vi. Once the tip is in the appropriate location, apply slight pressure to the tube and begin to rotate the tube gently in your fingers until the retro-orbital plexus has been punctured.

1. As soon as the sinus is punctured, blood will enter the tubing by capillary action (Figure 3).

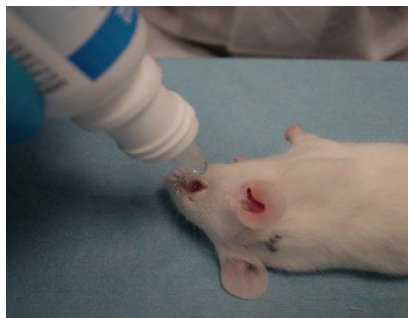


Figure 1. Apply Ophthalmic Anesthetic

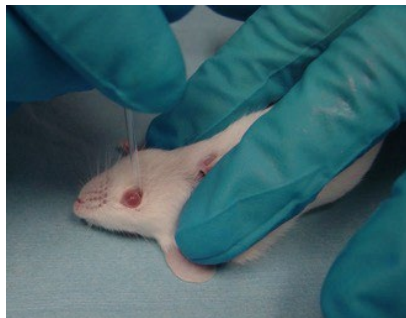


Figure 2. Capillary Tube Placement

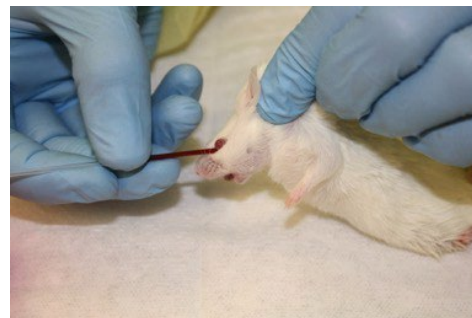


Figure 3. Blood Collection

- vii. If only a small amount of blood is required, the tube may be sealed using clay capillary tube.
 1. Place the end used to collect the blood sample into the sealant.
- viii. For larger volumes, allow the capillary tube/Pasteur pipette to completely fill and drip from the end of the tube into a larger blood collection tube.
- ix. After collecting the selected amount of blood, gently withdraw the tip of the tube/pipette from the eye in the same direction it was inserted.
- x. Apply direct pressure to the eye using a piece of gauze for a minimum of one minute, or until the bleeding ceases around the orbit of the eye.
- xi. For survival blood collections, apply a small amount of ophthalmic ointment from the medial canthus to the lateral canthus of the eye.
- xii. Monitor the animal's recovery following survival blood collection before returning the animal to its cage.
- xiii. Animals should be monitored twice weekly after each retro-orbital bleed.
- d. For terminal blood collections, euthanize the animal immediately upon completion of blood collection.

V. Variations

- a. Lateral or medial canthus can be accessed.

VI. Potential Adverse Events, Mitigation, or Treatment

- a. Potential adverse effects which may require veterinary intervention
 - i. Anesthetic respiratory distress
 - ii. Eye infection or ulceration
 - iii. Peri-orbital swelling, redness and/or hematoma formation
 - iv. Blindness

v. Death

VII. References

- American Association of Laboratory Animal Science. Laboratory Animal Technician Training Manual. (Memphis, TN: Drumwright and Co, 2007)
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