

SOP: Retro-Orbital Blood Collection in the Rat

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 plexus as a survival or terminal procedure in the rat.

Considerations

1. The presence of a plexus rather than a sinus in the rat can lead to greater orbital tissue damage than in the mouse. Retro-orbital (RO) bleeding in the rat with recovery should be only used under general anesthesia and only in circumstances where other blood sampling techniques are not feasible (e.g., peripheral veins used for dosing).
2. Please refer to the *Guidelines for Injections in Rodents and Rabbits, Virginia Tech Office of the University Veterinarian* for recommended volumes and needles sizes.
3. 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 be 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 and Hygiene

- a. Ensure appropriate PPE is used to protect technician from accidental exposure to blood and other body fluids, such as:
 1. Gloves
 2. Eye protection
 3. Mask
 4. 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)



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- 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
 - a) Intramuscular, subcutaneous, or intraperitoneal injection
- d. Topical anesthetic (e.g., Proparacaine Hydrochloride Ophthalmic Solution)
- e. Ophthalmic ointment
- f. Gauze pads

IV. Detailed Procedure

- a. Frequency
 - 1. A minimum of two weeks is recommended between sampling to allow for tissue repair.
 - 2. No more than one bleed per eye plus a terminal sample should be taken due to animal welfare.
- b. Anesthesia
 - 1. Retro-orbital sampling in the rat must be conducted under general anesthesia.
 - 2. Once anesthetized, 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
 - 1. Anesthetize the rat with the selected anesthetic agent prior to sample collection procedures.
 - 2. Apply topical anesthetic solution to eye to be sampled as described above ([Figure 1](#)).
 - 3. Place the rat in ventral recumbency once the rat has reached an appropriate level of anesthesia.
 - 4. Using one hand, secure the rat's head using the thumb and forefinger. It is acceptable to move the rat to lateral recumbency to obtain a better view of the eye being sampled.
 - 5. 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 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.
 - 6. 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 sinus has been punctured. As soon as the sinus is punctured, blood enters the tubing by capillary action ([Figure 3](#)).
 - 7. If only a small amount of blood is required, the tube may be sealed using capillary tube sealant by placing the end used to collect the blood sample into the sealant.

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8. For larger volumes, allow the capillary tube/Pasteur pipette to completely fill to drip from the end of the tube into a larger blood tube.
 9. 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.
 10. 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.
 11. For survival blood collections, apply a small amount of ophthalmic ointment from the medial canthus to the lateral canthus of the eye.
 12. Monitor the rat's recovery following survival blood collection before returning the rat to cage.
 - i. Rats should be checked for post-operative peri-orbital lesions approximately 30 minutes after sampling, and again within two hours of sampling.
 - ii. Rats should be monitored twice weekly after each retro-orbital bleed.
- d. For terminal blood collections, euthanize the rat immediately upon completion of blood collection.



Figure 1. Apply Topical Anesthetic



Figure 2. Insert Capillary Tube

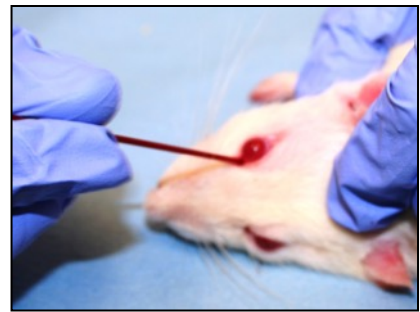


Figure 3. Blood Collection

V. Variations

None

VI. Potential Adverse Events, Mitigation, or Treatment

- a. Potential adverse effects which may require veterinary intervention
 1. Anesthetic respiratory distress
 2. Eye infection or ulceration
 3. Necrotic dacryoadenitis of the Harderian gland
 4. Peri-orbital swelling, redness and/or hematoma formation
 5. Blindness
 6. Death

VII. References

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

Charles River SOP 1661-1 – *Retro-Orbital Blood Sampling in Rodents*

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