



CARE 408.01 Retro-Orbital Bleeding

The intent of this standard operating procedure (SOP) is to describe the procedure for obtaining blood from the retro-orbital sinus of mice or the retro-orbital plexus of rats. This SOP is intended for use by all personnel that plan to use this technique for blood sampling. This procedure is approved by the Cornell Institutional Animal Care and Use Committee (IACUC) and the Cornell Center for Animal Resources and Education (CARE). Any exemption must be submitted for approval to the IACUC, prior to its application.

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1. Introduction

- a. Retro-orbital blood collection in rodents can provide moderate to large amounts of blood when performed by well trained personnel; however, severe injuries may occur to the animal if this procedure is not done properly.
- b. Satisfactory alternatives to retro-orbital bleeding are available and must be used whenever possible. The use of retro-orbital bleeding must be justified in the protocol and approved by the IACUC.
- c. Because rats have a plexus rather than a sinus, retro-orbital bleeding may result in greater tissue damage than in the mouse; therefore, alternative blood collection sites are strongly recommended for the rat.
- d. CARE must observe and approve the technique of personnel that will use this procedure.

2. Materials

- Blood collection tubes (e.g. capillary tubes or Pasteur pipette)
- Blood collection containers (e.g., microfuge tubes, cryo-vials)
- Gauze sponges
- General anesthetic agents (see [CARE SOP 101 Rodent Anesthesia](#))
- Topical Ophthalmic anesthetic
- Topical Ophthalmic ointment
- Heat source (to keep mice warm when using anesthetic agents)

3. Procedure

- a. Establish that the animal is adequately anesthetized (see [CARE SOP 101 Rodent Anesthesia](#)).
 - i. Use general anesthetic, apply ophthalmic ointment to both eyes as per CARE SOP 101.
 - ii. If not using general anesthesia, apply topical ophthalmic anesthetic (e.g., proparacaine 1%) to the eye being used for blood collection.
- b. Place the animal on the table in lateral recumbency. Scruff the animal with the thumb and forefinger of the non-dominant hand firmly enough to pull the skin taut around the eye.
- c. Insert a collection tube into the medial canthus of the eye under the nictitating membrane at approximately a 30 degree angle to the nose.
 - i. Direct the hematocrit tube gently in a ventrolateral direction while rotating the capillary tube (rolling it between the thumb and forefinger of the dominant hand).
 - ii. Position the animal in such a way that the end of the hematocrit tube in the medial canthus is higher than the end which will drip into the blood collection container.
 - iii. A slight thrust may be needed to puncture the tissue and enter the plexus or sinus.
 - iv. Blood will begin to flow into the capillary tube. If you need to obtain more blood than the volume of the capillary tube, the blood may be allowed to run through the tube and drip into the collection container.

NOTE: Do not attempt both eyes at the same session if damage is done to eye or failed to collect blood from the eye.
- d. Follow CARE SOP 101 Rodent Anesthesia for administration, animal monitoring and reporting.
- e. Check the animal once a day for at least 3 days for complications as a result of this procedure. Record these checks in the animal's record.
- f. Injury to the eye is indicated by squinting, bulging, swelling, or opacification of the eye, or orbital discharge following the procedure.
 - i. If the eye is damaged from a previous procedure that eye may not be used again for blood collection.
 - ii. Perform no more than one retro-orbital bleed per week.
 - iii. Alternate right and left eyes so that each eye is used no more than twice a month.
 - iv. No more than 3 procedures may be performed per eye in a lifetime.
 - v. Call a CARE Vet immediately if damage to the eye is apparent. The CARE vet will determine if the eye can be treated, or if the animal should be euthanized.
- g. Observe guidelines for blood collection volume (see CARE SOP 403 Recommended Blood Collection Volume and Frequency)

4. Safety

- a. See CARE SOP 707 Animal Related Injury
- b. See CARE SOP 711 Sharps Precautions
- c. See CARE SOP 713 Hygiene-Hand Washing
- d. Working with rodents can trigger related allergies (refer to CARE Allergens web page).
- e. Check with the facility manager regarding requirement for personal protective

equipment and refer to CARE SOP 715.

f. Refer to CARE zoonosis web page for zoonotic information specific to rodents.

5. Contingencies

- Emergency CARE veterinary assistance available 24/7: pager 1-800-349-2456.
- Nonemergency assistance: 607-253-4378 or care@cornell.edu.

6. References

CARE SOP 101 Rodent Anesthesia:

<http://www.research.cornell.edu/care/documents/SOPs/CARE101.pdf>

CARE SOP 403 Recommended Blood Collection Volume and Frequency:

<http://www.research.cornell.edu/care/documents/SOPs/CARE403.pdf>

CARE SOP 707 Animal Related Injury:

<http://www.research.cornell.edu/care/documents/SOPs/CARE707.pdf>

CARE SOP 711 Sharps Precautions

<http://www.research.cornell.edu/care/documents/SOPs/CARE711.pdf>

CARE SOP 713 Hygiene-Hand Washing

<http://www.research.cornell.edu/CARE/documents/SOPs/CARE713.pdf>

CARE SOP 715 Personal Protective Equipment

<http://www.research.cornell.edu/CARE/documents/SOPs/CARE715.pdf>

Allergens Prevention web page

<http://www.research.cornell.edu/Care/documents/OHS/AllergyPreventionFactSheet.pdf>

Zoonosis Web page: <<http://www.research.cornell.edu/care/zoonoses.html>>

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L. Guanzini, Jan. 2004 C. Barriere June 2008	July 2005	June 2009	M. Martin	CARE 408.01