

NHGRI ANIMAL CARE AND USE COMMITTEE (ACUC) GUIDELINE AND PROCEDURES FOR RETRO-ORBITAL BLEEDING IN MICE*

The NHGRI ACUC has determined that IN THE HANDS OF A SKILLED PRACTITIONER, retro-orbital bleeding is a humane method to obtain blood samples from mice. Investigators should be aware that this procedure may cause transient pain and distress and appears to members of the lay community and others to be unpleasant to the animal. Training by the Animal Program Director (APD), or his/her designee, is absolutely required to achieve proficiency in retro-orbital bleeding. Authorization for an investigator to perform retro-orbital bleeding will be granted by the ACUC only after the APD has certified that an individual has become proficient at the procedure. Alternative methods* of blood collection such as saphenous or tail vein puncture are available, and investigators are encouraged to consider these as alternatives to retro-orbital bleeding. Refer to the ARAC Guidelines for Survival Bleeding of Mice and Rats for additional information <http://oacu.od.nih.gov/ARAC/survival.pdf>. Although this guideline recommends rotating orbits for successive blood collection from the retro-orbital sinus, the NHGRI ACUC does not make this same recommendation unless the individual demonstrates proficiency in both orbits. Collection of blood from the opposite orbit is sometimes an awkward undertaking and may increase, rather than decrease, the possibility of injuring the animal. The NHGRI ACUC believes that it is safer and more humane to collect blood from the orbit you are accustomed to.

General Information

- All blood sampling (including frequency of sampling and volume of sample) must be approved in the Animal Study Proposal.
- Ideally, mice should be at least three weeks of age.

Procedure

- Manually restrain mouse by grasping near base of tail with one hand and grasping the nape of the neck with the opposite hand. Place tail between fingers to secure and control animal.
- Apply one drop of topical ophthalmic anesthetic (e.g., proparacaine, tetracaine) to eye to be used. Wait a few seconds. Gently blot away excess with clean gauze, being careful not to scratch the cornea. (An alternative to topical anesthesia is general anesthesia.)
- Place hematocrit tube or Pasteur pipette at the canthus of eye. Sterile hematocrit tubes or Pasteur pipettes are recommended for use, but are required for immune compromised animals. It is imperative that you know the volume of the tube you are using to collect blood and that you do not exceed the approved volume for the weight of the animal. For example, the volume of a standard microhematocrit tube is 70 μ l and the volume of a Pasteur pipette to the tapered section (shoulders) is 250 μ l.
- With a gentle rotating motion, insert tube through membrane.
- Continue rotating tube on back of orbit until blood flows.**
- In most cases, no measures need to be taken to ensure good hemostasis. Should excessive bleeding occur, apply gentle pressure with a gauze pad, being careful to avoid scratching the cornea.

Guideline 00.1

*See http://www.uib.no/vivariet/mou_blood/Blood_coll_mice.html for the use of the saphenous vein as an alternative to retro-orbital bleeding.

***No more than 10% of the blood volume should be removed at one sampling. Blood volume of a mouse is ~8% of the body weight. (For example a 25 gm mouse has a blood volume of ~2 ml – not more than 200 µl could be removed at a single bleeding without scientific justification and approval by the NHGRI ACUC.*

Mice should not be bled anymore frequently than every 3 weeks unless smaller volumes are removed.

Approved by the NHGRI ACUC 1/28/00

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