



A. PURPOSE

To provide guidance on appropriate methods for retro-orbital bleeding.

B. GUIDELINES

Retro-orbital bleeding is a method of blood collection whereby the retro-orbital sinus in mice or the retro-orbital plexus in rats is penetrated with a capillary tube. Retro-orbital sampling has a greater potential than other blood collection routes to result in complications. Therefore, this method should not be considered the first choice for obtaining blood. Contact UAC Veterinary Services for advice on the selection and performance of blood collection for your species and research needs.

The IACUC may approve the use of retro-orbital bleeding. However, as part of that approval, the following must occur:

Training

For IACUC approval of this method, protocol participants must be properly trained and capable of consistently applying this technique humanely and effectively.

- If anesthesia is **not** used, **all** protocol participants using this technique must be trained by a UAC veterinarian or designee to ensure proper technique.
- If anesthesia is used, at least **one** protocol participant must be trained by a UAC veterinarian or designee. In this case, that individual will then be responsible for training additional protocol participants in the appropriate methodology.
- The principal investigator may also choose to have all protocol participants trained by a UAC veterinarian or designee.

Protocol participants transferring from another institution may provide proof of training from that institution to the veterinary trainer. The protocol participant will be required to display competency in the method to a UAC veterinarian or designee.

Anesthesia

Mice: General anesthesia is recommended, if compatible with the experimental design. If retro-orbital bleeding is conducted without general anesthesia, a topical ophthalmic anesthetic (e.g. proparacaine or tetracaine drops) must be applied prior to the procedure, as this decreases post-procedural pain.

Rats: In rats, the presence of a venous plexus rather than a sinus can lead to greater orbital tissue damage than in the mouse. As the rat is a larger animal with other acceptable methods of bleeding, use of the retro-orbital method must be scientifically justified. Additionally, as it is considered a more painful procedure than in mice, general anesthesia must be used unless scientific justification is provided and approved by the IACUC. In addition, use of a topical ophthalmic anesthetic prior to the procedure is required.

If there is the potential for the topical ophthalmic anesthetic to contaminate the blood sample, this should be



described for IACUC review.

Procedures

- A micro-hematocrit capillary tube must be used to collect the blood to reduce complication such as infection
- After bleeding, slight pressure on the eyeball with a piece of gauze should be used to aid in hemostasis.
- If general anesthesia is used, animals must be appropriately monitored until recovered from anesthesia.

Blood collection parameters

- The maximum amount of blood that may be withdrawn is 1% of the animal's body weight every two weeks.
- For more than one blood draw, the eyes must be alternated.
- Blood can only be collected once every 14 days from each eye to allow the tissue to heal.
- Blood can only be collected a maximum of two times per eye.
- If blood is not obtained, do not retry bleeding from the same eye OR the other eye and contact UAC or designated lab personnel for retraining.

Post-procedural care

- Animals should be monitored 2-3 times in the week following each retro-orbital bleed to check for any signs of ocular trauma.
 - Squinted eye with discharge
 - Shrunken appearing eye
 - Evidence of self-trauma such as ulceration of the eyelid or around the eye
 - Corneal abnormalities such as opacities or roughness to the surface of the eye
- If bleeding does not stop within 5 minutes, if injury to the eye occurs during the procedure, or if the eye becomes inflamed or infected, a UAC veterinarian must be contacted in a timely manner.