

Mouse Retro Orbital Bleeding

It is recommended that mice are anesthetized for retro-orbital (RO) bleeding. Mice do not need to be in a surgical plane of anesthesia but need to be sedated deeply enough to immobilize the animal completely. This aids in proper performance of the technique and minimizes the possibility of ocular injury. If you do not use anesthesia to perform RO bleeding please consult [IACUC Guidance 606](#) for topical anesthetic requirements. All protocol participants that will use this technique without anesthesia must receive training from UAC veterinary staff prior to performing this technique.

Necessary Supplies

- Small heparinized capillary tubes
- Blood collection tube / Eppendorf tube
- Isoflurane anesthetic equipment.
- Cotton Tip applicators / Gauze to provide hemostasis

Technique

1. You will be entering the venous sinus that lays behind the orbit to obtain your blood sample. Review the anatomy to be familiar with the area (**Image 1**). Anesthetize the animal in the induction chamber until it is fully immobilized.
2. Lay the animal on its side. Maintenance on 1-3% isoflurane is typically needed until your level of proficiency allows you to perform the procedure before the animal wakes up from anesthesia..
3. Retract the eyelids, forcing the globe slightly out of the orbit so that it protrudes and generates a space behind the globe to access the sinus. This will ensure that you do not insert the capillary tube into the globe. (**Image 2**)
4. Enter at the medial/nasal corner of the eye with the capillary tube at a 45 angle to the midplane, avoiding the globe. Apply light pressure to tube and a rotating motion to puncture the sinus, blood will flow when successful. (**Image 3**)
5. After bleeding, apply slight pressure on the eyeball with a cotton tip applicator or piece of gauze to aid in hemostasis.
6. If general anesthesia is used, animals must be appropriately monitored until recovered from anesthesia.

Notes

- Use a new clean capillary tube per animal
- The maximum amount of blood that may be withdrawn is 1% of the animal's body weight every two weeks.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.

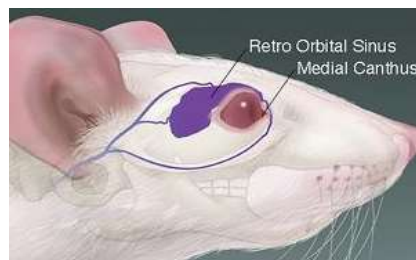


Image 1- Anatomical position of the Retro Orbital Sinus.



Image 2- Eyelid retraction to induce slight proptosis of the globe.



Image 3- Capillary insertion and blood sampling collection.