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## IG043: RETRO-ORBITAL SAMPLING

Retro-orbital sampling is used to collect blood in volumes up to ~200 microliters from laboratory mice. This method allows for rapid, consistent collection of moderate blood volumes from the venous plexus located in the retrobulbar space (the area behind the globe of the eye) when conducted by trained personnel. Animals should be placed under general anesthesia, and topical anesthesia may be applied (e.g., proparacaine drops) to the eye prior to sampling, to minimize discomfort after the procedure.

The CAR Veterinary staff should be contacted prior to the start of your protocol for training on this technique. In the hands of a trained operator, adverse complications from retro-orbital bleeds are rare and distress to the animal is minimal due to care taken during anesthesia and handling. **CAR Veterinary Staff are to be notified if ocular/periocular injuries are observed.**

Experiments that require *exceptions* to these guidelines should be discussed with the CAR veterinarian prior to inclusion in the protocol and prior to the start of the experiment.

### Retro-orbital blood collection expectations:

- Retro-orbital blood collection **must be done under general anesthesia.**
- There should be no ocular/periocular complications noted prior to blood collection.
- Collection must be done into sterile hematocrit tubes.
- Adequate hemostasis (e.g., gentle manual pressure with sterile gauze to the closed eye) following the procedure is expected to prevent hematoma formation and ocular damage.
- Ophthalmic ointment should be applied to the sampled eye following collection.
- For repeat sampling, there should be at least 7 days between collection from the same eye to allow for retro-orbital healing and minimization of the inflammatory response.
  - If additional blood is needed, alternate collection sites (saphenous, tail tip, etc.) should be incorporated into the sampling protocol, in consultation with the CAR vet staff.
  - Alternating between eyes during subsequent collections is equivalent to sampling from each eye **once every 14 days**; this allows time for appropriate healing before using the same retro-orbital site for blood collection again.
- After sampling:
  - The animal must be checked **at least once daily for a minimum of 3 days** post-sampling to monitor for general appearance around the sampled eye, grooming habits, skin color, and overall appearance and behavior.
  - Document observations, looking for clinical signs associated with ocular injury, like scratching of eyes, cornea lesions, squinting or bulging of eye, discharge, or swelling around the eye.

### Retro-orbital injection expectations:

- Retro-orbital blood injection **must be done under general anesthesia.**
- To prevent trauma, needle length should not exceed ½ inch. A 27-gauge needle (or smaller) or an insulin syringe with needle attached should be used (see Yardini reference below).
- For adult mice, the injection volume should not exceed 200 microliters per eye (10 microliters for neonates).

- For injection of drugs or cells, there should be a 2-day interval between injections. If additional injections are given, there should be no ocular/periorbital abnormalities noted prior to additional injections.

**REFERENCES:**

Fried JH, Worth DB, Brice AK, Hankenson FC. Type, Duration, and Incidence of Pathologic Findings Following Retro-orbital Bleeding of Mice by Experienced or Novice Personnel. *J Am Assoc Lab Anim Sci*; 54(3): 317-327, 2015.

Yardeni T, *et al.* Retro-orbital injection in mice. *Lab Anim (NY)*, 2011; 40:155-160.

Suckow, MA.; Danneman, P. ; Brayton, C. The Laboratory Mouse. Suckow, MA., editor. Boca Raton, FL: CRC; 2001.