

GUIDELINES FOR RETRO-ORBITAL SAMPLING

After review of the current literature and industry practices, the IACUC at the University of Pittsburgh has determined retroorbital bleeding in rodents to be a painful procedure. Even in the hands of a skilled operator there is potential for pain and distress which must be addressed in the research protocol when performing retroorbital bleeding in rodents.

- In the hands of an unskilled operator, retro-orbital sampling has a greater potential than other blood collection routes to result in complications.
- Not amenable to frequent repeated sampling from the same orbit (10 days to 2 weeks recommended between successive bleeds. If weekly sampling is necessary orbits must be alternated).
- The presence of a plexus rather than sinus in the rat can lead to greater orbital tissue damage than in the mouse.
- Can be used in both rats and mice by penetrating the retro-orbital plexus/sinus with a glass capillary.
- Rapid - large number of animals can be bled within a short period of time.
- Obtainable volume: medium to large.
- Good sample quality. Potential contamination with topical anesthetic should be taken into account.

Qualifications for use in rodents:

Alternative techniques for blood collection other than retro-orbital bleeding must be considered and used whenever possible. Scientific justification as to why other less painful methodologies would not be sufficient for investigative needs must be provided in the submitted IACUC protocol.

Investigators must be proficient in the technique and have a plan in place to provide training and document proficiency of individuals within the lab. Contact the DLAR if additional expertise for training lab personnel on retro-orbital bleeding is required.

Protocols proposing to perform this procedure in awake (fully conscious) animals will be called for full committee review.

Retroorbital bleeding can not be performed in awake mice unless documented and referenced scientific justification is provided in the submitted IACUC protocol.

Rats must be fully anesthetized due to the presence of a plexus rather than a sinus which can lead to greater orbital tissue damage than in the mouse.

Mice must be chemically restrained with either a) general anesthesia, or b) a sedative dose of anesthesia combined with topical/local anesthesia. (The combined use of a topical anesthetic along with general anesthesia is considered optimal, but is not mandatory.)

A sedative dose of anesthesia must be enough to maintain the animal in lateral recumbency with minimal purposeful movement. If full anesthesia is used there must be no response to toe pinch or other environmental stimuli.

Topical anesthesia must be administered 1-2 minutes before blood collection to allow for desensitization of the cornea and mucous membranes.

Topical anesthesia alone although not preferred, will be considered as an alternative if scientific justification is provided as to why accompanying sedation or general anesthesia is not acceptable.