

# Standard Operating Procedure for Retro-Orbital Injection in Mice

## Purpose

Retro-orbital injections are an accepted intravenous route for administration of some compounds and the initiation of some parasitic infections in rodents. This technique requires hands-on training in both the injection technique and general anesthesia methods. Documentation of proficiency is required for all persons utilizing the retro-orbital route for injections.

## Equipment

Nitrile gloves (worn when handling Isoflurane)	Isoflurane
Bell jar anesthesia (see the SOP for inhalation anesthesia)	Cotton ball
1 cc TB syringe with a 26g – 30g needle	

## Procedure Description

1. Prepare the bell jar by soaking a cotton ball in Isoflurane anesthetic and placing it below the platform in the jar to prevent contact with the animal. Allow the anesthetic to vaporize and fill the interior of the jar before placing the animal into the chamber. Anesthetize the mouse using Isoflurane in a bell jar of a size appropriate for the size of the animal in a chemical fume hood.
2. After removal from the anesthetic, place the mouse in lateral recumbancy with the eye to be injected facing up.
3. Retract the skin toward the body causing the eye to protrude.
4. Insert the needle bevel up into the medial canthus of the eye at a 45 degree angle to the nose into the vessels behind the eye ball.
5. There should be a small “flash” of blood into the syringe when slight back pressure is applied to the plunger.
6. Gently inject the agent into the retro-orbital vessels.
7. Withdraw the needle and apply light pressure to the eye to control bleeding.

## Limitations

1. The injection volume is not to exceed 100 microliters per eye.
2. Only one injection per eye per day.
3. At least a one day interval between injections.
4. No more than two injections total per eye in survival procedures.
5. Non-survival injection volumes up to 500 microliters allowed in anesthetized mice.
6. Recommend a topical ophthalmic anesthetic (tetracaine) when multiple injections are to be performed.

## Cautions

1. If the needle is placed too deeply or too aggressively the thin bone of the skull behind the eye can be pierced and the agent will be injected into the skull.
2. The needle must have minimal movement once inserted into the retro-orbital plexus or the vessels will be ruptured causing bleeding and loss of the agent into the tissues behind the eye.
3. When retracting the skin special care must be taken not to apply pressure on the trachea and cut off the animal’s airway.
4. The needle must be placed behind the eye and not intraocularly.
5. Mice that experience swelling, conjunctivitis, or other sequelae related to injection trauma will be evaluated by the Attending Veterinarian and treated or euthanized according to his recommendation.

## References:

1. Steel, C., Stephens, A., Hahto, S., Singletary, S., Ciavarra, R. Comparison of the lateral tail vein and the retro-orbital sinus as routes of intravenous drug delivery in a transgenic mouse model. *Lab Anim.* **37**, 26-31 (2008).
2. NYU Medical Center, Guidelines for Retro Orbital Injection in Mice. [www.med.nyu.edu/dlar/policies/retro\\_orbital\\_injections.html](http://www.med.nyu.edu/dlar/policies/retro_orbital_injections.html)
3. Washington University at St. Louis. Retroorbital Injection Technique, [ourworld.compuserve.com/homepages/TheBroons/en4.htm](http://ourworld.compuserve.com/homepages/TheBroons/en4.htm)