

**Arizona State University
Institutional Animal Care and Use Committee
STANDARD INSTITUTIONAL GUIDELINE**

RETRO-ORBITAL INJECTION - MICE

Description of procedure:

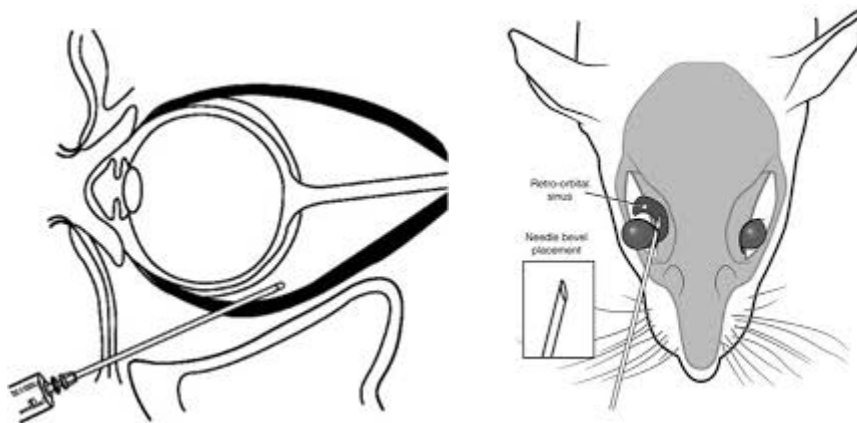
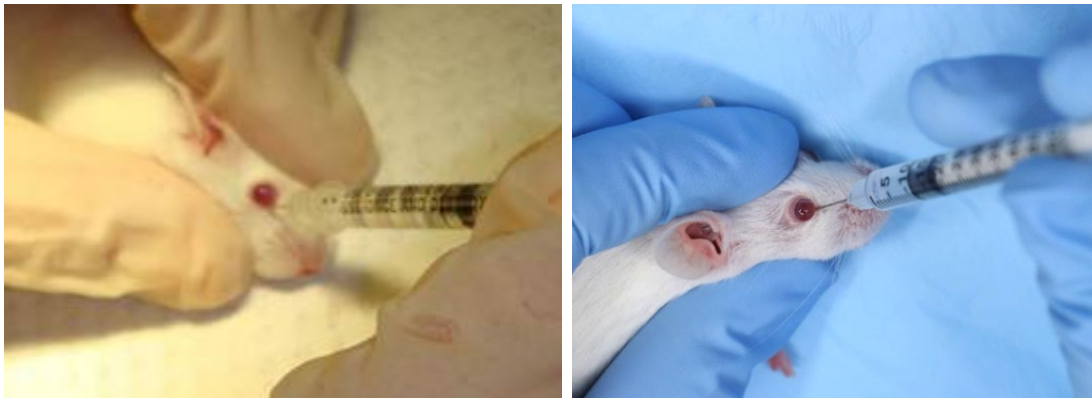
Retro-orbital injection is an acceptable route for the intravenous delivery of non-irritating compounds when the person doing the injection has been properly trained. Anesthesia is required for retro-orbital injection because the location of the needle insertion requires that the mouse not move to avoid ocular injury. The procedure, including anesthesia and recovery time, takes less than five minutes when performed proficiently.

Cell suspensions must be filtered or agitated prior to injection to prevent cell clumping. If injecting cancer cells, the needle must be wiped clean prior to injection and the suspension withdrawn slightly to prevent cells from inadvertently being deposited in the surrounding tissues. The needle should be cleaned by lightly wiping it on sterile gauze that is on a flat surface. To avoid needle sticks and contamination of the needle, neither the needle nor the gauze should be touched with fingers. The maximum volume of injectate is limited to 200 μ L, with an ideal volume of 100 μ L. A mouse can receive no more than one injection per day. When more than one injection is required, alternate between eyes and allow at least 24 hours between injections. Using a 27 gauge or smaller needle on a 0.5 mL insulin syringe is recommended to reduce the potential for eye trauma. A standard sharp syringe needle is preferred over a blunt-tipped needle as it leads to less tissue distortion and damage.

Stepwise instructions:

1. Anesthetize the mouse with an appropriate anesthetic.
 1. Inhalant anesthetic, such as isoflurane, is preferred. When properly trained, the injection takes less than 30 seconds. By the time the injection is complete and the mouse is placed back in the cage, it should be starting to recover from anesthesia.
 2. Injectable anesthetics (e.g., a ketamine cocktail) can be used if additional planned manipulations require a longer duration of anesthesia.
2. Position the mouse on its side.
3. Use the gloved index finger of the non-dominant hand to draw back the skin above the eye and the thumb to draw back the skin below the eye (see image below). Applying downward pressure to both fingers will cause the eye to protrude slightly. Rest the fingers on the skull to avoid compression of the trachea and surrounding blood vessels.
4. Hold the syringe in the dominant hand, in a way such that the hand and fingers will not have to be repositioned to inject once the needle has been inserted.
5. Insert the 27-30 gauge needle (bevel side pointed down, away from the eyeball) at approximately a 30-45° angle to the eye, lateral to the medial canthus, through the conjunctival membrane (see diagram below). There is a bit of resistance that causes the

- eye to retreat back into the sinus until the needle pierces through the conjunctiva. The needle will be positioned behind the globe of the eye in the retro-bulbar sinus.
6. Inject slowly into the retro-bulbar sinus. Do not aspirate before injection, as this will collapse the vessels.
 7. Remove the needle gently to prevent injury to the eye.
 8. Apply a small dab of eye lubricant to the eye.
 9. Close the eyelid and apply mild pressure to the injection site with a gauze sponge.
 10. Place the mouse back into the home cage on top of a paper towel.
 11. Monitor the mouse during the recovery from anesthesia until it is consistently moving around.
 12. Examine the injection site for swelling or other visible trauma.
 13. If no adverse effects are observed and the mouse is fully ambulatory, it can be returned to its housing rack.



References:

1. Steel, C., Stephens, A., Hahto, S., Singletary, S., Ciavarrá, R. 2008. 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.
2. Yardeni T, Eckhaus M, Morris D, Huizing M, Hoogstraten-Miller S. 2011. Retro-orbital injection in mice. *Lab Anim.* **40**, 155-160.
3. JoVE Science Education Database. Lab Animal Research. Compound Administration IV. JoVE, Cambridge, MA, 2020.

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