Johns Hopkins University
Animal Care and Use Committee

Retro-orbital Injections: Mice

PURPOSE:

Retro-orbital injection is a specialized technique used to inject drugs, non-irritating compounds, or cells intravenously using the retro-orbital sinus.

INTRODUCTION:

Retro-orbital injections provide an alternative to tail or saphenous vein injections. They are technically easier to perform but carry a risk of ocular injury if performed incorrectly. The animal is to be anesthetized for the procedure to prevent damage due to movement of the animal. The use of the retro-orbital route for injection should be scientifically justified compared to less-invasive sites that do not require anesthesia. The drugs, compounds, and cells must be non-tumorigenic. Injection by the retro-orbital route requires the technician be trained and experienced in the injection technique and general anesthesia methods.

PROCEDURE:

A. Volumes and Frequency

- **Adults:** recommended volume is 100-150µL per injection, with a maximum of 200 µL or 1% of body weight, whichever is lower, for a survival procedure.
- **Neonates**: maximum of 10 µL per injection, or 1% of body weight, whichever is lower.
- **Frequency:** No more than one retro-orbital injection per day. Alternate between eyes if giving more than one injection per animal. Maximum number of injections for any individual animal should not exceed two injections per eye, unless otherwise scientifically justified in the protocol.

B. Anesthesia – This is a category D procedure. If adding retro-orbital injection by amendment, mark the *Modify Pain Category* option on the amendment form.

The procedure does not take long to perform, and therefore a relatively short acting anesthetic drug, such as isoflurane, is recommended.
Consider the anesthetic options below.

1) Gas anesthetic drugs:

a) The "Drop Method." See the ACUC guidelines, *Anesthesia-Gas: Drop Method*.
b) For a system that uses anesthesia machines and vaporizers:
   i) The recommended doses for isoflurane are: 3-5% for induction and 1-3% for maintenance.
   ii) See the ACUC guidelines for *Anesthesia-Gas: Use and Vaporizer Calibration*.

2) Injectable anesthetic drugs:
   a) For drugs and doses, see the *RAR formulary*
   b) To prevent corneal damage due to drying, apply ophthalmic ointment to the eye that is not undergoing retro-orbital injection. After injection, ophthalmic ointment should also be applied to that eye, unless doing so would interfere with the research outcomes.

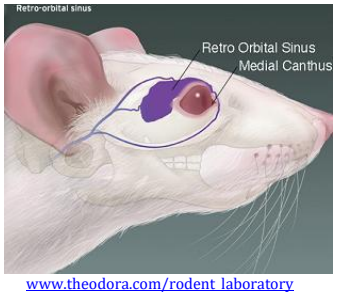
C. Analgesia

Pre-emptive analgesia should be applied for this procedure. Use either a local anesthetic such as proparacaine hydrochloride ophthalmic 0.5% (1 drop per eye, applied approximately 1 minute prior to the procedure, with analgesia lasting ~15 minutes after application) or a systemic analgesia such as buprenorphine (see the *RAR formulary*). The use of local anesthetic does not replace the need for general anesthesia.

D. Injection

1) Use aseptic technique *Guide for the Care and Use of Laboratory Animals: Eighth Edition, Copyright 2011, NAS | OLAW Page 118.*
2) Confirm an appropriate depth of anesthesia by toe pinch.
3) Cell suspensions must be filtered or agitated prior to injection to prevent cell clumping.
4) Hold the mouse with the head tilted slightly back to expose the eye. Using the index finger and thumb draw back the skin above and below the eye causing the eye to protrude slightly, see the images below.
5) Insert the needle, 27–30gauge, bevel down, at a 45° angle, into the medial canthus of the eye under the nictitating membrane or third eyelid.
6) Do not aspirate.
7) Deliver the drug, compound, or cells slowly to avoid tissue damage.
8) Once the material has been injected, withdraw the needle.
9) If bleeding occurs, close the eyelid and apply slight pressure with sterile gauze to ensure hemostasis.
10) Care is taken not to scratch the cornea.

Retro-orbital sinus illustration



www.theodora.com/rodent_laboratory

Adult injection

Arizona State University, IACUC Guideline

Neonate injection

www.theodora.com/rodent_laboratory

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E. Anesthesia Recovery Monitoring

1) To protect the animal from hypothermia, place them on a water recirculating heating pad or provide an overhead heat lamp.
2) During recovery from anesthesia, the following clinical parameters must be monitored and documented at 10-to-15-minute intervals until the animal is fully recovered. Ensure the following:
    a) If bleeding occurs, hemostasis is maintained by closing the eyelid and holding gentle manual pressure with sterile gauze for several seconds, after which the site is checked for continued swelling/bleeding or other signs of trauma.
    b) Adequate respiration: not too shallow, or too rapid
    c) Ability to maintain sternal recumbency.
3) The animal should never be returned to the housing facility until fully ambulatory.

F. Post-procedure care and monitoring for adverse effects following each injection

Consult a veterinarian if signs of distress or injury are observed.

- Squinting of the eye
- Corneal signs such as ulceration or opaqueness
- Signs of infection such as discharge or redness
- Peri-orbital swelling
- Eye appears shrunken
- Evidence of self-trauma

G. Humane Endpoints

Animals should be euthanized if: the eyeball is acutely damaged, treatment of an injured/infected eye is unsuccessful, and/or bilateral blindness occurs.

Sources:
pmc.ncbi.nlm.nih.gov/articles/PMC3158461/
Standard Operation Procedure for Retro-Orbital Injection in Rodents
Standard Operating Procedure #2
THE INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)
SIG-Retro-Orbital-Injections-in-Mice-8.24.2023.pdf

Approved: