Retrobulbar Injection & Retro-Orbital Bleeds

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July 2014
Retro-Orbital Sinus Injection

• Is an injection into the ophthalmic venous sinus (mice) or plexus (rats), or a retrobulbar injection

• Is suited for the injection of non-irritating substances into darkly pigmented mice, hamsters, or mice and rats with no discernible tail veins available for injection

• Distribution of substances injected retro-orbitally is similar to that of the intravenous route with high blood levels achieved rapidly
Retro-Orbital Injection (ROI)

- Retro-orbital injection allows multiple injections (must alternate eyes) and causes minimal distress if done correctly.
- This procedure is to be done only on approved protocols listing this route or in extreme circumstances with veterinary staff approval.
- Anesthesia is always required for injection; Isoflurane is recommended to speed recovery.
RO Injection

• Injection volumes should not exceed 0.1 to 0.3 ml in mice
• no more than two injections (one per eye) should be performed on one animal, unless the protocol states otherwise
RO Injection

- A 28 to 25g needle for mice (28-23g in rats) with loaded syringe attached is introduced retro-orbitally via the medial canthus.
- As one advances the needle into position, the bone beneath can be felt and used as a guide.
RO Injection

- The bevel should be positioned towards the bone of the orbit.
- Once placement has been achieved, indicated by a slight “bump” as the tip reaches the slight depression felt where the ophthalmic veins come together.
RO Injection

- The plunger may be depressed and the material delivered.
- Slight downward pressure will result in the veins being punctured for an intravenous injection.
RO Injection

• The technician will need to apply lubricant to the eye following injection to prevent the cornea from drying/injury

• Too much pressure will result in the arteries being ruptured or the bones fractured and the compound may be lost in the resulting hemorrhaging
RO Injection

- Proper placement and injection technique will result in a visible ‘bulging’ or blanching of the eye with no leakage of material
RO Injection

• Upon delivery, the needle may simply be backed out and the eye will return to its normal position.

• If the eye ruptures, appears damaged or dried out following injection(s) then veterinary care must be sought and the animal treated or euthanized as directed.
Retro-Orbital Sinus Blood Collection in Mice

• The retro-orbital site for blood sampling has been associated with a high potential for inducing orbital and ocular pathology, therefore other routes should always be considered.
  – increased observation frequency after retro-orbital sampling should occur.

• The method has been associated with greater incidence of sample hemolysis and increased levels of corticosterone and glucose.
Retro-Orbital Bleeds

• Signs that would indicate problems associated with ocular pathology:
  – swelling or injury of the eye, hematoma behind the globe, opacity of the lens, overt guarding of the eye, corneal opacity or ulceration, excessive tearing, exophthalmos (bulging), self trauma, conjunctivitis.

• If complications manifest, animals will be treated/euthanatized in accordance with the attending veterinarian’s recommendations.
The retro-orbital sinus (mouse) and plexus (rat) is a system of dilated venous channels at the back of the orbit. Blood can be collected from this area in anesthetized animals using a sterile hematocrit tube. Anesthesia is always required; Isoflurane is recommended.
RO Bleeds

- Once anesthetized, the animal is placed on a flat surface, and the handler’s thumb is used to apply pressure to the external jugular vein just behind the lower jaw.

- The index finger of the same hand is used to pull the upper eyelid back and help ‘pop’ the eye up.
• The other hand is then used to introduce a sterile hematocrit capillary tube along the inner corner of the eye (medial canthus) beside the eyeball.

• There should be no movement of the head during the procedure.

• Damage to the optic nerve and other intra-orbital structures, which can lead to deficits in vision and even blindness.
• Pressure is exerted from the thumb and forefinger just behind the eye and the skin pulled back to allow the eyeball to protrude.

• The tube is positioned, and gently inserted through the conjunctiva towards the back of the eye sliding along the orbit.

• Fracture of the fragile bones of the orbit and neural damage by the micropipette; and penetration of the eye globe itself with a loss of vitreous humour.
RO Bleeds

- Rotation of the tube gently while advancing assists positioning, penetration of conjunctiva and rupturing of the sinus and allows blood to flow freely when properly inserted.
RO Bleeds

- Tilting the head slightly downward may improve flow.
RO Bleeds

- Bleeding will stop when the hematocrit tube is withdrawn, pressure to the jugular is released and the eyeball is allowed to fall back into its normal position.
RO Bleeds

- **Light** pressure applied to the closed eyelids will assist with hemostasis.
- **Gently** shut the eye as the tube is removed by squeezing the lids together.
RO Bleeds

- Immediate indication of damage as evidenced by the eyeball not returning to a normal position following the bleed or observance of nasal bleeding should be brought instantly to the attention of the veterinary staff.
RO Bleeds

Use **heparinized** capillary tubes to prevent clotting during collection.

- *No more than 10% of the blood volume should be removed at one sampling.*
- The blood volume of a mouse is approximately 8% of the body weight.
- Example: 25g mouse = ~2ml bld volume = bleed of ≤ 200μl max

Each micro hematocrit tube holds ~ 75μl
RO Bleeds

• This procedure may be done repeatedly
  – should not be done in excess of six times per animal
  – no more than three alternating bleeds per eye

• Collection volume of 10ml/kg, at an interval of once every 2 weeks will not be exceeded unless otherwise approved

• Additional information can be found at: