UNIVERSITY OF DELAWARE
OFFICE OF LABORATORY ANIMAL MEDICINE

Retro-Orbital Injection in Mice SOP #PRO-015

Introduction:
This is an alternative injection route to tail vein for intravenous injections. The injectable solution is limited to 200 µl or less per injection. Injections are not to be repeated in one day, unless you alternate eyes. Injections are limited to one injection per eye per day.
Training is required prior to performing this procedure.

Equipment required:
- Isoflurane Anesthesia Machine
- ½ cc syringe and 28 gauge needle (needle not to exceed 1/2” to prevent trauma)
- Proparacaine Hyrdochloride Ophthalmic Solution 0.5%

Procedure:
1. Anesthetize the rodent using isoflurane. NOTE: Neonatal rodents do not require anesthesia for this procedure – their size allows easy restraint without anesthesia. Neonatal is defined as 1-2 days of age. A dissecting microscope is necessary for neonates.
2. Place a drop of the proparacaine liquid into the eye you are going to inject and allow it to sit for at least a minute.
3. Position the rodent on their side with a heat source safely underneath the animal. Restrain the mouse with the thumb and middle finger of the non-dominant hand, pulling back the loose skin over the nape and behind the ears. If you are right hand dominant, it’s easiest to have the animal in left lateral recumbancy.
4. Use the 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. The eye will protrude slightly.
5. Insert the needle at about a 45° angle to the eye, through the conjunctival membrane. There is a slight amount of resistance which causes the eye to regress back into the sinus, until the needle passes through the membrane. The needle will be positioned behind the globe of the eye.
6. Inject into the retrobulbar sinus.
7. Remove the needle gently to prevent injury to the eye.
8. Close the eyelids and apply mild pressure to the injection site with a gauze sponge.
9. Examine the animal during the recovery period, especially the injection site. If there are no adverse effects observed, return the animal to their home cage.

NOTE: If there is any swelling around the eye or a mass developing at the injection site – please notify OLAM staff immediately for medical attention.