Description of procedure:

Retro-orbital injection is an acceptable alternative to tail vein injection for the intravenous delivery of compounds, provided the individual is properly trained. When administering a retro-orbital injection, anesthesia is required. When performed proficiently, the procedure, including anesthesia and recovery time, takes less than five minutes.

The volume of injectate is limited to 200µL. Cell suspensions must be filtered or agitated prior to injection to prevent cell clumping. If injecting cancer cells, the needle must be cleaned off prior to injection and the suspension withdrawn slightly to prevent cells from inadvertently being deposited in the surrounding tissues.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, whether gloved or not. 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. Do not exceed two injections per eye in a mouse.

Stepwise instructions:

1. Anesthetize the mouse with an appropriate inhalant anesthetic (typically isoflurane).
2. Position the mouse on its side. Restrained it with the thumb and middle finger of the non-dominant hand, pulling back the loose skin over the shoulders and behind the ears (see picture below).
3. 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.
4. Insert the 25-30 gauge needle (bevel side away from the eyeball) at approximately a 45° angle to the eye, lateral to the medial canthus, through the conjunctival membrane (see diagram below). There is a degree of resistance, which 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. A standard sharp, syringe needle is preferred over a blunt-tipped needle as it results in reduced tissue distortion and damage.
5. Inject slowly into the retro-bulbar sinus.
6. Remove the needle gently to prevent injury to the eye.
7. Close the eyelid and apply mild pressure to the injection site with a gauze sponge.
8. Monitor the mouse during the recovery from anesthesia.
9. Examine the injection site for swelling or other visible trauma.
10. If no adverse effects are observed, return the mouse to its home cage immediately and monitor it until it is moving about.
References:
2. NYU Medical Center, Guidelines for Retro Orbital Injection in Mice. 
   www.med.nyu.edu/dlar/policies/retro_orbital_injections.html
3. Washington University at St. Louis. Retroorbital Injection Technique, 
   http://www3.nd.edu/~ndflsc/RetroOrbitalinjSOP08.pdf

[Developed February 2014 by Zoe Browning, Clinical Veterinarian]