Although described below, orbital venous sinus blood collection requires anesthesia, careful training, and justification to the IACUC. The facial vein provides similar volumes of blood, and is preferred when possible. General anesthesia must be provided when collecting from this site. Under general anesthesia the rodent is grasped so that its back rests on the palm of your non-dominant hand with its head toward your thumb. The thumb is placed just lateral to the trachea so that the jugular vein on the same side as the eye from which you are collecting blood is occluded and the fur on the animals head is drawn into the palm of your hand. This causes the animal’s eye to proptose (bulge) slightly. Be careful not to occlude the trachea! A 50uL sterile microhematocrit tube is directed into the medial canthus (junction of eyelids closest to the animal's nose) of the eye rotating slightly as the tube is directed to a point directly behind the globe. Sufficient pressure must be applied to cut through the fibrous layer that surrounds the sinus. Blood flows through the tube and occasionally around the tube once the sinus has been penetrated. After blood collection, the tube is removed and the eyelid closed and a dry cotton pad is applied over the eye with gentle pressure to prevent retroorbital hemorrhage. Be sure the eyelid is closed before applying the cotton pad to prevent damage to the cornea. In general blood should not be collected from the same eye more than 2 times, allowing at least 2 weeks between collections. An antibiotic ophthalmic ointment must be applied following bleeding.

While this procedure can be performed well by those with experience, there are serious potential adverse effects: (i) retroorbital hemorrhage resulting in hematoma and excessive pressure on the eye, which is almost certainly painful for the animal; (ii) any pressure required to stem persistent bleeding (e.g. by pressing on the eye) or pressure from a hematoma can lead to corneal ulceration, keratitis, pannus formation, rupture of the globe and micro-ophthalmia; (iii) damage to the optic nerve and other intra-orbital structures, which can lead to deficits in vision and even blindness; (iv) 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. Many of these unwanted sequelae may stay undetected, being located deep within the orbit. [Ref oJ. Appl. Toxicol. 21, 15–23 (2001)]