SAN DIEGO STATE UNIVERSITY

IACUC Guidelines

RODENT BLOOD COLLECTION METHODS

IACUC approved: October 14, 2019

BACKGROUND

Blood is removed from animals and substances are administered to animals for a variety of reasons. While commonplace, these procedures are not without the potential for pain and distress. It is desirable to remove blood from animals and/or administer substances to animals in the most humane and efficient way to minimize any pain, distress or discomfort experienced by the animal (1, 2). Individuals, including investigators, technical personnel, trainees and visiting investigators, who perform these procedures must be qualified through training or experience to accomplish these tasks in a humane and scientifically acceptable manner (3). Inappropriate blood sampling could result in hypovolemia and shock, anemia, trauma, pain, morbidity, or mortality.

RESPONSIBLE INDIVIDUALS

Only experienced staff may perform these procedures.

MATERIALS

Materials required for each method are described under those headings. Gloves, lab coat, other PPE as directed.

PROCEDURE

For all methods of blood collection:

1. Referring to the table in Appendix A, review the recommended volume of collection. Be sure the volume to be collected does not exceed those recommended in Appendix A. See also "Veterinary Guidelines for Blood Removal" for more information.

2. Gather the supplies necessary for the blood collection method employed. Note that materials such as syringes, needles, and lancets should be sterile and handled using aseptic technique.

3. Use restraint appropriate to the species and type of blood collection. Do not attempt to collect blood from an aggressive animal or one that cannot be safely restrained. Such animals may require anesthesia for blood to be safely collected.

4. Be familiar with the local anatomy of the sampling site such that you are aware of areas to avoid such as organs, glands and bony structures.

5. Assure hemostasis by applying direct pressure over the collection site. Be sure bleeding has ceased before returning animal to home cage and monitor for any signs of complications.

6. More information including still picture and video tutorials are available on the AALAS (American Association for Laboratory Animal Science) Website within the Learning Library. Free login is required. Go to Lesson 10 Blood Collection and IV Injection.
   Working with the Laboratory Mouse
   .

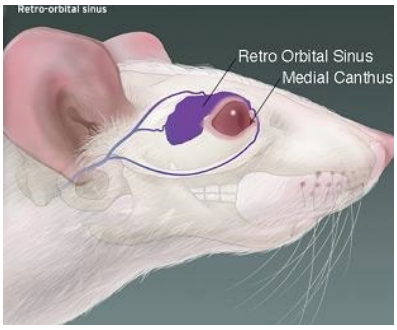
7. DO NOT perform these blood collection techniques unless and until you have received a hands-on supervised training session conducted by qualified personnel proficient in the technique. Such personnel can include an investigator with an approved protocol on which they are listed as qualified to perform the technique, OLAC personnel, or SDSU campus veterinarians.

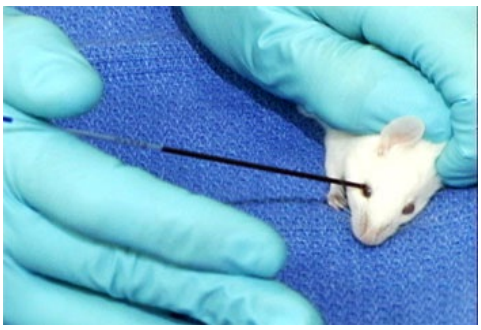
8. If any complications are observed such as bleeding, trauma, damage to tissue, lameness, overall signs of illness in the animal or unexpected mortality notify the OLAC manager and/or Campus Veterinarians.

Retro-orbital Blood Collection:

Also referred to as peri-orbital, posterior-orbital, and orbital venous sinus bleeding. Retro-orbital bleeding should typically be performed as a **terminal** procedure. Adverse effects reported for this procedure are described in the summary below.

Where its use is unavoidable, retro-orbital bleeding should only be used under general anaesthesia. Because of the severity of the adverse effects that can occur with this technique, even in skilled hands, it is essential that it is conducted only by staff members competent (practiced) in the technique.






1. The animal must be anesthetized to perform this technique. In addition, a topical ophthalmic anesthetic such as Opticaine or Tetracaine is applied to the eye prior to performing blood collection.

2. The equipment needed to perform this technique includes a glass tube such as a hematocrit tube, Natelson capillary tube, or glass pipette; gauze, an eppendorf tube or other collection tube if needed and a bulb that can be attached to the tube to move the blood out of the tube and into the collection vial. If necessary all tubes and vials can be treated with an anticoagulant if appropriate.

3. Blood is collected from the venous sinus. The mouse is restrained, the neck gently scruffed and the eye made to bulge. A capillary tube/pipette is inserted medially, laterally or dorsally. Blood is allowed to flow by capillary action into the capillary tube/pipette. The sample obtained is a mixture of venous blood and tissue fluid, and is not representative of venous blood.

4. Blood flow can be stopped by applying gentle finger pressure to the soft tissue. A finger should be placed over the closed eyelid for approximately 30 seconds. Application of a sterile ophthalmic ointment to the eye after sampling may aid in the return of the eye to normal physiological status. The mouse should be checked for post-operative peri-orbital lesions approximately 30 minutes after blood sampling and on at least one more occasion within two weeks of the sampling to ensure proper healing.

5. Sequential sampling is not recommended as histological changes, abnormal clinical signs, and evidence of discomfort have been reported. Ideally, no more than one bleed per eye should be taken - where a second retro-orbital bleed is required this should be performed as a terminal procedure. In rare circumstances sequential sampling may be used. In such instances only one orbit should be sampled from at any one sampling time, and adequate time should elapse between sampling to allow peri-orbital tissue repair to take place. An interval of two weeks between bleeds should allow damaged tissue to repair in most cases.

Summary

Number of samples	It is recommended that only one sample be taken.
Sample volume	Up to 0.2 ml with recovery; Up to 0.5 ml non-recovery.
Equipment	Isoflurane, glass capillary tube or Pasteur pipette, ophthalmic ointment
Staff resource	One person is required to take the blood sample.
Other	Careful monitoring for adverse effects is necessary both peri- and post-operatively.
Adverse effects	Retro-orbital haemorrhage resulting in haematoma and excessive pressure on the eye Corneal ulceration, keratitis, pannus formation, rupture of the globe and micro-ophthalmia caused by proptosis of the globe Damage to the optic nerve and other intra-orbital structures which can lead to deficits in vision and blindness Fracture of the fragile bones of the orbit and neural damage by the micro-pipette Penetration of the eye globe itself with a loss of vitreous humour

SUBMANDIBULAR BLOOD COLLECTION IN MICE

Description of procedure:

Also called the Superficial Temporal Vein Bleed or Cheek Bleed, this is the preferred blood collection technique for mice. This method allows for maximum allowable sample volume with minimal trauma to the animal. Multiple samples can be taken daily by alternating sides. This technique does not require the use of anesthesia and can be performed on conscious animals. When hemolysis of sample is a concern, this is the preferred method, as little hemolysis results from proper collection and processing.

Supplies necessary:

- 23 - 18 gauge needles or lancet
- Gauze
- Blood collection tube

Procedure:

1. Restrain the mouse with the non-dominant hand by grasping the loose skin over the shoulders and behind the ears; the skin should be taut over the mandible
2. Puncture the vein with a needle slightly behind the mandible, but in front of the ear canal. A swift lancing motion is used to puncture the vessel. Only the tip of the needle should enter the vessel to a shallow depth of 1-2 mm. Blood will flow immediately.
3. Allow blood to fall freely into collection tube, do not touch the tube to the face as this could apply pressure and stop blood flow. Additionally, "scraping" of blood off the face with the tube will cause hemolysis.
4. When the sample has been collected apply gentle pressure to the blood collection site with a gauze pad until bleeding has stopped.
5. The animal may be returned to their home cage and monitored for several minutes until recovered. If larger amounts of blood are collected, fluid may be replaced by injecting .2-.5ml of warm sterile saline subcutaneously.

To determine the appropriate location of the puncture site, measure a straight line from the bottom of the mandible to the caudal portion of the ear (image 1) and from the top of the eye to the point of the shoulder (image 2). Puncture the vein where these two lines intersect (image 3).



Image 1



Image 2

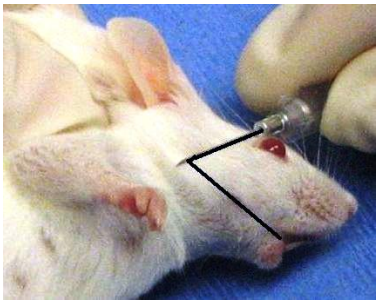


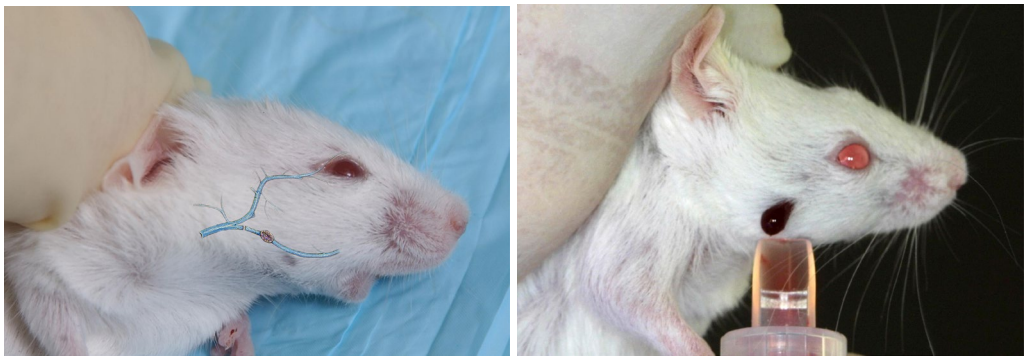
Image 3



The pictures above demonstrate the vessel anatomy, lancet placement and collection of blood for the submandibular blood collection technique. A detailed description of the technique can be found in Reference #s 5, 8, and 9. In addition, the Medipoint website has recommendations for lancet sizes and video descriptions of the technique: http://www.medipoint.com/html/animal_lancets.html

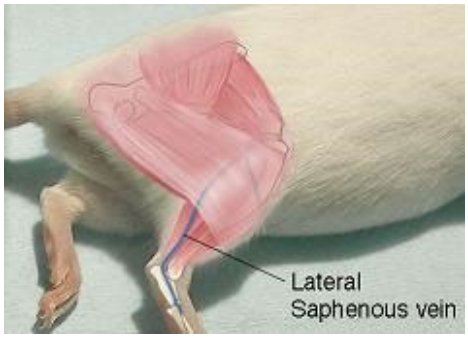
Adverse effects to be considered:
Hemorrhage from the ear canal or nasal cavity, hematoma formation

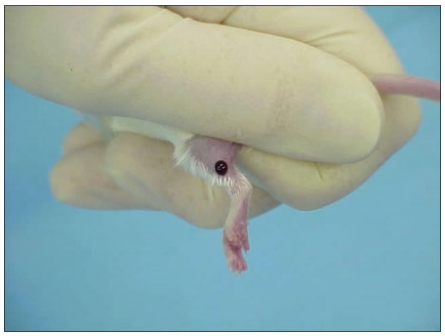
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Saphenous Vein Blood Collection:



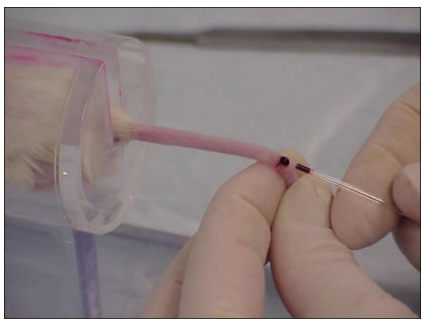
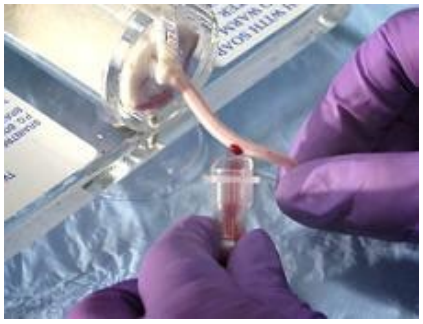



1. The anatomy and collection method are demonstrated in the above pictures.

2. The equipment needed to perform this technique includes clippers, alcohol wipe, restraint tube, small gauge needle (25-27g), and a collection vial or tube.

3. A detailed description of this technique can be found in reference #s 5,6,7 and 9. A slide show or video is available at the following site: https://norecopa.no/films-and-slide-shows/mouse

Tail Vein Blood Collection:


1. The technique for tail vein blood collection is depicted in the above pictures. In this technique a small nick is made in the vein with a scalpel and the blood collected into a collection vial or tube (as depicted in the first 2 pictures) or alternatively a small butterfly catheter can be placed into the vessel as depicted in the third picture.

2. Only small volumes can be collected using this method. A detailed description can be found in reference # 6.

3. The equipment needed for this method includes a scalpel or small guage (25-27g) butterfly catheter, guaze and a collection vial or tube.

REFERENCES

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2. Removal of blood from laboratory mammals and birds. A report of the BVA/FRAME/RSPCA/UFAW Joint Working Group on Refinement. Laboratory Animals. 1993. 27:1-22.

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4. Guidelines for Survival Bleeding of Mice and Rats. National Institutes of Health. http://oacu.od.nih.gov/ARAC/Bleeding.pdf

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7. Hem, A., A.J. Smith, P. Solberg. Saphenous vein puncture for blood sampling of the mouse, rat, hamster, gerbil, guineapig, ferret and mink. Laboratory Animals Ltd. Laboratory Animals. 1998. 32:364-368.

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APPENDIX A

Table 2. Ranges of Blood Sample Volume for Mice, Hamsters, and Rats

Species	Body Weight (g)	Circulating Blood Volume (ml)*	1% Sample Volume (ml) Per 24 hours	10% Sample Volume (ml) per 2-3 weeks
Mouse	20	1.26-1.60	0.013-0.016	0.13-0.16
	25	1.58-2.00	0.016-0.020	0.16-0.20
	30	1.89-2.40	0.019-0.024	0.19-0.24
	35	2.21-2.80	0.022-0.028	0.22-0.28
	40	2.52-3.20	0.025-0.032	0.25-0.32
Hamster	50	3.90	0.039	0.39
	75	5.85	0.058	0.58
	100	7.80	0.078	0.78
	125	9.75	0.098	0.98
	150	11.70	0.117	1.17
Rat	125	6.25-8.75	0.063-0.088	0.63-0.88
	150	7.50-10.50	0.075-0.105	0.75-1.05
	200	10.00-14.00	0.100-0.140	1.00-1.40

	250	12.50-17.50	0.125-0.175	1.25-1.75
	300	15.00-21.00	0.150-0.210	1.50-2.10
	350	17.50-24.50	0.175-0.245	1.75-2.45

Adapted from Guidelines for Survival Bleeding of Mice and Rats (4).
Mice: Circulating blood volume 63-80ml/kg (20-40g)
Hamster: Circulating blood volume 78 ml/kg (50-150g)
Rats: Circulating blood volume 50-70 ml/kg (125-350g)