

UC SANTA BARBARA

Institutional Animal Care and Use Committee

Santa Barbara CA 93106-5062

Tel: (805) 893-5855

Fax: (805) 893-2005

Email: iacuc@lifesci.ucsb.edu

<https://www.research.ucsb.edu/compliance/animal-subjects/>

| | |
|--------------------|---|
| IACUC GUIDELINE: | BLOOD AND TISSUE COLLECTION |
| DATE IMPLEMENTED: | January 21, 2011 |
| REVISION APPROVED: | March 16, 2012; January 18, 2013; April 19, 2013; February 24, 2015; July 23, 2015; November 20, 2015; November 13, 2019; March 1, 2023 |

Purpose:

This guideline establishes the expectations of the IACUC on the performance of animal tissue and blood collection procedures. If the procedures described below are not appropriate for your specific animal species, or your unique experimental requirements, then you need to describe your specific blood or tissue collection procedures in your animal use protocol.

1. Terminal Blood or Tissue Collection

Antemortem blood or tissue collection, even during terminal procedures, must be described in an approved animal protocol. Similarly, animals procured for the specific purpose of cell, tissue or body fluid harvest require an approved animal protocol. However, cells or tissues acquired from animals euthanized as part of an unrelated experiment do not require IACUC approval, assuming that the conditions listed below are met. Sharing tissues in this manner is considered to be an effective application of reduction of animal use.

Pre-Conditions for Tissue Sharing:

- The animals were euthanized by trained personnel using a euthanasia method described in their approved animal protocol and meeting the recommendations of the American Veterinary Medical Association Guidelines on Euthanasia.
- The animals do not pose a risk of injury or infection for the personnel using the harvested cells or tissues. Specifically, cells or tissues from animals exposed to toxic or biohazardous agents should not be shared.
- The harvested cells or tissues do not pose a risk of rodent pathogen transmission. Specifically, cells or tissues harvested from rodents outside of your animal colony (e.g. different vivarium) should not be transplanted in animals housed in your colony.

2. Vascular Perfusion for the Purpose of Tissue Collection

Research needs may require tissues to be collected from animals that are perfused with a fixative or physiological solution to ensure preservation of the tissues for histological examination, or to collect fresh tissue samples without blood, respectively. The usual route for administration of this vascular perfusion is transcatheter, and the animal must be fully anesthetized by the administration of euthanasia solution or anesthetic agent prior to initiating the perfusion procedure. Death in this two-step procedure is ensured by the exsanguination and/or removal of vital organs caused by the perfusion and/or tissue collection. There should be no delay, or any other potentially painful procedures performed, between verifying achievement of drug-induced anesthesia and performance of the terminal step (perfusion and tissue collection). Tissue collection performed in this manner is therefore considered a method of euthanasia (i.e. USDA pain category C), and not a non-survival surgery (i.e. USDA pain category D). In cases where a fixative is used (e.g. buffered formalin), the fixative must be collected and/or properly disposed.

3. Terminal Blood Collection

Blood collection that is performed on a dead animal does not need to be described in an animal protocol. Blood collection that either exceeds the maximum blood volume recommendations of the table below, or that is done for the purpose of causing exsanguination, must only be performed on a fully anesthetized animal that is not allowed to recover from anesthesia (i.e. it's a terminal procedure). Cardiac puncture is often used in cases where maximal volumes of blood are required to be obtained. Cardiac puncture for blood collection is ONLY permitted as a terminal procedure in a fully anesthetized animal.

4. Blood Sampling Volumes and Recovery Periods

The blood sampling method must be performed in an aseptic manner (i.e. using sterile instruments), and must be listed in the animal protocol. The following table, derived from published reference standards¹, should be used to determine the maximum volume of blood that can be removed from an animal **within a 24-h period**. The limits are dependent on the animal's circulating blood volume (CBV) and body weight. The body weight of your animal won't always match the weight in the table due to differences in the age, strain, and gender of the animal; therefore, multiply the species-appropriate CBV by the animal's body weight and by the percentage (7.5 – 15%) of the CBV to be collected in order to calculate the maximum sampling volume. As a general rule, if only a single blood collection is planned, or if multiple blood collections are planned in a 24-h period, then the cumulative total of the blood sampling volume (in ml) should $\leq 1\%$ of the animal's body weight (in g). For example, on a 20g mouse the cumulative total of blood collected would be ≤ 0.2 ml. In this example, a 2-week recovery period is recommended before blood can be collected from the animal again. Collection of blood sampling volumes greater than those recommend by the table below requires scientific justification and IACUC approval.

| Species | Circulating Blood Volume | Animal's Body Weight | Maximum Blood Volume Removed in 24hr Period | Allow This Much Recovery Time Before Next Blood Collection |
|---------|--------------------------|----------------------|---|--|
|---------|--------------------------|----------------------|---|--|

| | (CBV) | | | | |
|--------|----------|-------|-------------|----------------|---------|
| Mouse | 72 mL/kg | 20 g | 7.5% of CBV | 0.11 mL | 1 week |
| | | | 10-15% | 0.14 – 0.22 mL | 2 weeks |
| Rat | 64 mL/kg | 300 g | 7.5% | 1.44 mL | 1 week |
| | | | 10-15% | 1.94 – 2.88 mL | 2 weeks |
| Rabbit | 56 mL/kg | 3 kg | 7.5% | 12.6 mL | 1 week |
| | | | 10-15% | 16.8 – 25.2 mL | 2 weeks |

5. Retro-Orbital Blood Collection in Mice and Rats

The collection of blood from the retro-orbital sinus is a controversial procedure that many find objectionable for aesthetic reasons; however, if done properly it is a safe and humane method for collecting small blood samples. Extreme care must be taken not to abrade the cornea, obstruct breathing, damage nerves, or penetrate the globe itself (Fig. 1; rat orbital region schematic). It should also be noted that the approach to the sinus can be made from a lateral or medial canthus. The rat has a dorsal venous plexus so it may be less likely to cause damage if approached from a dorsal aspect, although published studies have demonstrated no significant difference between sites.² It was determined, however, that the *skill* of the operator and the *frequency* with which one performs orbital sinus blood sampling are important factors in reducing the number and severity of complications.² Generally, the collection of blood from the retro-orbital sinus should be performed as *infrequently* as possible and on very short-term studies or on those longer-term studies that require infrequent sample collection.

The collection of blood from the retro-orbital sinus should only be performed by highly skilled and trained individuals with proven proficiency in performing retro-orbital blood collection to minimize corneal lesions, ocular discharge, and other ocular problems that can be caused by improper technique.² Upon request, the Attending Veterinarian or designee can provide the necessary training, including training in alternative methods of blood collection.

The collection of blood from the retro-orbital sinus must be performed under anesthesia, and mice or rats must be closely monitored for 48 hrs afterwards for signs of adverse effects (see 4th bullet point). The IACUC recommends the following practices to minimize the potential for pain or distress:

- A small capillary tube (100µL) or a similar pipette is recommended for this procedure.
- The procedure is not recommended for frequent repeat sampling from the same orbit, because it takes two to four weeks to repair the tissue damage caused by this procedure³. Therefore, the collection of blood from the retro-orbital sinus should be limited to one sampling per orbit, every two weeks, or in situations of serial blood collections where the animal is not recovered from anesthesia.

- Appropriate aftercare should be instituted to ensure a quick and uneventful recovery. Aftercare should include gentle compression of the affected closed eye with a gauze pad until blood flow stops, and general observation for overt clinical signs of distress. Ophthalmic ointments are generally not recommended, because attempts by animals to clean them away by scratching or wiping tend to further traumatize the eye.
- All abnormalities such as untoward behavior, protruding eye, shrunken eye, ocular discharge, or uncontrolled bleeding should be reported to the veterinarian.

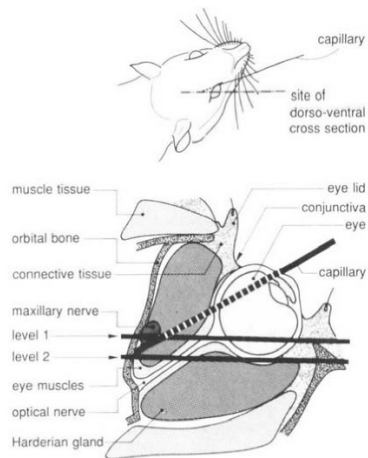


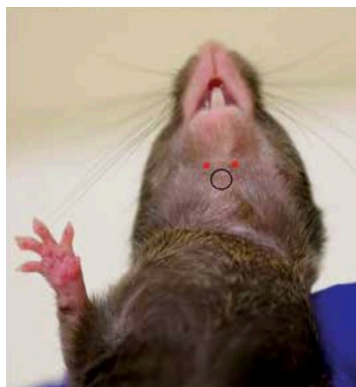
Figure 1.

Since this is a potentially painful or distressful procedure, alternate methods of blood collection should be considered. One should weigh the potential advantages and drawbacks of each procedure when designing a study, for example: Species to be bled, and size of the animal to be bled. How will the collected blood sample be used? Does hemolysis matter? Will the sample size be adequate for the assay methodology? What is the frequency of collection? The stress of restraint and the corresponding physiological response should also be taken into consideration when designing a study. The following are frequently described alternative survival blood collection methods:

- **Catheters:** The implantation of intravenous (IV) catheters generally requires the administration of longer-acting anesthetics. It also necessitates a recovery period prior to the initiation of any studies where drug-drug interaction or induction is a concern. The surgical procedure of implanting IV catheters takes time and after implantation the catheters require maintenance (flushing, etc.) to prevent clotting. The catheters must be protected or they can be chewed or scratched off, and catheterized animals with exteriorized ports may need to be singly housed. Catheters, however offer several advantages: they provide an easily accessible route to administer IV compounds and many serial blood samples can be collected with relative ease with minimal disruption to the animal.
- **Tail vein sampling:** Small blood samples can be collected with a needle and syringe from the tail vein, however hemolysis is common and unless anesthetized, the animals will need to be restrained during the procedure. The stress of restraint and the

corresponding physiological response should also be taken into consideration when designing a study.

- **Tail cutting/clipping:** Still a common practice in some laboratories, but the risk of infection, necrosis and granuloma formation make this a less desirable alternative from a purely scientific perspective. A relatively recent refinement of this method calls for the creation of a small (2mm) incision rather than tail-tip amputation, which according to the authors is more "animal-friendly."⁴
- **Saphenous vein sampling⁵:** Reasonable (0.1 – 0.5 ml) volumes of blood can be collected from mice or rats using this method. Serial sampling is possible. The procedure can be performed quickly and without anesthesia, but skin preparation (hair removal) and atypical animal restraint (placing the mouse or rat in restraining tube or stockinette so that only the hind leg is accessible) are required.
- **Facial vein sampling⁶ (mice only):** The mice are scruffed, and the facial vein is punctured in the mandibular region (cheek) using a 21 gauge needle introduced only to the depth of the bevel. Reasonable volumes (0.2 ml) of blood can be collected from mice using this method. The procedure can be performed quickly and without anesthesia, and without any hair removal or atypical animal restraint. Certain strains of mice (e.g., DBAs) tend to be more susceptible to stress when undergoing this procedure and may experience syncope. It is also possible to get bleeding from the ear if the bundle is punctured too high above the jaw.
- **Submental (chin) sampling^{7, 12} (mice only):** The technique is easier to learn and master and safer than other nonterminal methods (retroorbital or submandibular/facial vein). The mouse is manually restrained with a scruff hold (grasping the skin of the nape of the neck between the thumb and index finger) to identify the anatomic landmarks (black circle) and blood collection (red dots) on the submental/chin region (see picture below). The vein is then punctured with a 5-mm lancet or needle and the blood is allowed to drip into the collection tube (0.1 - 0.2 ml). Bleeding will usually stop after releasing the scruff hold, but if it doesn't then apply gentle pressure to the blood collection site with gauze.



- **Jugular vein sampling⁸ (rats only):** Medium to large (1 – 3 ml) volumes of blood can be collected from rats using this method. The procedure can be performed on an anesthetized or manually-restrained rat. The latter requires two people to perform –

one to restrain the animal and one to collect the blood. The animals recover rapidly from the procedure with few long-term complications, and the blood sample quality is superior to other methods, that is, retro-orbital method.

- **Dried Blood Spot (DBS)**^{9, 10}: This is not a sample collection method, but rather a sample analysis method, which has advantages compared with traditional whole blood or plasma sampling techniques. Specifically, DBS uses a very small sample volume (30 – 60 µls), which permits more frequent serial sampling without compromising the health of the animal, and may reduce the number of animals per study. The blood sample, collect by any method even a small tail prick or nick, is allowed to dry on the sampling card, and it's then removed with a hole or biopsy punch and subjected to chromatographic (LC-MS or MS) analysis or radioimmunoassay.¹¹ As such, the diagnostic instrumentation requirements are not trivial, but the advantages in terms of animal welfare are significant and therefore the method is worthy of consideration as refinement to conventional blood sampling techniques.

References:

1. Diehl KH, Hull R, Morton D, Pfister R, Rabemanpianina Y, Smith D, Vidal JM, and Van de Vorstenbosch C. A good practice guide to the administration of substances and removal of blood, including routes and volumes. *J. Appl. Toxicol.* 21:14-23. 2001
2. Van Herck H, Baumans V, Brandt C, Hesp A, Sturkenboom J, van Lith HA, van Tintelen G, and Beynen AC. Orbital sinus blood sampling in rats as performed by different animal technicians: the influence of technique and expertise. *Lab. Anim.* 32, 377-386. 1998
3. Van Herck H, Baumans V, Van der Craats NR, Hesp AP, Meijer Gw, Van Tintelen G, Walvoort HC, and Beynen AC. Histological changes in the orbital region of rats after orbital puncture. *Lab. Anim.* 26:53-58. 1992
4. Fluttert M, Dalm S, and Oitzl MZ. A refined method for sequential blood sampling by tail incision in rats. *Lab. Anim.* 34:372-378. 2000
5. Hem A, Smith A, and Solberg P. Saphenous vein puncture for blood sampling of the mouse, rat, hamster, gerbil, guinea pig, ferret and mink. *Lab. Anim.* 32, 364-368. 1998
6. Harikrishnan VS, Hansen AK, Abelson KSP, and Sørensen DB. A comparison of various methods of blood sampling in mice and rats: Effects on animal welfare. *Lab. Anim.* 52(3):253-264. 2017
7. Regan RD, Fenyk-Melody JE, Tran SM, Chen G, and Stocking KL. Comparison of submental blood collection with the retroorbital and submandibular methods in mice (*Mus musculus*). *J Am Assoc Lab Anim Sci.* 55(5):570-576. 2016
8. Toft MF, Petersen MH, Dragsted N, and Hansen AK. The impact of different blood sampling methods on laboratory rats under different types of anaesthesia. *Lab. Anim.* 40(3):261-274. 2006
9. Bioanalytical Systems website - <http://www.basinc.com/services/DBS.php>
10. Burnett JEC. Dried blood spot sampling: practical considerations and recommendation for use with preclinical studies. *Bioanalysis* 3(10):1099-1107. 2011
11. Millot MR, James JS, Merali Z, and Plamondon H. A refined blood collection method for quantifying corticosterone. *Lab. Anim.* 41(3):77-83. 2012

12. George AJ, Harmsen BJ, Ford JA, Tadepalli SR, and Horton ND. Evaluation of submental blood collection in mice (*Mus musculus*). *J Am Assoc Lab Anim Sci.* 62(1):92-98. 2023