UNIVERSITY STANDARD

Title

UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL
STANDARD FOR RODENT BLOOD WITHDRAWAL AND TAIL BIOPSY

Introduction

PURPOSE
The standards and procedures described below provide guidance to all researchers and animal handlers for performing blood withdrawals and tail biopsies in mice and rats.

SCOPE OF APPLICABILITY
All personnel engaged in the hands on collection of blood and/or tail tissue from rats and mice.

The UNC-CH IACUC expects that anyone involved in animal work at the University will comply with this Standard and the University Policy on the Care and Use of Vertebrate Animals for Research, Training and Teaching Purposes. Requests for exceptions to this Standard must be reviewed and approved by the IACUC and/or DCM Management.

TRAINING
Your Laboratory Animal Coordinator (LAC) if qualified, may be able to train others in this technique, or you can contact the IACUC at 919-966-5569 (iacuc@med.unc.edu) to arrange for training.

See the IACUC Mouse Handling & Techniques and Rat Handling & Techniques packets for more detailed instructions and diagrams.

Standard

- **Acute or Single Blood Withdrawal:** The maximum survival amount of an acute blood withdrawal is 1% of the lean body weight. [e.g.; For a 20 gram adult mouse, no more than 200 µl (4 X 50 µl micro capillary tubes), may be withdrawn]
- **Chronic Blood Withdrawal:** For sequential blood sampling (over a period of time), the maximum aggregate survival blood withdrawal for most mammals is 1.5% of lean body weight over a 14-day period. The above guideline for acute or single blood withdrawal still holds at any given time point within this 14-day period.
- To facilitate blood collection, warm the rodent first. When using the tail veins or artery, you may dip the tail in warm water (45°C). The entire animal can be warmed with a carefully placed heat lamp for 5-10 minutes or by placing the housing cage on a
circular water pad. Alternatively, alcohol may be used initially as a vasodilator, but it should not be used on broken skin.

Table 1: Approximate Blood Sample Volumes for a Range of Body Weights

<table>
<thead>
<tr>
<th>Body weight (g)</th>
<th>*CBV(µl)</th>
<th>1% CBV (µl) every 24 hrs†</th>
<th>7.5% CBV (µl) every 7 days†</th>
<th>10% CBV (µl) every 2 - 4wks†</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>1100 - 1400</td>
<td>11- 14</td>
<td>82 - 105</td>
<td>110 - 140</td>
</tr>
<tr>
<td>25</td>
<td>1370 - 1750</td>
<td>14 - 18</td>
<td>100 – 130</td>
<td>140 -180</td>
</tr>
</tbody>
</table>

* Circulating blood volume
† Maximum sample volume for that sampling

** adapted from NIH Guidelines for Survival Bleeding of Mice and Rats

Methods:

1. **Submandibular:**

A relatively simple way to obtain blood (usually only used to collect blood from mice) is to puncture the area behind the hinges of the jawbones. The superficial temporal vein is a large vessel positioned behind the eye, which can be traced backward to the temporal vein, the maxillary vein, and finally, the jugular vein.

Scrub the mouse and pierce the skin in the relevant area. A mouse bleeding lancet is strongly recommended for use. However, an 18 gauge needle may also be used.

2. **Saphenous Vein:**

This method of obtaining blood is often used when a series of small samples is required.

Restrain the animal and shave the caudal surface of the thigh. The saphenous vein can be seen in this area. It is advantageous to apply a lubricant to prevent wicking. Place a tourniquet above the knee and enter the vein with a needle. This method of blood withdrawal does not require anesthesia, however, the method of restraint is cumbersome.

3. **Tail Artery / Vein (Tail Nick Bleed):**

Tail veins and artery can be used for serial bleedings. Use the central tail artery or lateral tail veins. Anesthesia is not required for tail vein nick bleed or tail artery bleed.

Tail Nick Bleed: When bleeding from either of the lateral tail veins, a 22-gauge needle or lancet is inserted into the vein, midway up the tail. You may collect blood with micro capillary tubes, or a micropipette.

Tail Artery Bleed: Most common in rats. Blood may be withdrawn from the ventral tail artery using a 22-gauge (or smaller) needle; let the blood drip into the collection vessel. A tourniquet
placed at the base of the tail will facilitate bleeding. Anesthesia is not required but is highly recommended for ease of collection.

4. **Tail Clip Bleed and/or Tail Biopsy for Genotyping:**

Performed on unanesthetized or anesthetized animals depending on amount of tissue needed (see below):

- **Anesthesia is optional for the removal of up to 4mm from the tail tip. It is strongly recommended that no more than 2mm be removed at a time.** Anesthesia may be used as a means of animal restraint and its use must be described in the approved protocol.
- Depending on the age of the animal, removal of greater than 4mm from the tail tip may involve cutting into the vertebral column. Therefore, anesthesia is always required when removing this much tail, irrespective of the age of the animal. The use of anesthesia must be described in the approved animal care application.
- **Requests to perform tail biopsies or successive tail cuts totaling greater than 4mm without anesthesia must be scientifically justified and must receive IACUC approval prior to implementation.**

The IACUC has approved the tail cut method for both rats and mice to obtain blood and/or tissue. This method must be described in the animal use application and approved by the IACUC prior to use. See procedure below.

1. Place animal in approved animal restrainer. (Experienced handlers may be able to perform technique in habituated animals with light or no restraint).
2. Remove any bedding material or feces from the tail.
3. Place the animal on a clean work surface.
4. Using a fresh scalpel blade, cut 1-2 mm of the distal tail at an angle perpendicular to the work surface.
5. Apply gentle pressure proximal to the collection site to occlude venous return and ease collection.
6. Apply gentle digital pressure to the wound for 30-45 seconds with a clean gauze pad to stop bleeding, before placing animal back in its home cage. For persistent bleeding, apply a silver nitrate stick, styptic powder or a cautery pen to the wound to stop bleeding.
7. Serial blood samples can be obtained over a short time frame by gently removing the scab without performing an additional cut.
8. Only the fleshy portion of the tail tip should be cut. Cutting into the vertebrae is NOT permitted. As only a small portion of the tail does not contain vertebrae, the use of the tail cut procedure should be limited.

5. **Retro Orbital Bleeding:**

Retro-orbital or orbital sinus/plexus bleeding is permitted in rats, mice, gerbils, guinea pigs, hamsters. The IACUC will permit orbital sinus bleeding when it is scientifically justified, performed with appropriate technique and anesthesia. Veterinary staff experience indicates that this method
may lead to orbital damage, blindness and potentially death if not performed correctly. The IACUC encourages the primary use of the submandibular, tail artery or veins; specifically the nick or cut techniques. These methods are less likely to harm the animal and may be used repeatedly for bleeding.

LACs may not train in this technique so training and certification must be obtained from IACUC Training and Compliance team or DCM veterinary services.

**Alternating eyes for each bleeding is mandatory, and a week must separate each bleeding (unless the final bleed is performed as a terminal procedure – animal does not recover from anesthesia). A maximum of two (2) bleedings per eye is permitted. Maximum volume withdrawn within a two week period is 1.5% body weight. Orbital sinus bleeding requires training and must be performed on anesthetized animals only with IACUC approval.**

“Following blood collection, the eyelids should be held closed for a few seconds to allow the punctured blood vessel to clot. It is also common practice to place a small amount of ophthalmic ointment into the eye following this procedure.” excerpt from Laboratory Animal Technician Training Manual.

6. **Cardiac Puncture: Always a terminal procedure conducted under anesthesia or immediately following euthanasia!**

Cardiac puncture as a method of blood withdrawal permitted in all species provided the following conditions are met:

1. Animal is under a surgical plane of anesthesia or deceased when procedure is conducted.
2. Animal is NOT allowed to recover from anesthesia following the puncture.
3. If the animal is euthanized prior cardiac puncture, training and certification in the technique is not required.
4. A needle is inserted into the heart and blood is extracted until a sufficient volume is collected or the animal is exsanguinated. This procedure must be followed by a physical euthanasia method.

**EXCEPTIONS**
Requests for exceptions to this Standard must be reviewed and approved by the IACUC and/or DCM Management.

**Definitions**

**IACUC:** Institutional Animal Care and Use Committee  
**DCM:** Division of Laboratory Animal Medicine  
**OACU:** Office of Animal Care and Use  
**University Standard:** The minimum acceptable limits or rules used to achieve Policy implementation, enforceable by the IACUC.
Blood Withdrawal: Removal of blood, usually by venipuncture

Related Requirements

EXTERNAL REGULATIONS AND CONSEQUENCES

UNIVERSITY POLICIES, STANDARDS, AND PROCEDURES

For more detailed guidance, please refer to the University Policy on the Care and Use of Vertebrate Animals for Research, Training and Teaching Purposes.

Contact Information

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<thead>
<tr>
<th>Subject</th>
<th>Contact</th>
<th>Telephone</th>
<th>Email</th>
</tr>
</thead>
<tbody>
<tr>
<td>IACUC Protocol and Training</td>
<td>IACUC</td>
<td>919-966-5569</td>
<td><a href="mailto:iacuc@med.unc.edu">iacuc@med.unc.edu</a></td>
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<tr>
<td>Veterinary Services</td>
<td>DCM</td>
<td>919-966-2609</td>
<td></td>
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</tbody>
</table>

Important Dates

- Effective Date and title of Approver: 07/26/2002; UNC IACUC
- Revision and Review Dates, Change notes, title of Reviewer or Approver: 01/16/03, 07/24/04, 10/21/05, 06/14/06/03/13/09, 02/08/13, 12/1/2017; UNC IACUC

Approved by: UNC IACUC

Dr. Mitchell Picker
UNC IACUC Chair 12/2017