Purpose Bred Mice and Rats in Research, Testing and Teaching

Section 5 (part 1): Using Experimental Methodologies

Using Experimental Methodologies

Scenario:

You have always collected blood from awake rodents via the retro-orbital sinus. Now, the IACUC is expecting you to anesthetize the animals first or use an alternate collection site. What blood collection methods are acceptable for "awake" rodents?

Recall The Guide to the Care and Use of Laboratory Animals; it was developed to assist researchers in conducting experiments which follow the highest scientific, humane, and ethical standard. To meet these standards, choices and alternatives must be examined. This training section reviews methods which are currently deemed acceptable for rodent research. Please consult the DLAR if you would like more information on any technique. Remember, if a procedure would hurt you, it would also hurt an animal.

This rodent training section—which is segmented into three parts—will enable you to

- Select appropriate injection sites
- Understand approved surgery guidelines
- Understand the need to provide analgesia
- Apply the tumor burden policy
- Recognize signs of pain and distress
- Identify research hazards

- Identify blood collection sites based on required volume
- Determine when & how to use pharmaceutical and non-pharmaceutical grade drugs
- Select the appropriate method of euthanasia

Using Experimental Methodologies

Part I

Blood Collection Sites Based on Required Volume

- The standard for acceptable blood withdrawal amounts is based on the percentage of total CBV. CBV should be determined from known species-specific volume: weight values and not calculated based on flat % of body weight.
- A maximum survival bleed not exceeding 15% of CBV is allowable once monthly if fluid replacement is administered.
- Bleedings performed weekly should not exceed 7.5% CBV
- Bleedings performed once every 2 weeks should not exceed 10% CBV

For assistance with calculations and techniques, consult the University of Pittsburgh Policy for Regulating the Volume of Experimental Blood Sample Withdrawals in Laboratory Animals or contact the DLAR.

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Part I

Blood Collection Sites Based on Required Volume

- Appropriate blood collection sites include the: lateral tail vein, the ventral/dorsal tail artery, the saphenous vein, and the jugular vein. The University of Pittsburgh IACUC allows blood collection from the retro-orbital sinus or by terminal cardiac puncture only in anesthetized animals. Tail clipping may require anesthesia based on the age of the animal. More detailed information regarding rodent bleeding techniques can be found on the IACUC's website at "Collecting Blood Samples."

This photograph demonstrates blood collection from the retro-orbital sinus of an anesthetized mouse.

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Many protocols not only call for blood sampling but also require injecting various chemicals or treatments. While working with animals, even for non-survival (acute/terminal) procedures **you must use pharmaceutical grade injectables unless otherwise specified in an approved IACUC protocol.** The definition of "Pharmaceutical Grade" encompasses chemical agents that have been purified and buffered by a company; and are designated for medicinal purposes in humans and animals. Non-pharmaceutical grade agents may be less pure and non-sterile. Impurities may affect research results. All non-pharmaceutical grade agents must be sterilized and kept in sterile containers. **Storage containers must be labeled with the PI name, drug name, its concentration, and expiration date.**

Appropriate injection sites

For rodents, consider the following table:

Site	Location	Mouse Max ml	Rat Max ml
Subcutaneous(sq)	Scruff of neck	2 - 3	5-10
Intramuscular (im)	Quadriceps/Caudal Thigh	0.05	0.3
Intraperitoneal (ip)	Abdominal lower quadrant	2 – 3	5 - 10
Intravenous (iv)	Lateral tail vein	0.2	0.5

Remember to choose the correct needle size; 25 – 26 gauge needles are typically suitable for adult mice. For assistance, contact the DLAR Veterinary Services. Experienced staff members will be happy to provide you with training. Some examples of appropriate injections sites are given on the following pages.

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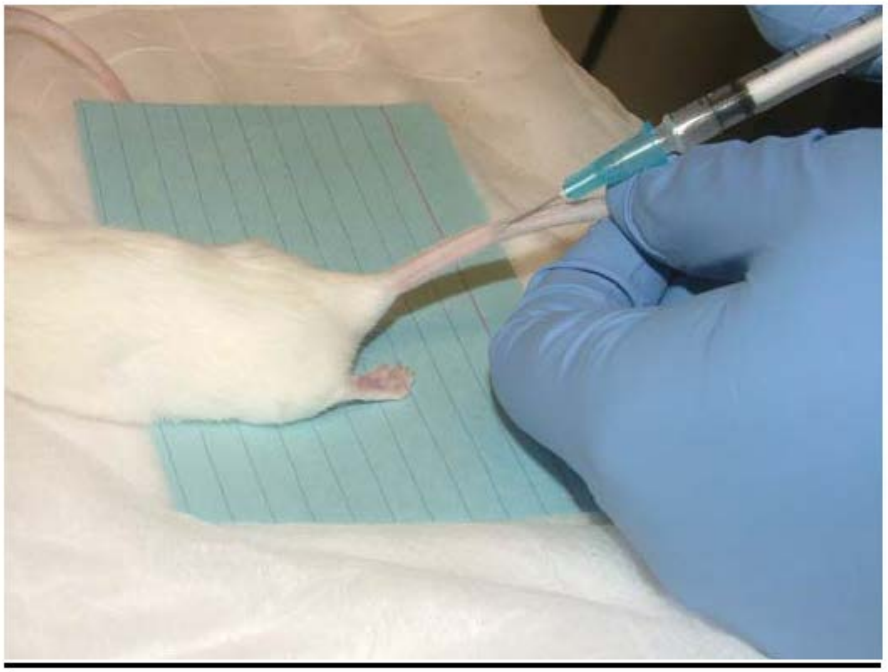
Intraperitoneal (IP)



Intravenous (IV)

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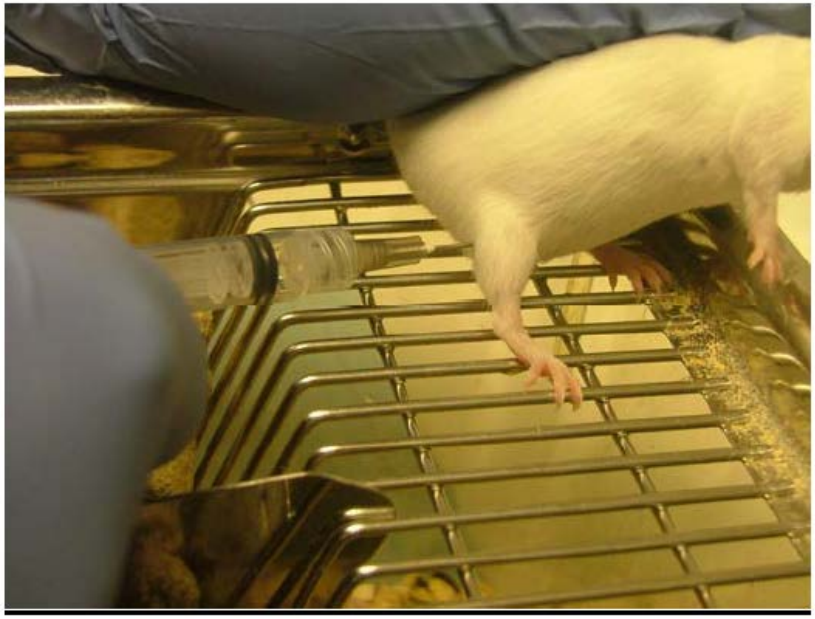
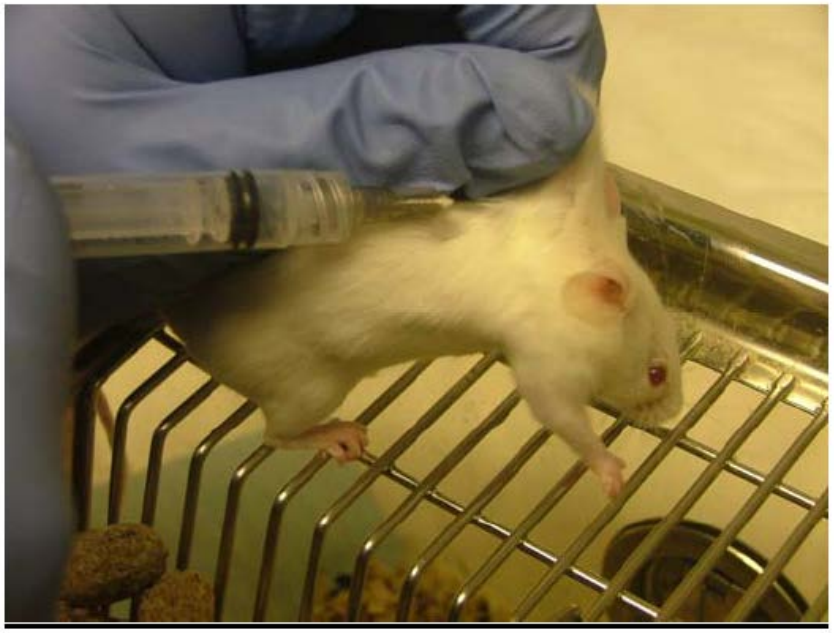


Intramuscular (IM)



Subcutaneous (SQ or SC)

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There are policies involving the injection of inflammatory agents such as Complete Freund's adjuvant in research. Having considered the appropriate procedures for blood sampling and administering chemical agents to rodents, our focus will now turn to tumor induction.

While working with "tumor-burdened" rodents, meaning rodents which have been given tumors as part of a research study, it is important to carefully monitor these animals on a daily basis. Any adverse changes in health and behavior must be documented and forwarded to the principal investigator, support personnel, and attending veterinarian. Adverse changes may include—but are not limited to—weight loss, decreased food and water intake, lethargy, labored breathing, hypothermia, and tumor growth which interferes with an animal's normal behavior and condition (mouse—20 mm diameter maximum; rat—40 mm diameter maximum). Pay particular attention to the organ system "most likely to be affected by the tumor type."

- Remember that "Death as an End-Point" is not sanctioned by the IACUC—all animal experiments must provide for a humane sacrifice before the animal experiences pain and distress. More detailed information relating to the Tumor Burden Policy can be found on the IACUC web site.

Before continuing with Section II of this training session, it would be helpful to review Section I by taking the section quiz.