



GUIDELINES: Rodent Blood Collection

Objective:	To establish guidelines for survival bleeding of mice and rats
Author:	Attending Veterinarian, Laboratory Animal Resources
Date:	February 1, 2024

I. Overview

These guidelines have been developed to assist investigators in their choice and application of survival rodent bleeding techniques. This is based on peer-reviewed publications as well as data and experience accumulated at (and shared by) the NIH.

II. Training

It is the responsibility of both the investigator and IACUC to ensure the use of techniques and procedures that result in the least pain and distress to the animal, while adequately addressing the needs of the experimental design.

With any procedure, training is critically important. Training opportunities and resources, including access to experienced investigators and veterinary staff for assistance and supervision, must be made available to new personnel by the PI and are also available through Laboratory Animal Resources. Researchers are not to work unsupervised until they have been deemed proficient (and the IACUC has received proper documentation).

NOTE: For specific questions regarding rodent blood collection techniques, contact the Attending Veterinarian, or the Director, Laboratory Animal Resources for discussion and training.

III. Review and Approval

- A. The Investigator, in consultation with the Attending Veterinarian and veterinary staff, should decide which method of blood withdrawal to use.
- B. Volumes greater than recommended should be justified in the protocol AND appropriate fluid and/or cellular replacement provided.
- C. Exceptions to these guidelines (e.g., increase in blood volume to be collected or retro-orbital bleeding without use of anesthesia), should be scientifically justified in the Protocol and approved by the IACUC.
- D. The procedures utilized must be reviewed and approved by the IACUC (via standard protocol or amendment submission processes) prior to their implementation.
- E. Factors to consider in choosing the blood withdrawal technique/method for the protocol:
 - The species to be bled.
 - The size of the animal to be bled and the estimated total blood volume.
 - The type of sample required (e.g. serum, whole cells, etc.).

- The quality of the sample required (sterility, tissue fluid contamination, etc.)
- The quantity of blood required.
- The frequency of sampling.
- The health status of the animal being bled.
- The training and experience of the individual collecting blood.
- The effect of restraint or anesthesia on the blood parameter measured.

IV. Blood Volumes

The acceptable quantity and frequency of blood sampling is dependent on the circulating blood volume of the animal and the red blood cell (RBC) turnover rate‡. Blood sample ranges, based on body weight, are provided in **Table 1**.

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

Body weight (g)	*CBV (ml)	1% CBV (ml) every 24 hrs†	7.5% CBV (ml) every 7 days†	10% CBV (ml) every 2 – 4 wks†
20	1.10 - 1.40	.011 - .014	.082 - .105	.11 - .14
25	1.37 - 1.75	.014 - .018	.10 - .13	.14 - .18
30	1.65 - 2.10	.017 - .021	.12 - .16	.17 - .21
35	1.93 - 2.45	.019 - .025	.14 - .18	.19 - .25
40	2.20 - 2.80	.022 - .028	.16 - .21	.22 - .28
125	6.88 - 8.75	.069 - .088	.52 - .66	.69 - .88
150	8.25 - 10.50	.082 - .105	.62 - .79	.82 - 1.0
200	11.00 - 14.00	.11 - .14	.82 - 1.05	1.1 - 1.4
250	13.75 - 17.50	.14 - .18	1.0 - 1.3	1.4 - 1.8
300	16.50 - 21.00	.17 - .21	1.2 - 1.6	1.7 - 2.1
350	19.25 - 24.50	.19 - .25	1.4 - 1.8	1.9 - 2.5

* Circulating blood volume

† Maximum sample volume for that sampling frequency

‡RBC life span of the mouse: 38-47 days. RBC life span of the rat: 42-65 days.

NOTE: Volumes greater than recommended should be justified in the protocol and appropriate fluid and/or cellular replacement provided.

For example: The approximate circulating blood volume of rodents is 55 to 70 ml/kg of body weight. Of the circulating blood volume, approximately 10% of the total volume can be safely removed every 2 to 4 weeks, 7.5% every 7 days, and 1% every 24 hours.

References

NIH Animal Research Advisory Committee (ARAC), "Guidelines for Blood Collection in Mice and Rats" (https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/b2_blood_collection_in_mice_and_rats.pdf)

Revision History

Approved February 22, 2021

Administrative changes September 17, 2022

Revised February 1, 2024