University of Notre Dame IACUC Policy on the Frequency of Blood Sampling in Rodents

Purpose

To ensure the humane use of animals in research, limits on the frequency of blood collection from various sites in rodents is necessary. Serial survival sampling is done routinely to check antibody titer levels, percent parasitemia, and for genotyping. The acceptable quantity and frequency of blood sampling is dependent on the circulating blood volume of the animal. The approximate circulating blood volume of rodents is 55 to 70 ml/kg of body weight[1]. Of the circulating blood volume, approximately 10% of the total volume can be safely removed every 2 to 4 weeks and 1% every 24 hours. Volumes greater than recommended will be reviewed by the IACUC. The investigator must provide scientific justification in the animal protocol for blood collection volumes. The following table is a guide for recommended volumes[2]:

Body Weight (grams)	Circulating Blood Volume (mL)	approx. 1% (mL)	approx. 10% (mL)
20 g	1.10 – 1.40	0.014	0.14
25 g	1.37 - 1.75	0.018	0.18
30 g	1.65 - 2.10	0.021	0.21
35 g	1.93 - 2.45	0.025	0.25
40 g	2.20 - 2.80	0.028	0.28
125 g	6.88 - 8.75	0.088	0.88
150 g	8.25 - 10.50	0.10	1.0
200 g	11.00 - 14.00	0.14	1.4
250 g	13.75 - 17.50	0.18	1.8
300 g	16.50 - 21.00	0.21	2.1
350 g	19.25 - 24.50	0.24	2.4

Although the body replaces blood volumes quickly, various components of the circulating blood are replaced more slowly. Therefore it is recommended that blood sampling protocols allow for replacement of volume and cellular components, necessitating a minimum frequency when withdrawing the maximum volume.[2]

Mice

Route	Volume*	Frequency	Instructions
Retro-orbital	150 - 300µL	once every 2-4 weeks	alternating eyes
Retro-orbital	<75 µL	once a week up to 8 weeks	alternating eyes
Retro-orbital	<50 µL	every other day for 1 week	alternating eyes
Mandibular [3]	150 - 300µL	once every 2-4 weeks	alternating sides
Mandibular [3]	<75 µL	4 x in 24 hr once a week	alternating sides
Mandibular [3]	10 - 14 µL	daily for 4 days	alternating sides
Jugular	150 - 300µL	once every 4 weeks per side	alternating sides
Saphenous lateral	150 - 300µL	once every 2-4 weeks	alternating hind legs
Saphenous lateral	<75 µL	once a week up to 8 weeks	alternating hind legs
Tail stick	150 - 300µL	once every 2-4 weeks	1st stick at most caudal point working cranially for subsequent samples
Tail stick	<75 µL	once a week up to 8 weeks	1st stick at most caudal point working cranially for subsequent samples
Tail snip	<75 µL	4 x in 24 hr once a week	tail tip is removed <5 mm, scab removed for subsequent sampling
Tail snip	10 - 14 µL	daily for 4 days	tail tip is removed <5 mm, scab removed for subsequent sampling

* based on 10% body weight and must be calculated for individual animals

Rats

Route	Volume*	Frequency	Instructions
Retro-orbital	0.75 - 2.5 mL	once every 2-4 weeks	alternating eyes
Retro-orbital	0.5 mL	once a week up to 8 weeks	alternating eyes
Jugular	0.75 - 2.5 mL	once every 4 weeks / side	alternating sides
Saphenous medial	0.75 - 2.5 mL	once every 2-4 weeks	alternating hind legs
Saphenous medial	0.5 mL	once a week up to 8 weeks	alternating hind legs
Tail stick	0.75 - 2.5 mL	once every 2-4 weeks	1st stick at most caudal point working cranially for subsequent samples
Tail stick	0.5 mL	once a week up to 8 weeks	1st stick at most caudal point working cranially for subsequent samples
Tail stick	0.1 -0.25 mL	once every 24 hours up to 4 weeks	1st stick at most caudal point working cranially for subsequent samples
Tail snip	0.1 -0.25 mL	4 x in 24 hr once a week	tail tip is removed <8 mm, scab removed for subsequent sampling
Tail snip	0.1 -0.25 mL	daily for 4 days	tail tip is removed <8 mm, scab removed for subsequent sampling

* based on 10% body weight and must be calculated for individual animals

Precautions

Retro-orbital bleeds: Repeated retro-orbital sampling can result in damage to the optic nerve and reduction in blood supply to the eye. This can result in exophthalmia and blindness. It is best to avoid frequent sampling utilizing this route. Animals with this condition will be euthanized or the eye appropriately treated. Retro-orbital blood sampling should always be done with appropriate anesthesia.

Mandibular bleeds: Repeated sampling in the mouse can lead to scaring and hematoma formation. To avoid these problems in frequent sampling:

1. Alternate sides
2. Align the lancet with against the striations of the muscles in the animal's face so that when it enters the facial muscles it goes between the striations instead of across them. The muscles run fairly parallel to the bottom of the jaw line.
3. Apply firm pressure at the puncture site to ensure hemostasis and reduce hematoma formation.
4. If Blood comes out of the ear of your mouse, you made the puncture a little high and towards the ear. The sample is usable and the mouse will not suffer any negative effects.

Jugular bleeds: The jugular vein is not recommended for repeated sampling. Hemostasis must be assured prior to returning the animal to the cage.

Saphenous bleeds: The saphenous vein in mice is easiest to access on the lateral aspect of the leg. A tourniquet must be used to raise the vessel. In the rat access is on the medial surface at and below the knee. This site should not be used for large volume samples. The rat is likely to have hematoma formation if adequate digital pressure is not applied to the puncture site.

Tail stick: This is easiest accomplished in the rat. A needle can be inserted into the vein or a puncture made.

Tail snip: This is a useful technique for frequent sampling of small quantities of blood but snips should not remove more than a total of 5 mm of the original tail length in a mouse and less than 8 mm in a rat.

1. McGuill MW, Rowan AN. 1989. Biological effects of blood loss: implications for sampling volumes and techniques. *ILAR News* 31:5-202.
National Institutes of Health – Office of Animal Care and Use, Guidelines for the Survival Bleeding of Mice and Rats, 9/3/2008.
2. http://oacu.od.nih.gov/ARAC/Bleeding.pdf
3. Golden Rod Lancets, Submandibular bleeding techniques, Frequently Asked Questions,
http://www.medipoint.com/html/howoftenfrequent_questions.html