

HACKENSACK UNIVERSITY MEDICAL CENTER
Research Department Policy Manual

Name: Policy on the Frequency of Blood
Sampling in Rodents

Policy # IACUC 125

Effective Date: 3/2013

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GENERAL

Purpose: To set up guidelines for safe withdrawal/sampling of circulating blood from laboratory animals that take into account the species specific blood volume to body weight ratio measured in milliliters of blood to kilogram of body weight. This policy is to be used as a quick reference guide to highlight general principles and considerations with regard to blood sampling in laboratory animals in the Department of Biological Resources. The guidelines are designed to ensure that any pain, distress or discomfort is kept to a minimum. . Minimization of such adverse effects is important for scientific as well as ethical and legal reasons, since they can cause biological changes which may affect the blood sample, and hence the validity of the research results and the number of animals used to achieve the scientific objective.

Policy: To adhere to the guidelines set forth by this policy which is to protect and promote the welfare of all animals. These guidelines are for naïve, normal, healthy animals. Please note that animals that are young, aged, stressed, have undergone experimental manipulations, or are suffering from cardiac or respiratory disease may not tolerate this amount of blood loss.

Protocols that exceed the volumes referenced in this policy must have prior approval by The Institutional Animal Care and Use Committee (IACUC). The investigator must provide scientific justification in the animal protocol as to the need to exceed the volumes and will have to provide a plan for fluid therapy.

Administration: The IACUC is responsible for the review and revision of this policy

PROCEDURE

The animal will need to be physically restrained to prevent any movement that would result in the laceration of the blood vessel or other potentially serious complications. Blood may be collected from awake animals appropriately restrained provided the person performing the procedure is skillful. Physical restraint can be done using arms and hands or with an approved restraining device(s). Sedation may be required for some animals or particular routes chosen for bleeding. Certain chemical combinations may also make peripheral blood vessels easier to utilize. Anesthesia is required if blood collection is being performed either via the retro-orbital sinus

or by cardiac puncture due to the distress and pain which can be caused and for the serious complications associated with these routes.

Things to be aware of when planning blood collection include:

- Amount of blood being collected
- Appropriate restraint of animal
- Route of blood withdraw

Blood collection volumes (general guidelines):

- 1 percent of body weight = maximum volume per collection every two weeks.
- 3 percent of body weight = amount expected from exsanguinations.
- 5-10 percent of body weight = total blood volume.

Single blood draw: A maximum of 1% of the animal's body weight may be removed as a single blood draw. For example:

- 0.15 ml from a 15 gram mouse
- 50 ml from a 5 kg cat
- 400 ml from a 40 kg dog.

Approximately 14 days are needed for the average healthy adult animal to completely recover from this blood loss. Although the blood volume is restored within 24 hours, two weeks are needed for all blood constituents to return to normal. As a rule, an animal will replace blood constituents at a rate of 1 ml/kg/day.

Multiple Blood Draws: If blood must be drawn more frequently than once every two weeks, 0.5% of the animal's body weight may be removed each week. This volume may be divided into several draws.

In mice, collection via medial saphenous vein is preferable to orbital sinus bleeding. Collecting blood by lacerating ear or tail vessels is prohibited in all species. These procedures are more painful than needle puncture and there is greater risk of lacerating an artery with subsequent hemorrhage.

Regardless of the method of collection used, an animal may not be returned to its cage until complete homeostasis at the collection site has been achieved. Homeostasis may be achieved using gauze and direct pressure. Up to several minutes of pressure may be required following arterial puncture. Safe guidelines for blood

sample volumes for laboratory animals and domestic species are given in the attached table.

REFERENCES

National Institutes of Health, Office of Animal Care and Use, Animal Research Advisory Committee. (9/12/12). Guidelines for the Survival Bleeding of Mice and Rats. Retrieved from http://oacu.od.nih.gov/ARAC/documents/Rodent_Bleeding.pdf

Diehl K.H., et al. "A Good Practice Guide to the Administration of Substances and Removal of Blood, including Routes and Volumes." J Appl Toxicol (2001) 21:15-23

Wolfensohn, Sarah & Lloyd, Maggie. Handbook of Laboratory Animal Management and Welfare, 3rd Edition. Blackwell Publishing Ltd, 2003.

Guidelines for Recommended Volumes

Species	Reference weight	Typical Sites of Collection ⁽¹⁾	Blood volume Average (ml/kg) ⁽²⁾	Circulating Blood Volume (CBV) (mls)	Safe volume at 10% CBV for single bleed (ml) ⁽³⁾	Safe volume at 1% CBV for multiple bleed (ml) ⁽⁴⁾	Bleed out volume (ml)
Mouse	18 – 40 grams	cardiac(*), orbital sinus (anesthetized required), tail vein, saphenous vein	72 - 80	Male 1.5 - 2.4 Female 1.0 - 2.4	0.1 - 0.2	0.01 -0.02	Male 0.8 - 1.4 Female 0.6 - 1.4
Rat	250 – 500 grams	as with mouse, plus jugular (anesthesia required), tail veins and artery, dorsal metatarsal vein.	50 - 70	Male 29 - 33 Female 16 - 19	Male 2.9 - 3.3 Female 1.6 - 1.9	Male 0.29 - 0.33 Female 0.16 - 0.19	Male 13 - 15 Female 7.5 - 9
Hamster	85-150 grams		78	Male 6.3 - 9.7 Female 7.1 - 11.2	Male 0.6 - 0.9 Female 0.7 - 1.1	Male 0.06 – 0.09 Female 0.07 – 0.11	Male 2.9 - 4.5 Female 3.3 - 5.2
Gerbil	55 – 100 grams		66 - 78	Male 4.5 - 7 Female 3.8 - 6	Male 0.4 - 0.7 Female 0.4 - 0.6	Male 0.04 – 0.07 Female 0.04 – 0.06	Male 2.2 - 3.5 Female 1.9 - 2.9
Guinea pig	700 – 1200 grams	Cardiac (*), jugular vein (anesthesia required), lateral saphenous.	69 - 75	Male 59 - 84 Female 48 - 63	Male 6 - 8 Female 5 - 6	Male 0.6 – 0.8 Female 0.5 – 0.6	Male 29 - 42 Female 24 - 31
Rabbit	1000 – 6000 grams	cardiac(*), marginal ear vein, jugular (no anesthesia required).	44 - 70	58.5 - 585	5 - 50	0.5 – 5.0	31 - 310
Dog (beagle)	-	Cephalic, saphenous veins, femoral and jugular veins	85	900 - 1170	90 - 110	9.0 – 11.0	-
Cat	-	Cephalic, saphenous veins, femoral and jugular veins	47 - 65	140 - 200	14 - 20	1.4 – 2.0	-
Pig	-	jugular vein, anterior vena cava, ear veins	65	13,200 - 15,000	1320 - 1500	132 - 150	-

* Cardiac puncture for blood collection must be performed as a terminal event

¹ Other routes can be approved by IACUC

² A blood volume estimate for a single species may not reflect differences among individual breeds or variations due to age, size, or illness

³ Single bleed of 10% total blood volume every two to four weeks.

⁴ Multiple bleeds at shorter intervals, a maximum of 1.0% of an animal's total blood volume can be removed every 24 hours; the effects of stress, site chosen and anesthetic used, must be carefully considered.