



Indiana University

Office of the Vice President for Research
Office of Research Compliance

Institutional Animal Care and Use Committee (IACUC) Office of Research Compliance (ORC)

IACUC Policy for Blood Collection in Laboratory Animals

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Responsible University Office:
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Policy Owner:
Bloomington Institutional Animal Care and Use Committee
(BIACUC)

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Policy Statement

This document provides information on blood collection methods for common laboratory animals. All procedures must be approved by the Institutional Animal Care and Use Committee (IACUC). The method of blood collection to be used, the intervals between blood collection procedures, and the volume of blood to be removed **must** be listed in the approved protocol specific to each study. Contact the LAR veterinary staff (ext. 5-2356) or lar@iu.edu if training is needed on specific blood collection methods and techniques.

Procedures

The LAR veterinary staff provides the following criteria to determine the maximum safe amount of blood to be withdrawn. It is recommended that no more blood is withdrawn than is absolutely necessary. Remember to calculate beforehand the minimum amount of blood necessary to perform all tests and assays, and that the serum fraction is about ½ of the total blood volume. When calculating blood volumes based on body weights (see below), remember that body weights in kilograms (kg) will convert to blood volumes in liters, and weights in grams (g) will convert to volumes in milliliters (ml).

Approximate Blood Volume

- 5-10% of body weight= total blood volume
 - The circulating blood volume can generally be estimated as **55-70 ml/kg** of total body weight. However, care should be taken in these calculations as the % of total blood volume will be lower (-15%) in obese and older animals.
 - See table at end of this document for some specific blood volumes.

Blood Collection Volumes

- **1% of body weight = maximum volume withdrawn over 14 days, without requiring supplemental fluids.** This applies for total blood collections as well as repeated collections. For irregular sampling schedules, calculate the total amount needed over a 14 day span.
- 0.07% of body weight = amount that can be taken daily without requiring supplemental fluids.
- 4-5% of body weight = amount expected at exsanguination.

Single Blood Draw

Maximum of 1% of body weight can be removed as a single blood draw every 14 days, without requiring administration of supplemental replacement fluids. Withdrawing the minimum amount of blood necessary is strongly recommended. Examples:

- 0.15 ml from a 15 g mouse
- 3 ml from a 300 g rat
- 30 ml from a 3 kg rabbit

Multiple Blood Draws

- If the total volume withdrawn over a **14 day** period is **less than 1% BW**, than no additional action needs to be taken.
- If the total volume withdrawn over a **14 day** period is **up to 2% BW, fluid volume replacement must be considered.** Withdrawing the minimum amount of blood necessary is strongly recommended. Examples:
 - Up to 0.15 ml withdrawn from a 15 g mouse over 2 weeks is OK
 - Up to 0.3 ml withdrawn from a 15 g mouse over 2 weeks, replace volume with 0.3 ml saline SC
 - Up to 3 ml from a 300 g rat over 2 weeks is OK
 - Up to 6 ml from a 300 g rat over 2 weeks, replace volume with 6 ml saline SC
 - Up to 30 ml from a 3 kg rabbit over 2 weeks is OK
 - Up to 60 ml from a 3 kg rabbit over 2 weeks, replace volume with 60 ml saline SC or IV
 - As a helpful guideline, daily blood draws under 0.07% BW will keep the total 2-week withdrawal under 1% BW.

Exsanguination

Approximately 50-75% of total blood volume (4-5% BW) can be obtained by terminal exsanguination. The animal must be deeply anesthetized, or recently euthanized, prior to exsanguination. Since the amount of blood obtained is substantially increased if the heart is

beating during the bleeding procedure, use of a surgical plane of anesthesia is required. The procedure for anesthesia and/or euthanasia must be described fully in the approved IACUC protocol. Examples:

- 0.6-0.75 ml from a 15 g mouse
- 12-15 ml from a 300 g rat
- 120-150 ml from a 3 kg rabbit

Fluid Replacement

If the volume of blood removed from an animal exceeds the maximum recommended blood collection volumes (i.e. >1% BW every 14 days), replacement of the removed volume of blood with warm (30-35°C) isotonic solution (e.g. 0.9% saline (normal, physiologic), lactated Ringer's solution) constitutes accepted veterinary practice. When this volume of blood is harvested, it should be withdrawn at a slow, steady rate, and the volume of solution to be infused should be administered similarly.

Monitoring

If too much blood is withdrawn too rapidly or too frequently without replacement (approximately 2% of the animal's body weight at one time), the animal may experience hypovolemic shock. If signs of shock are observed, contact LAR veterinary staff immediately. Signs of shock include:

- Fast and thready pulse
 - Pale dry mucous membranes
 - Cold skin and extremities
 - Restlessness
 - Hyperventilation
 - Sub-normal body temperature
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- If 15-20% of total blood volume is removed, cardiac output and blood pressure will be reduced.
 - If 30-40% of total blood volume is removed, death will result in at least 50% of animals.
 - If >40% of total blood volume is removed, death of the animal is expected.

Stressed, sick, or otherwise compromised animals may not tolerate the blood collection criteria noted above, which is for healthy animals.

By monitoring hematocrit (HCT or PCV) and/or hemoglobin (Hb) it is possible to evaluate if the animal has sufficiently recovered from single or multiple blood draws. Remember it may take up to 24 hours for hematocrit or hemoglobin to reflect a sudden or acute blood loss. In general, if the animal is anemic (below the normal PCV range for the species), or if the hemoglobin concentration is less than 10 g/dL, it is not safe to remove the volumes of blood listed above.

Blood Collection Sites and Methods

The following are frequently used sites for survival blood sampling from common laboratory animal species. Based on the requirements of the study, certain sites are preferable (*constitutes a preferred method in the table). Also, publications have indicated that the results from blood analysis (especially cellular indices) may vary based on the site of blood withdrawal; consult the literature for more information.

Species	Site	General Anesthesia?	Repeat bleeds (daily basis)	Expected volume
Mouse	Lateral tail vein	No	Yes	++(+)
	*Saphenous vein	No	Yes	++
	Distal tail transection (1-3 mm)	Required	Limited	+
	*Submandibular	No	Yes	++
	Jugular vein	Recommended	Yes	+++
	Retroorbital sinus [@]	Required (must justify if not used)	Yes	+++
Rat	*Saphenous vein	No	Yes	++(+)
	*Lateral tail vein	No	Yes	++(+)
	Jugular vein	Required	Yes	+++
	Sublingual vein	Required	Yes	+++
	Retrobulbar plexus [@]	Required (must justify if not used)	Yes	+++
	Penile vein	Required	Yes	+
Guinea Pig	Jugular vein	Recommended	Yes	+++
	*Saphenous vein	No	Yes	++(+)
	Nail bed	Recommended	No	+
	Marginal ear vein	No	Yes	+
	Cranial vena cava	Required	Yes	+++
	Penile vein	Required	Yes	+
Hamster [#]	Retro-orbital plexus [@]	Required	No	+++
	Jugular vein	Recommended	Yes	++
	Femoral vein	Recommended	Yes	+
	Saphenous vein	Recommended	Yes	++
Rabbit	Jugular vein	Recommended	Yes	+++
	*Marginal ear vein/central ear artery	No (local anesthesia recommended)	Yes	++
Bird	*Brachial wing vein	No	Yes	++
	Right jugular vein	Recommended	Yes	++
Fish	*Caudal vein	Recommended		

[#]Sublingual or gingival puncture can also be considered for hamsters (see references).

[@]Recommended for only single sampling from each eye.

+ Indicates quantity of blood collected with + being small quantity and +++ being large quantity.

Circulating blood volumes in common lab animal species (Heinz-Diehl, 2001)

Species	Mean blood volume (ml/kg)	Range of mean blood volume (ml/kg)
Mouse	79	63-80
Rat	64	58-70
Rabbit	56	44-70
Hamster	78	No information available

Table 2. Recommended maximum safe blood volumes collected over a two-week period.

Species	Body Weight	Total Circulating blood volume (ml)	10% blood volume (ml) ideal	15% blood volume (ml)	20% blood volume (ml) need fluid replacement
Mouse	15 g	0.95-1.20	0.10-0.12	0.14-0.18	0.19-0.24
	20 g	1.26-1.60	0.13-0.16	0.19-0.24	0.25-0.32
	25 g	1.58-2.00	0.16-0.20	0.24-0.30	0.32-0.40
	30 g	1.89-2.40	0.19-0.24	0.28-0.36	0.38-0.48
	35 g	2.21-2.80	0.22-0.28	0.33-0.42	0.44-0.56
	40 g	2.52-3.20	0.25-0.32	0.38-0.48	0.50-0.64
Rat	50 g	2.50-3.50	0.25-0.35	0.38-0.53	0.50-0.70
	100 g	5.80-7.00	0.58-0.70	0.87-1.05	1.16-1.40
	150 g	8.70-10.50	0.87-1.05	1.31-1.58	1.74-2.1
	200 g	11.6-14.0	1.16-1.4	1.74-2.1	2.32-2.8
	250 g	14.5-17.5	1.45-1.75	2.18-2.63	2.9-3.5
	300 g	17.4-21.0	1.74-2.1	2.61-3.15	3.48-4.2
	350 g	20.3-24.5	2.03-2.45	3.04-3.68	4.06-4.9
	400 g	23.2-28.0	2.32-2.8	3.48-4.2	4.64-5.6
	450 g	26.1-31.5	2.61-3.15	3.92-4.73	5.22-6.3
500 g	29.0-35.0	2.9-3.5	4.35-5.25	5.8-7.0	
Hamster	10 g	0.78	0.08	0.12	0.16
	15 g	1.17	0.12	0.18	0.23
	20 g	1.56	0.16	0.23	0.31
	25 g	1.95	0.20	0.29	0.39
	30 g	2.34	0.23	0.35	0.47
	35 g	2.73	0.27	0.41	0.55
	40 g	3.12	0.31	0.47	0.62
	45 g	3.51	0.35	0.53	0.70
	50 g	3.9	0.39	0.59	0.78

If up to 20% of the blood volume is withdrawn over a 2 week period of time, it is recommended to **replace the volume of blood withdrawn with an equal volume of sterile isotonic solution such as Lactated Ringers solution.**

Sanctions

Failure to comply with IACUC policies may result in noncompliance reports to the Institutional Official, the Office of Laboratory Animal Welfare (OLAW), the U. S. Department of Agriculture (USDA), and/or the suspension of animal use privileges. In addition, the availability of sponsored research funds may be affected when an investigator is found to be in violation of these policies.

Contacts

Subject	Contact	Phone	Email
Veterinary Concerns	LAR Veterinarians	855-2356	lar@indiana.edu
Policy	IACUC Administrator	855-5138	buiacuc@indiana.edu

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