

<b>Title: Blood Collection Guidelines, IACUC Administrative Policy P-0014-01</b>
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<b>Authorized by: Institutional Animal Care and Use Committee</b>
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## I. Purpose

- A.** This document is designed to provide general guidelines on blood collection methods for common laboratory animals. All procedures must be approved by the Institutional Animal Care and Use Committee (IACUC), however a protocol may reference these guidelines and describe in detail any deviations from them. For training on specific blood collection methods and techniques, please contact: [ANIMALCARE@cmich.edu](mailto:ANIMALCARE@cmich.edu)
- B.** It is recommended to take no more blood 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 will convert to volumes in milliliters (ml).

## II. Procedures

### A. Approximate Blood Volume

- i. 5-7% (mean of 6%) of body weight = **total** blood volume
  1. 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 sick, obese and older animals.
  2. See Table 2 at end of document for some specific blood volumes.

### B. Blood Collection Volumes

- i. 1% of body weight = maximum volume per collection **every 14 days**, without requiring supplemental fluids. This applies for single blood collections as well as repeated collections. For irregular sampling schedules, calculate the total amount needed over a 14 day span.
- ii. 0.07% of body weight = amount that can be taken **daily** without requiring supplemental fluids
- iii. 3% of body weight = amount expected at **exsanguinations** (approximately 9ml from a 300g rat).

### C. Single Blood Draw

- i. 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:
  1. 0.15 ml from a 15 g mouse
  2. 3 ml from a 300 g rat

#### D. Multiple Blood Draws

- i. If the total volume withdrawn over a 14-day period is less than 1% BW, then no additional action needs to be taken.
- ii. If the total volume withdrawn over a 14 day period will be 2% BW (or over), fluid volume replacement is necessary. Withdrawing the minimum amount of blood necessary is strongly recommended. Examples:
  1. Up to 0.15 ml withdrawn from a 15g mouse over 2 weeks is OK
  2. Up to 0.3 ml withdrawn from a 15g mouse over 2 weeks, replace volume with 0.3 ml saline SQ
  3. Up to 3 ml from a 300g rat over 2 weeks is OK
  4. Up to 6ml from a 300g rat over 2 weeks, replace volume with 6 ml saline SQ
  5. As a helpful guideline, daily blood draws under 0.07% BW will keep the total 2-week withdrawal under 1% BW.

#### E. Exsanguination/Terminal Blood Collection

- i. Approximately 50-75% of total blood volume (3% mean, 4-5% maximum of body weight) 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 recommended. The procedure for anesthesia and/or euthanasia must be described fully in the approved IACUC protocol. Example maximums:
  1. 0.60-0.75 ml from a 15g mouse
  2. 12-15 ml from a 300g rat

#### F. Fluid Replacement

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

similarly. Contact [ANIMALCARE@cmich.edu](mailto:ANIMALCARE@cmich.edu) for training on specific methods. Recommended route(s) of administration: 50% of volume each delivered IV and SubQ or IP.

#### G. Monitoring

- i. If too much blood is withdrawn too rapidly or too frequently without replacement, the animal may go into hypovolemic shock. If signs of shock are observed, contact the attending veterinarian immediately. Signs of shock include:
  1. Fast and thready pulse
  2. Pale dry mucous membranes
  3. Cold skin and extremities
  4. Restlessness
  5. Hyperventilation
  6. Sub-normal body temperature
- ii. If 15-20% of total blood volume is removed, cardiac output and blood pressure will be reduced.
- iii. If 30-40% of total blood volume is removed, death will result in at least 50% of animals.
- iv. If > 40% of total blood volume is removed, death of the animal is expected.
- v. Stressed, sick, or otherwise compromised animals may not tolerate the blood collection criteria noted above, which is for healthy animals.
- vi. 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.

#### H. Blood Collection Sites and Methods

- i. Table 1 lists the blood collection sites for common laboratory animal species. They are listed from most common/desirable to least common/desirable based on ease of collection from the site. For uncommon laboratory animal species, please contact the attending veterinarian Dr. Robert Werner at [werne2r@cmich.edu](mailto:werne2r@cmich.edu) or [ANIMALCARE@cmich.edu](mailto:ANIMALCARE@cmich.edu). For smaller species, the volume of blood attainable for each site is listed; however, the volumes are estimation and will also depend on the size, health, and hydration status of the animal as well as the experience and skill level of the person collecting the sample. Based on the goals and requirements of the study, certain sites may be preferable. Additionally, publications have indicated that

the results from blood analysis (especially cellular indices) may vary based on the site of blood collection; consult the literature for more information. In all cases, cardiac puncture may be used to obtain a single, large volume of blood from heavily anesthetized (terminal procedure only) or euthanized animal

**Table 1 – Frequently Used Sites For Blood Draws for Common Lab Animal Species**

Species	Site	Anesthesia	Repeat Bleeds*	Expected Volume
Mouse	Lateral tail vein	No	Yes	50-100 µl
	Saphenous vein	No	Yes	100-200 µl
	Submandibular vein	No	Yes	200-500 µl
	(discouraged) Distal tail transect./lancing (1mm)	Required**	Yes- limited	50-100 µl
	(discouraged) Retro-orbital sinus	Required	Yes- limited	200 µl
	Sublingual vein***	Required	Yes	500 µl
	Jugular vein	Recommended	Yes	
	Cardiac puncture (terminal only)	Required	Terminal	~ 1 ml
	Dorsal metatarsal/Pedal	No	Yes	< 100 µl
	Rat	Saphenous vein	No	Yes
Lateral tail vein***		No	Yes	200-400 µl
Dorsal metatarsal/Pedal		No	Yes	100-200 µl
Submandibular/Facial vein		No	Yes	200-500 µl
Jugular vein		Recommended	Yes	0.5- 2.0 ml
Sublingual vein***		Required	Yes	0.5-1.0 ml
(discouraged) Retro-orbital plexus		Required	Yes-limited	0.5-1.0 ml
Cardiac puncture (terminal only)		Required	Terminal	~3 ml
Ventral tail		Recommended	yes	100 µl
tail artery		Required	yes	0.5-1.0ml
Hamster	Lateral tarsal vein	No	Yes	100-200 µl
	Toenail clipping	No	Yes	100-200 µl
	Retro-orbital sinus	Required	Yes- limited	100-200 µl
	Jugular vein	Required	Yes	0.5- 2.0 ml
	Cardiac puncture (terminal only)	Required	Terminal	~3 ml
	Gerbil	Lateral tail vein	No	Yes
Toenail clipping		No	Yes	100-200 µl
Distal tail transection (1-3 mm)		Required	Yes (1-2 times only)	100-200 µl
Retro-orbital sinus		Required	Yes- limited	100-200 µl
Jugular vein		Required	Yes	0.5- 2.0 ml
Cardiac puncture (terminal only)		Required	Terminal	~3 ml
Guinea pig		Auricular vein	No	Yes
	Cephalic vein	No	Yes	50-100 µl
	Saphenous vein	No	Yes	400-500 µl
	Jugular vein	Recommended	Yes	2-3 ml
	Cranial vena cava	Recommended	Yes	2-3 ml
	Cardiac puncture (terminal only)	Required	Terminal	
	Rabbit	Marginal ear vein/central ear artery	Local anesthesia recommended	Yes
Lateral saphenous vein		No	Yes	
Cephalic vein		No	Yes	
Jugular vein		Recommended	Yes	
Cardiac puncture (terminal only)		Required	Terminal	

\*Please refer to section D above for details. \*\*Distal tail transection in adult mice requires the use of general anesthesia unless scientifically justified and approved in the IACUC protocol. \*\*\*Anesthesia is recommended for sublingual blood collection. \*\*\*\*Contact [ANIMALCARE@cmich.edu](mailto:ANIMALCARE@cmich.edu) for training.

Table 2 – Circulating blood volumes in common lab animal species (adopted from Heinz-Diehl, 2001 and Hawk et al. 2005)

Species	Mean blood volume (ml/g)	Range of mean blood volume (ml/g)
Mouse	.072	.063-.080
Rat	.064	.058-.070
Hamster	.078	
Gerbil	.067	
Guinea pig	.075	.067-.092
Rabbit	.056	.044-.070

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