

 University of Michigan Medical School	<h1 style="margin: 0;"><u>ULAM</u></h1> <p>Unit for Laboratory Animal Medicine</p>	Policy and Guidelines
Title: Blood Collection Guidelines		
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This document is designed to provide general information on blood collection methods for common laboratory animals. All procedures must be approved by the University Committee on the Use and Care of Animals (UCUCA). 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. For training on specific blood collection methods and techniques, please contact the UCUCA Office at 3-8028.

The ULAM veterinary staff provides the following criteria to determine the maximum safe amount of blood to be withdrawn. 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 (kgs) will convert to blood volumes in liters, and weights in grams will convert to volumes in milliliters (mls).

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 document for some specific blood volumes.

Blood Collection Volumes

- 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.
- 0.07% of body weight = amount that can be taken **daily** without requiring supplemental fluids
- 4-5% of body weight = amount expected at **exsanguinations**

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 gm mouse
 - 3 ml from a 300 gm rat
 - 50 ml from a 5 kg cat
 - 100 ml from a 10 kg monkey
 - 400 ml from a 40 kg dog

Multiple Blood Draws

- If the total volume withdrawn over a 14-day period is less than 1% BW, then no additional action needs to be taken.
- If the total volume withdrawn over a 14 day period is up to 2% BW (or over), 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 SQ
- Up to 3 ml from a 300 gm rat over 2 weeks is OK
- Up to 6ml from a 300 gm rat over 2 weeks, replace volume with 6 ml saline SQ
- Up to 200 ml from a 20 kg dog over 2 weeks is OK
- Up to 400 ml from a 20 kg dog over 2 weeks, replace volume with 400 ml saline SQ 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% 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 UCUCA protocol. Examples:

- 0.60-0.75 ml from a 15 gm mouse
- 12-15 ml from a 300 gm rat
- 200-250 ml from a 5 kg cat
- 400-500 ml from a 10 kg monkey
- 1600-2000 ml from a 40 kg dog

Fluid Replacement

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) 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 go into hypovolemic shock. If signs of shock are observed, contact the ULAM 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
- 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 gm/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]. 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 transaction (1-3 mm)	Required	Limited	+
	* Tail incision (small cut)	No	Yes	+
	* 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	+
GERBIL and HAMSTER	Jugular vein	Required	Yes	++(+)
	Lateral tarsal vein	No	Yes	+
	Caudal vena cava	Required	No	++
	Ear vein	No	Yes	+
	Retroorbital Sinus	Required (must justify if not used)	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	+
FERRET	* Jugular vein	Recommended	Yes	+++
	Lateral saphenous vein	No	No	+
	Caudal tail artery	Required	Yes	++
	Cephalic vein	No	Yes	++
RABBIT	Jugular vein	Recommended	Yes	+++
	* Marginal ear vein / central ear artery	No (but local anesthesia recc.)	Yes	++
CAT	* Jugular vein	No	Yes	+++
	Medial saphenous vein	No	Yes	++
	* Cephalic vein	No	Yes	++
	Dorsal pedal vein	No	No	+
DOG	* Jugular vein	No	Yes	+++
	* Lateral saphenous vein	No	Yes	++
	* Cephalic vein	No	Yes	++
	Dorsal pedal vein	No	Yes	+
SHEEP	* Jugular vein	No	Yes	+++
	Cephalic vein	No	Yes	+++
PIG	Marginal ear vein	No	Yes	++
	Cephalic vein	No	Yes	++
	Right jugular vein	Recommended	Yes	++
	* Anterior vena cava	Recommended	Yes	++
	Mammary vein	Recommended	Yes	++
	For blood collection in pigs, training and/or restraint can be used rather than anesthesia			
NONHUMAN PRIMATE	* Femoral vein	Required	Yes	+++
	Cephalic vein	Required	Yes	++
	Saphenous vein	Required	Yes	++
	Jugular vein	Required	No	++
	Brachial vein	Required	No	++
	For blood collection in nonhuman primates, training and/or restraint can be used rather than anesthesia			

BIRD	* Brachial wing vein	No	Yes	++
	Right jugular vein	Recommended	Yes	++
REPTILES and AMPHIBIANS	Consult ULAM veterinary staff for blood collection suggestions for individual species			
FISH	* Caudal vein	Recommended		

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	72	63-80
Rat	64	58-70
Rabbit	56	44-70
Dog (beagle)	85	79-90
Macaque (rhesus)	56	44-67
Macaque (cynomolgus)	65	55-75
Marmoset	70	58-82
Minipig	65	61-68

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