Blood Collection Guideline

1. Purpose

The purpose of this guideline is to provide recommended blood sampling volumes and guidance on a variety of acceptable blood collection techniques in rodents and bats.

1. Scope

This guideline applies to all personnel collecting blood samples from laboratory rodents and bats.

1. Guidance
	1. General Information
		1. Factors to consider when selecting the appropriate blood collection technique for research purposes include, but are not limited to:
			1. The species to be bled
			2. The size and age of the animal to be bled and the estimated total blood volume
			3. The type of the sample required (e.g., serum, whole blood cells, etc.)
			4. The quality of the sample required (sterility, tissue fluid contamination, etc.)
			5. The quantity of blood required (considering extraneous blood loss due to a selected method)
			6. The frequency of sampling
			7. The health status of the animal being bled
			8. The training and experience of the phlebotomist
			9. The size and type of capillary tube is appropriate
			10. The effect of the site, restraint, or anesthesia on the blood parameter measured.
		2. The acceptable quantity and frequency of blood sampling is dependent on the circulating blood volume of the animal and the red blood cell (RBC) turnover rate. The approximate circulating blood volume of adult animals varies with species and body weight. For purposes of calculating the maximum blood volume that may be sampled, the following reference mean total blood volume (TBV) values may be used:
			1. Mouse 72 ml/kg
			2. Rat 64 ml/kg
			3. Hamster 78 ml/kg
			4. Guinea pig 75 ml/kg
			5. Bats 95 ml/kg
		3. Approximately 10% of the total blood volume can be safely removed every 2 to 4 weeks, 7.5% every 7 days, and 1%

every 24 hours.

* + 1. The guidance provided below is for healthy adult animals. Animals that are young, aged, stressed, have cardiac or respiratory disease, or are otherwise compromised may not tolerate recommended amounts of blood removal.
		2. If the experimental design requires blood volumes and/or frequency of collection that fall outside the recommendations within this guideline, consultation with the AV and justification in the IACUC protocol is required.

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| **Table 1: Calculated Blood Sample Volumes for Species and Range of Body Weights** |
| **Species** | **Body weight (g)** | **\*CBV (ml)** | **~1% CBV *every 24 hrs.†*** | **~7.5% CBV *every 7 days†*** | **~10% CBV *every 2 - 4wks†*** |
| Mouse | 20 | 1.10 - 1.40 | 11 - 14 µl | 90 - 105 µl | 110 - 140 µl |
|  | 25 | 1.37 - 1.75 | 14 - 18 µl | 102 - 131 µl | 140 - 180 µl |
|  | 30 | 1.65 - 2.10 | 17 - 21 µl | 124 - 158 µl | 170 - 210 µl |
|  | 35 | 1.93 - 2.45 | 19 - 25 µl | 145 - 184 µl | 190 - 250 µl |
|  | 40 | 2.20 - 2.80 | 22 - 28 µl | 165 - 210 µl | 220 - 280 µl |
| Rat | 125 | 6.88 - 8.75 | 69 - 88 µl | 516 - 656µl | 690 - 880 µl |
|  | 150 | 8.25 - 10.50 | 82 - 105 µl | 619 - 788 µl | 820 - 1000 µl |
|  | 200 | 11.00 - 14.00 | 110 - 140 µl | 825 – 1050 µl | 1.1 - 1.4 ml |
|  | 250 | 13.75 - 17.50 | 138 - 175 µl | 1.0 – 1.3 ml | 1.4 - 1.8 ml |
|  | 300 | 16.50 - 21.00 | 165 - 210 µl | 1.2 – 1.6 ml | 1.7 - 2.1 ml |
|  | 350 | 19.25 - 24.50 | 193 - 245 µl | 1.4 – 1.8 ml | 1.9 - 2.5 ml |
| Bat | 40 | 3.60- 4.40 | 36 – 44 ul | 270 – 330 ul | 360 – 440 ul |
|  | 50 | 4.5-5.5 | 45 – 55 ul | 338 – 413 ul | 450 – 550 ul |
|  | 60 | 5.4-6.6 | 54 – 66 ul | 405 – 495 ul | 540 – 660 ul |
|  | \*Circulating blood volume (1ml = 1000µl) | †Maximum sample volume for that sampling frequency |

* 1. Collection site requirement and advantages / disadvantages:

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| **Collection Sites** | **Species** |  **ADVANTAGES** |  **DISADVANTAGES** |
| Submandibular Sampling  | Rodent  | * Preferred blood collection method
* Maximum allowable sample volume with minimal trauma.
 | * Anesthesia recommended
* Must be securely restrained
* Yields a large sample so should not be used for frequent small sampling
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| Tail Nick or Tail Vein Sampling  | Rodent  | * Anesthesia not required
* Multiple samples can be taken
* Vein is easily accessed
 | * Must be securely restrained
* Yields only small quantities
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| Sublingual Vein  | Rodent  | * Multiple samples can be taken
 | * Anesthesia required
* Must be securely restrained
* Yields a large sample so should not be used for frequent small sampling
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| Saphenous Sampling (medial or lateral approach)  | Rodent | * Excellent technique for serial blood sampling
* Moderate volume of blood can be collected
* Multiple samples can be taken by alternating sites
 | * Requires specialized training
* Specialized equipment required
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| Cardiac Puncture  | Bat Rodent | * Maximum volume of blood can be collected
 | * Requires deep anesthesia.
* Non-survival procedure only
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| Retro-orbital Sinus  | Rodent  | * Yields a greater volume of blood
* For multiple sampling, see IACUC standard procedure
 | * Requires anesthesia
* Involves risk of injury to the eye and surrounding structures and therefore use is discouraged.
* Use must be justified in the protocol.
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| Wing Vein Sampling  | Bat | * Excellent technique for serial blood sampling
* Moderate volume of blood can be collected
 | * Anesthesia required
* Must be securely restrained
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