WVU IACUC Guidelines:
Blood Collection – Maximum Volumes and Fluid Replacement

Purpose and Background

This document describes standards for blood collection in laboratory animals. All procedures ***must*** be described in the approved animal use protocol – including: 1) the method of blood collection to be used, 2) the intervals between blood collection procedures, and 3) the volume of blood to be removed, specific to each study. If an investigator wishes to deviate from this SOP, all changes ***must*** be outlined and justified in the animal use protocol. Approval of the protocol indicates approval of the deviation from the SOP for that protocol only.

Blood collection ***must*** be performed in a manner that does not compromise the animal's health and wellbeing. Personnel performing blood collection ***must*** be proficient and their training documented, if applicable. Collection of excessive blood volume can lead to diminished blood pressure, shock, and death. As such, hemostasis ***must*** be ensured.

If there are any questions or concerns that arise, veterinary staff are available for consultation.

Guidelines

1. **Single Blood Draw**
   a. 10% of the animal's total blood volume is the best practice single sample that can be taken once every two weeks. (see Table 1)
   b. An animal's total blood volume can vary but is generally estimated as approximately 6-8% of body weight (Table 2).
      i. 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. **Multiple Blood Draws**
   a. 15% of the animal's total blood volume can be taken through multiple samplings over a 2-week period.
   b. If 20% blood volume is collected over a 2-week period through multiple samplings, double fluid replacement ***must*** be administered (see #4 below).

3. **Exsanguination**
   a. The animal ***must*** be anesthetized or euthanized prior to terminal blood collection.

4. **Fluid Replacement**
   a. When blood is withdrawn above the maximum amount (10% blood volume), fluid replacement ***must*** be implemented.

b. Two times the blood volume removed should be replaced with appropriate isotonic fluids. For example, if 0.27 mL of blood is collected from a 25g mouse (representing 15% of blood volume), then 0.5 mL fluids ***must*** be administered.

5. **Hypovolemic Shock**
   a. Single blood collection samples above 15% of total blood volume are NOT recommended due to potential for hypovolemic shock.
   b. Clinical signs include: fast and thread pulse, pale dry mucous membranes, cold skin and extremities, restlessness, hyperventilation, sub-normal body temperature.

Table 1: Blood collection % and associated recovery periods

Single Sample Collection		*Multiple Sample Collection*	
% circulatory blood volume removed	Approximate recovery period	% circulatory blood volume removed	Approximate recovery period
7.5%	1 week	7.5%	1 week
10%	2 weeks	10-15%	2 weeks
15%	4 weeks	20%	4 weeks

Table 2: Total blood and sample volumes for species with specific body weight (i.e. volume estimates are based on the example body weight)

Species	Mean blood volume (ml/kg)*	Example Animal Weight	Total blood volume (TBV)	7.5% of TBV	10% of TBV (max. single sample)	15% of TBV	20% of TBV
Mouse	72	25 g	1.8 mL	0.14 mL	0.18 mL	0.27 mL	0.36 mL
Rat	64	250 g	16 mL	1.2 mL	1.6 mL	2.4 mL	3.2 mL
Avian	100 (avg.)	500 g	50 mL	3.75 mL	5 mL	7.5 mL	10 mL
Rabbit	56	3 kg	168 mL	12.6 mL	16.8 mL	25.2 mL	33.6 mL
Swine	65	45 kg	2.93 L	220 mL	293 mL	440 mL	585 mL
Cattle	60	533 kg	32 L	2.4 L	3.2 L	4.8 L	6.4 L
Goat	66	70 kg	4.62 L	347 mL	462 mL	693 mL	924 mL
Poultry	60	3 kg	180 ml	13.5 mL	18 mL	27 mL	36 mL
Sheep	66	80 kg	5.28 L	396 mL	528 mL	792 mL	1056 mL

* For mature, healthy animals with an adequate plane of nutrition.

<u>Table 3: Recommended sites for blood collection by species</u>

Species	Site of Collection
Mouse	Lateral tail vein*, saphenous vein, facial vein (submandibular & submental veins), sublingual vein (anesthesia required), jugular vein (anesthesia recommended), cardiac puncture (terminal only)
Rat	Same as mouse; dorsal metatarsal vein
Avian	Jugular vein, medial metatarsal vein, basilic vein ("wing" vein), cardiac puncture (terminal only)
Rabbit	Marginal ear vein / central ear artery (local anesthesia recommended), lateral saphenous vein, cephalic vein, jugular vein (anesthesia recommended), cardiac puncture (terminal only)
Swine	Marginal ear vein, right jugular vein, anterior vena cava (anesthesia recommended)
Ruminants	Jugular vein, tail vein (cattle)

Based on the goals and requirements of the study and skill level of personnel, certain sites may be preferable. Additionally, publications have indicated that the results from blood analysis (especially cellular induces) may vary based on the site of blood withdrawal; 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 animals.

*For tail vein injection in mice and rats, warm water can be used to cause vasodilation. Warm tap water ***must*** be used (do not heat water in microwave). Water temperature ***must*** be taken before placing the animals' tail in the water. A temperature of 32-37 degrees C is recommended. Temperature ***must not*** exceed 37 degrees C.

<u>References</u>

1. BVA/FRAME/RSPCCA/UFAW Joint Working Group on Refinement. 1993. Removal of blood from laboratory mammals and birds (first report). Laboratory Animals; 27:1-22.
2. Diehl KH et al. A good practice guide to the administration of substances and removal of blood, including routes and volumes. J Appl. Toxicol. 21:15-23 (2001).
3. Lee HB, Blaufox MD. Blood volume in the rat. J Nucl Med. 26(1):72-6 (1985).
4. Wayne State University Blood Collection: Maximum Volumes and Fluid Replacement
5. University of Michigan Guidelines on Blood Collection
6. LafeberVet Venipuncture in Birds