Guidelines for Regulating the Volume of Experimental Blood Sample Withdrawals in Animals

Purpose

The purpose of this guidance document is to provide institutional standards for conducting phlebotomy in common laboratory animal species and will cover standardized circulating blood volume in milliliters per kilogram as well as recommended blood collection frequency and volume.

- Research protocol submissions to the Virginia Tech (VT) Institutional Animal Care and Use Committee (IACUC) that involve blood collection volumes and frequencies within the ranges specified in this document need only indicate that they are in compliance with the Guidelines for Regulating the Volume of Experimental Blood Sample Withdrawals in Laboratory Animals.
- Research protocol submissions to the IACUC that propose volumes or frequencies outside of the ranges recommended in this document must provide a detailed description of the procedures and means of fluid replacement, justification of their necessity, and history of safety in the protocol submission.
- It is the responsibility of principal investigators to ensure that co-investigators, students, and technicians drawing blood from experimental animals receive training and demonstrate competency in techniques proposed in the study before allowing them to perform the techniques unsupervised.
- See Guidelines for Injections in Rodents and Rabbits for recommended needle sizes. See species specific SOPs for recommendations regarding needle sizes.

Restraint

Appropriate instruction for all species can be obtained from the Office of the University Veterinarian, 540-231-1767 or 540-231-8144.

- Dogs, cats, cows, horses, and sheep generally require only physical restraint for blood collection.
- Swine may require only physical restraint if they have been trained. Chemical restraint may be necessary for some animals.
- Rabbits, mice, rats, and other rodents may be placed in appropriate restraining devices or undergo chemical restraint as needed.

Phlebotomy

Circulating blood volume (CBV) should be determined from known species specific volume to weight values and not calculated based on flat percentage of body weight. See *Table 1* for estimated circulating blood volumes for multiple species.

- A maximum survival bleed not exceeding 15% of CBV is allowable once every three weeks (i.e. plan for a three week recovery period between bleeds of this volume)
- A maximum survival bleed not exceeding 10% of CBV is allowable once every two weeks (i.e. plan for a two week recovery period between bleeds of this volume)
- Bleedings performed weekly should not exceed 7.5% of CBV (i.e. plan for a 7 day recovery period between bleeds of this volume)
- Animals being bled daily may have 1% of CBV taken (i.e. plan for a 24 hour rest period between bleeds of this volume)
- Exceptions to these numbers are possible with fluid replacement therapy

Example: *10kg dog will undergo a single blood draw once per day for 5 days (See Table 1 for Circulating Blood Volumes and examples for other species).*

Dog Estimated Circulating Blood Volume = 86ml/kg

10kg dog: CBV = 10kg x 86ml/kg = 860ml

Endorsed by the IACUC: December 18, 2013
Edited and Approved by the IACUC: December 16, 2015
Effective Date: December 18, 2013

Maximum daily withdrawal (without fluid supplementation) = 0.01 x 860ml = 8.6ml

Monitoring

Adhere to monitoring parameters indicated in each IACUC approved animal use protocol.

Terminal Blood Withdrawal

Terminal Bleeds are only allowed on animals under general anesthesia, and the animal's death must be verified at the end of the bleed. An alternative euthanasia method is recommended after the blood withdrawal.

Table 1. Circulating Blood Volumes and Blood Withdrawal Volume Examples for Various Animal Species.

Species (Weight)	Circulating Blood Volume (ml/kg)	Total Blood Volume (ml)	1% CBV (ml) Every 24 hrs	7.5% CBV (ml) Every 7 days	10% CBV (ml) Every 2 weeks	15% CBV (ml) Every 4 weeks
Mouse (25g)	79	1.9	0.02	0.14	0.19	0.29
Gerbil (70g)	67	4.7	0.05	0.35	0.47	0.71
Hamster (150g)	78	11.7	0.12	0.88	1.2	1.75
Rat (250g)	64	16	0.16	1.2	1.6	2.4
Guinea Pig (500g)	75	37.5	0.38	2.8	3.75	5.6
Ferret (1.5kg)	75	112.5	1.13	8.4	11.3	16.9
Rabbit (4kg)	56	224	2.2	16.8	22.4	33.6
Cat (4kg)	55	220	2.2	16.5	22	33
Dog (10kg)	86	860	8.6	64.5	86.0	129
Sheep (55kg)	60	3300	33	247.5	330	495
Pig (90kg)	65	5850	58	440	585	878
Goat (105kg)	70	7350	73	551	735	1103
Cow (600kg)	55	33,000	330	2475	3300	4950
Horse (500kg)	76	38,000	380	2850	3800	5700

Birds

- As with other species, the avian blood volume is approximately 6-8% of body weight.
- Healthy Passeriformes and Psittaciformes can lose 10% of the blood volume, or the equivalent of 1% of the body weight, without ill effects providing sufficient recovery time is allowed (Table 2).

Table 2. Birds and Other Non-Traditional Laboratory Animals Without Known CBV

Maximum blood draw (% body weight in grams = milliliters blood x 10)	Recovery time
1%	4 weeks
0.75%	2 weeks
0.5%	1 week
0.05%	Daily

Example: *25g songbird will undergo a single blood draw.*

Maximum withdrawal in a 4-week period of time:

25g x 1% = 0.25 grams

0.25 grams = 250 milligrams = 250 microliters

Frogs

- While the literature suggests a range of blood that can be safely removed in healthy frogs is 50% of the blood at one time (about 5% of the body mass), it is recommended that collection be limited to 60-80ml/kg body weight.

Fish

- The volume for repeated blood sampling of zebrafish at intervals should be ≤ 0.4% of body weight per week or ≤ 1% per 2 weeks (Zhang et al 2013).
- Use Non-traditional lab animal guidelines provided above for other fish species.

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