



Johns Hopkins University Animal Care and Use Committee

Multiple Blood Draws

PURPOSE: In many protocols, multiple blood draws are an important part of collecting and analyzing data. To help prevent problems associated with multiple blood collections, guidelines have been developed regarding the amount and frequency of blood collection. The aim of these guidelines is to reduce the risk of research related anemia, etc. and the affects these can have on experimental results.

PROCEDURE: There are several references that suggest the maximum amount of blood that can safely be drawn at a single survival blood draw is 1% of the animal's body weight. If kilograms are used for the calculation, the volume result will be in liters. To convert to mls, multiply the final answer by 1000. For example, a 25 kg baboon can safely have 0.25 liters (or 250 mls) of blood drawn at a single blood draw (1% or $0.01 \times 25 = 0.25$). If grams are used for the calculation, the volume result will be in milliliters. For example, a 200 gram rat can safely have 2 mls of blood drawn at a single blood draw ($200 \times 0.01 = 2$).

If the recommended maximum blood draw is performed, there are several published suggestions on how much time should elapse between blood draws ranging from 2 weeks to 4 weeks. It only takes about 24 hours for the blood volume to be restored, but it can take a lot longer for the erythrocytes, platelets, coagulation factors, etc. to be replenished. If the maximum amount of blood is drawn on one occasion, we recommend waiting about 3 weeks between blood draws to reduce the risk of anemia or other abnormalities. For experiments that do not require the suggested maximum blood draw, blood can safely be drawn more frequently. The opposite also applies. If an experiment requires that blood is drawn and analyzed at certain time points, the guidelines can help determine the safe amount of blood that can be drawn at each time point.

Below is a table with guidelines that have been developed to assist investigators in their experiments. These guidelines are based on the amount of blood that is drawn and the frequency of blood collection. Remember, the blood volume calculation will be in liters or milliliters, depending on the units of weight that are used (kg = liters, gm = mls).

Max blood draw (body weight)	Recovery time
1%	3 weeks
0.75%	2 weeks
0.5%	1 week
0.05%	Daily

Regarding the daily blood draw, this is the safe maximum per day that can be drawn. So, if there are several draws within one day, the safe maximum total amount of blood drawn in a 24 hour period is 0.05% of body weight. For example, for a 7 kg rhesus, the maximum amount of blood that should be drawn within 24 hours is 3.5 mls (0.05% x 7). If you need to test the blood every 8 hours, 1.7 mls of blood can be drawn each time. This will result in 3.5 mls drawn within 24 hours.

The table below gives the maximum amount of blood that can be drawn based on a percentage of **body weight** applicable to most rodents.

weight (g)	.05% (ml)	0.5% (ml)	0.75% (ml)	1% (ml)
20	.010	.100	.150	.200
25	.012	.120	.187	.250
30	.015	.150	.225	.300
35	.017	.170	.262	.350
40	.020	.200	.300	.400
125	.065	.650	.937	1.25
150	.075	.750	1.12	1.50
200	.100	1.00	1.50	2.00
250	.125	1.25	1.87	2.50
300	.150	1.50	2.25	3.00
350	.175	1.75	2.62	3.50

Another way to calculate the amount of blood that can be drawn is to use circulating blood volume. Circulating blood volume (CBV) is approximately 60 ml/kg or 6% of body weight in kilograms or grams. When using 6% of body weight, the final result will be in liters if kilograms are used. To get mls, multiply by 1000. If grams are used, the final result will be in mls.

If using circulating blood volume (CBV), the maximum amount of blood that can be safely drawn at a single survival blood draw is 15% of CBV. For example, a 200 gram rat will have a circulating blood volume of about 12 mls (60 ml/kg x 0.2 kg) or (0.06 (6%) x 200). 15% of the circulating blood volume would be 0.15 (15%) x 12 = 1.8 mls. This is the amount of blood that can safely be drawn at a single time and there should be 3 weeks in between blood draws of this volume.

Max blood draw (CBV)	Recovery time
15%	3 weeks
10%	2 weeks
7.5%	1 week
0.75%	Daily

The table below gives the maximum amount of blood that can be drawn based on a percentage of the **circulating blood volume** applicable to most rodents.

weight (g)	CBV (ml)	0.75% (ml)	7.5% (ml)	10% (ml)	15% (ml)
20	1.2	.009	.09	.12	.18
25	1.5	.010	.10	.15	.22
30	1.8	.013	.13	.18	.27
35	2.1	.016	.16	.21	.31
40	2.4	.018	.18	.24	.36
125	7.5	.056	.56	.75	1.12
150	9.0	.068	.68	.90	1.35
200	12.0	.090	.90	1.2	1.80
250	15.0	.112	1.12	1.5	2.25
300	18.0	.135	1.35	1.8	2.70
350	21.0	.157	1.57	2.10	3.15

All research related blood draws must be fully detailed in an approved protocol or amendment.

If you have questions about this guideline, please contact one of the clinical veterinarians at 410-955-3273.

References:

1. Removal of blood from laboratory mammals and birds. First report of the BVA/FRAME/RSPCA/UFAW joint working group on refinement. *Laboratory Animals* (1993) 27, 1-22.
2. McGill MW, Rowan AN. Biological Effects of Blood Loss: Implications for Sampling Volumes and Techniques. *ILAR* Fall 1989: 31(4) 5-18.
3. Hoff J. Methods of Blood Collection in the Mouse. *Lab Animal* Nov 2000: 29 (10) 47-53.
4. NIH Guidelines for Survival Bleeding of Mice and Rats.
http://oacu.od.nih.gov/ARAC/documents/Rodent_Bleeding.pdf