University of Pittsburgh Policy for Regulating the Volume of Experimental Blood Sample Withdrawals in Laboratory Animals

Blood withdrawal from laboratory animals is subject to Institutional Animal Care and Use Committee (IACUC) review and approval prior to implementation. This Policy provides both Investigators and IACUC members with guidelines to determine acceptable blood volume amounts in conjunction with sampling frequencies that can safely be withdrawn from laboratory animals as part of research manipulation, as well as appropriate recovery periods from such bleeds. Additional information is also available in the Rodent Bleeding Techniques SOP http://www.iacuc.pitt.edu/sop/restricted/BloodCollect.pdf

Animal welfare is a primary consideration when blood sampling is conducted, and the animal's physiological response and its impact on data interpretation and validity must be considered. Primary issues associated with blood withdrawals include the hemodynamic/circulatory impact of large-quantity survival bleeds as well as the potential hematopoietic consequences (i.e.- anemia) of ongoing samplings. Amounts specified in these guidelines are based on accepted and published standards in the field of laboratory animal science (1-7). Withdrawal volumes cited apply to healthy, adult animals. The use of immature or debilitated subjects for research blood collection requires consultation with the veterinary staff. Additionally, obese animals will typically have a smaller circulating volume per body weight than those in normal lean condition.

As with all policies, requests for variation will be considered by the IACUC. However; these must be based on strong scientific justification for exceeding recommended levels and a detailed plan for additional physiological support & monitoring is required. Anecdotal observations of lack of overt clinical signs in conjunction with higher than recommended withdrawal amounts are not justification for exceeding stated limits.

Determination of circulating blood volume (CBV):

CBV should be determined from known species-specific volume:weight values and not calculated based on flat % of body weight

The standard for acceptable blood withdrawal amounts is based on the percentage of total CBV. CBV levels for laboratory animal animals have been reported in the literature using a number of methodologies, including measuring various radiolabelled blood components and the use of marker dyes and enzyme dilutions. However, a relatively wide variation exists in the reported values which may be related to study population differences in breed, strain, age, body condition, etc. Accepted volumes for common species used in research at the University of Pittsburgh are listed below and should be utilized in calculations for arriving at protocol requested blood amounts. Determination of values to be used for species not listed or not currently published will be done in conjunction with DLAR veterinary staff consultation. Although rule-of-thumb estimates also exist for determining CBV, generally being based on blood:body weight ratios (often cited are 5-7 or 6-8%), the use of these in making specific volume determinations should be avoided as they may significantly over or under-estimate the acceptable amount of blood to be taken.

Estimated Circulating Blood Volumes (CBV) in common Laboratory Animal species

Macaque (rhesus)	55 ml/kg
Macaque (cynomolgus)	65 ml/kg
Baboon	60 ml/kg
Marmoset	70 ml/kg
Dog	85 ml/kg
Cat	55 ml/kg
Ferret	60 ml/kg
Pig	70 ml/kg
Sheep	60 ml/kg
Cows (non-lactating)	60 ml/kg
Rat	65 ml/kg
Mouse	75 ml/kg
Guinea pig	70 ml/kg
Hamster	80 ml/kg
Rabbit	55 ml/kg

Single maximum survival bleeding:

A maximum survival bleed not exceeding 15% of CBV is allowable once monthly if fluid replacement is administered

Although some working standards suggest a maximum of 10% (Joint Working Group on Refinement, 1993), most literature suggests that single survival bleeds done acutely can be safely performed **when not exceeding 15% of total circulating blood volume**. When taking samples at this maximum level, **the required recovery period before further bleeding is 1 month**. Additionally, animals undergoing maximum acute survival bleeds should be carefully monitored physiologically during the event and **fluid volume replacement is required. When technically possible, such replacement should be administered IV. If fluid replacement volume cannot be administered, single survival withdrawals should be limited to 10% CBV.**

Example: 10 Kg Rhesus macaque: CBV = 10 kg x 55 ml/kg = 550 ml. Maximum one time survival withdrawal (with fluid supplementation) = .15 x 550 = 82.5 ml

Example: 25 gram mouse: CBV = .025 kg x 75 ml/kg = ~1.9 ml. Maximum one time survival withdrawal (with fluid supplementation) = .15 x 1.9 = .285 microliters = ~.3 ml

Survival bleedings of greater frequency:

Bleedings performed weekly should not exceed 7.5% CBV
Bleedings performed once every 2 weeks should not exceed 10% CBV

In general, **weekly volumes of 7.5% circulating blood volume may be taken**. Volumes of up to **10% may be taken every two weeks**. Animals being bled daily (typically involving indwelling vascular catheters) may have 1% of circulating blood volume taken, although this may be exceeded if washed RBCs are returned along with fluid volume.

Less commonly, high volume blood withdrawal is done as multiple samplings over a 24 hour interval (such as in toxicokinetic studies). Under these circumstances, weekly withdrawal limits remain the same (i.e. 7.5%), whereas bleeds performed once every 2 weeks in such a fashion may take up to 15% of CBV. Multiple samplings over 24 hours may take a maximum of up to 20% CBV, with a recovery interval of 3 weeks.

Some protocols vary over the course of the study as to frequency of bleeding and investigators may wish to put in an acceptable "standard" number. **An overall conservative value considered acceptable for multiple (≥ 2) bleedings per month for most large animal species is 12 ml/kg maximum for the month** (although again, for some species and bleeding frequencies, this number may calculate significantly higher).

Terminal/non-survival bleeds:

As much blood as can be obtained is permissible in terminal, non-survival bleeds.

Terminal/non-survival bleeds must be conducted under general anesthesia. Although there is no limit to the amount of blood that can be taken, ~50-75% of the animal's CBV is generally the maximum volume that can be anticipated. Administration of IV fluids before and during exsanguination to expand and maintain blood pressure may increase this yield.

References:

1) *Journal of Applied Toxicology* 21, 15-23 (2001) A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes.

2) *ILAR Journal* 31(4) (1989) Biological Effects of Blood Loss: Implications for Sampling Volumes and Techniques

3) *Nonhuman Primates in Biomedical Research Biology and Management Academic Press* 1995, Fluid (Blood) Collection - p 271

4) *Schalm's Veterinary Hematology* (2010), John Wiley and Sons

5) *The UFAW Handbook on the Care and Management of Laboratory and Other Research Animals* (2010), John Wiley and Sons

6) *Lab Animals* 27(1) 1-22 (1993) Removal of blood from laboratory mammals and birds. First report of the BVA/FRAME/RSPCA/UFAW Joint Working Group on Refinement

7) *The Laboratory Mouse* (2001), CRC Press LLC, p 114-116

RHESUS MONKEYS

MAXIMUM ALLOWABLE BLOOD WITHDRAWAL VOLUMES						
Body Wt (Kg)	**Body Wt (lbs)**	**Total Circulating Blood Volume (CBV) in mls**	**Maximum allowable survival bleed (mls) with fluid volume replacement (~15% of CBV)**	**Maximum allowable survival bleed (mls) without fluid volume replacement (~10% of CBV)**	**Total maximum volume (mls) allowable weekly (~7.5% CBV)**	**Total maximum volume (mls) allowable every other week (~10% CBV)**
1	2.2	55	8.25	5.5	4.125	5.5
2	4.4	110	16.5	11	8.25	11
3	6.6	165	24.75	16.5	12.375	16.5
4	8.8	220	33	22	16.5	22
5	11	275	41.25	27.5	20.625	27.5
6	13.2	330	49.5	33	24.75	33
7	15.4	385	57.75	38.5	28.875	38.5
8	17.6	440	66	44	33	44
9	19.8	495	74.25	49.5	37.125	49.5
10	22	550	82.5	55	41.25	55
11	24.2	605	90.75	60.5	45.375	60.5
12	26.4	660	99	66	49.5	66
13	28.6	715	107.25	71.5	53.625	71.5
14	30.8	770	115.5	77	57.75	77
15	33	825	123.75	82.5	61.875	82.5
16	35.2	880	132	88	66	88
17	37.4	935	140.25	93.5	70.125	93.5
18	39.6	990	148.5	99	74.25	99

Based on determined species blood volume of: 55 ml/kg

CYNOMOLGUS

MAXIMUM ALLOWABLE BLOOD WITHDRAWAL VOLUMES						
Body Wt (Kg)	**Body Wt (lbs)**	**Total Circulating Blood Volume (CBV) in mls**	**Maximum allowable survival bleed (mls) with fluid volume replacement (~15% of CBV)**	**Maximum allowable survival bleed (mls) without fluid volume replacement (~10% of CBV)**	**Total maximum volume (mls) allowable weekly (~7.5% CBV)**	**Total maximum volume (mls) allowable every other week (~10% CBV)**
1	2.2	65	9.75	6.5	4.875	6.5
2	4.4	130	19.5	13	9.75	13
3	6.6	195	29.25	19.5	14.625	19.5
4	8.8	260	39	26	19.5	26
5	11	325	48.75	32.5	24.375	32.5
6	13.2	390	58.5	39	29.25	39
7	15.4	455	68.25	45.5	34.125	45.5
8	17.6	520	78	52	39	52
9	19.8	585	87.75	58.5	43.875	58.5
10	22	650	97.5	65	48.75	65
11	24.2	715	107.25	71.5	53.625	71.5
12	26.4	780	117	78	58.5	78
13	28.6	845	126.75	84.5	63.375	84.5
14	30.8	910	136.5	91	68.25	91
15	33	975	146.25	97.5	73.125	97.5
16	35.2	1040	156	104	78	104
17	37.4	1105	165.75	110.5	82.875	110.5
18	39.6	1170	175.5	117	87.75	117

Based on determined species blood volume of: 65 ml/kg

BABOON

MAXIMUM ALLOWABLE BLOOD WITHDRAWAL VOLUMES						
Body Wt (Kg)	Body Wt (lbs)	Total Circulating Blood Volume (CBV) in mls	Maximum allowable survival bleed (mls) with fluid volume replacement (~15% of CBV)	Maximum allowable survival bleed (mls) without fluid volume replacement (~10% of CBV)	Total maximum volume (mls) allowable weekly (~7.5% CBV)	Total maximum volume (mls) allowable every other week (~10% CBV)
2	4.4	120	18	12	9	12
3	6.6	180	27	18	13.5	18
4	8.8	240	36	24	18	24
5	11	300	45	30	22.5	30
6	13.2	360	54	36	27	36
7	15.4	420	63	42	31.5	42
8	17.6	480	72	48	36	48
9	19.8	540	81	54	40.5	54
10	22	600	90	60	45	60
11	24.2	660	99	66	49.5	66
12	26.4	720	108	72	54	72
13	28.6	780	117	78	58.5	78
14	30.8	840	126	84	63	84
15	33	900	135	90	67.5	90
16	35.2	960	144	96	72	96
18	39.6	1080	162	108	81	108
20	44	1200	180	120	90	120
22	48.4	1320	198	132	99	132
24	52.8	1440	216	144	108	144
26	57.2	1560	234	156	117	156
28	61.6	1680	252	168	126	168
30	66	1800	270	180	135	180

Based on determined species blood volume of: 60 ml/kg

MARMOSET

Body Wt (gram)	Total Circulating Blood Volume (CBV) in mls	Maximum allowable survival bleed (mls) with fluid volume replacement (~15% of CBV)	Maximum allowable survival bleed (mls) without fluid volume replacement (~10% of CBV)	Total maximum volume (mls) allowable weekly (~7.5% CBV)	Total maximum volume (mls) allowable every other week (~10% CBV)
200	14.00	2.10	1.40	1.05	1.40
225	15.75	2.36	1.58	1.18	1.58
250	17.50	2.63	1.75	1.31	1.75
275	19.25	2.89	1.93	1.44	1.93
300	21.00	3.15	2.10	1.58	2.10
325	22.75	3.41	2.28	1.71	2.28
350	24.50	3.68	2.45	1.84	2.45
375	26.25	3.94	2.63	1.97	2.63
400	28.00	4.20	2.80	2.10	2.80
425	29.75	4.46	2.98	2.23	2.98
450	31.50	4.73	3.15	2.36	3.15
475	33.25	4.99	3.33	2.49	3.33
500	35.00	5.25	3.50	2.63	3.50

Based on determined species blood volume of: 70 ml/kg

FERRET

MAXIMUM ALLOWABLE BLOOD WITHDRAWAL VOLUMES						
Body Wt (Kg)	**Body Wt (lbs)**	**Total Circulating Blood Volume (CBV) in mls**	**Maximum allowable survival bleed (mls) with fluid volume replacement (~15% of CBV)**	**Maximum allowable survival bleed (mls) without fluid volume replacement (~10% of CBV)**	**Total maximum volume (mls) allowable weekly (~7.5% CBV)**	**Total maximum volume (mls) allowable every other week (~10% CBV)**
1	2.2	60	9	6	4.5	6
1.25	2.75	75	11.25	7.5	5.625	7.5
1.5	3.3	90	13.5	9	6.75	9
1.75	3.85	105	15.75	10.5	7.875	10.5
2	4.4	120	18	12	9	12
2.25	4.95	135	20.25	13.5	10.125	13.5
2.5	5.5	150	22.5	15	11.25	15
2.75	6.05	165	24.75	16.5	12.375	16.5
3	6.6	180	27	18	13.5	18
3.25	7.15	195	29.25	19.5	14.625	19.5
3.5	7.7	210	31.5	21	15.75	21
3.75	8.25	225	33.75	22.5	16.875	22.5
4	8.8	240	36	24	18	24

Based on determined species blood volume of: 60 ml/kg

PIG

MAXIMUM ALLOWABLE BLOOD WITHDRAWAL VOLUMES						
Body Wt (Kg)	**Body Wt (lbs)**	**Total Circulating Blood Volume (CBV) in mls**	**Maximum allowable survival bleed (mls) with fluid volume replacement (~15% of CBV)**	**Maximum allowable survival bleed (mls) without fluid volume replacement (~10% of CBV)**	**Total maximum volume (mls) allowable weekly (~7.5% CBV)**	**Total maximum volume (mls) allowable every other week (~10% CBV)**
10	22	700	105	70	52.5	70
11	24.2	770	115.5	77	57.75	77
12	26.4	840	126	84	63	84
13	28.6	910	136.5	91	68.25	91
14	30.8	980	147	98	73.5	98
15	33	1050	157.5	105	78.75	105
16	35.2	1120	168	112	84	112
17	37.4	1190	178.5	119	89.25	119
18	39.6	1260	189	126	94.5	126
19	41.8	1330	199.5	133	99.75	133
20	44	1400	210	140	105	140
21	46.2	1470	220.5	147	110.25	147
22	48.4	1540	231	154	115.5	154
23	50.6	1610	241.5	161	120.75	161
24	52.8	1680	252	168	126	168
25	55	1750	262.5	175	131.25	175
26	57.2	1820	273	182	136.5	182
28	61.6	1960	294	196	147	196
30	66	2100	315	210	157.5	210
32	70.4	2240	336	224	168	224
34	74.8	2380	357	238	178.5	238
36	79.2	2520	378	252	189	252
38	83.6	2660	399	266	199.5	266
40	88	2800	420	280	210	280
42	92.4	2940	441	294	220.5	294
44	96.8	3080	462	308	231	308
46	101.2	3220	483	322	241.5	322
48	105.6	3360	504	336	252	336
50	110	3500	525	350	262.5	350

Based on determined species blood volume of: 70 ml/kg

SHEEP

MAXIMUM ALLOWABLE BLOOD WITHDRAWAL VOLUMES						
Body Wt (Kg)	**Body Wt (lbs)**	**Total Circulating Blood Volume (CBV) in mls**	**Maximum allowable survival bleed (mls) with fluid volume replacement (~15% of CBV)**	**Maximum allowable survival bleed (mls) without fluid volume replacement (~10% of CBV)**	**Total maximum volume (mls) allowable weekly (~7.5% CBV)**	**Total maximum volume (mls) allowable every other week (~10% CBV)**
20	44	1200	180	120	90	120
22	48.4	1320	198	132	99	132
24	52.8	1440	216	144	108	144
26	57.2	1560	234	156	117	156
28	61.6	1680	252	168	126	168
30	66	1800	270	180	135	180
32	70.4	1920	288	192	144	192
34	74.8	2040	306	204	153	204
36	79.2	2160	324	216	162	216
38	83.6	2280	342	228	171	228
40	88	2400	360	240	180	240
42	92.4	2520	378	252	189	252
44	96.8	2640	396	264	198	264
46	101.2	2760	414	276	207	276
48	105.6	2880	432	288	216	288
50	110	3000	450	300	225	300
52	114.4	3120	468	312	234	312
54	118.8	3240	486	324	243	324
56	123.2	3360	504	336	252	336
58	127.6	3480	522	348	261	348
60	132	3600	540	360	270	360
62	136.4	3720	558	372	279	372
64	140.8	3840	576	384	288	384
66	145.2	3960	594	396	297	396
68	149.6	4080	612	408	306	408
70	154	4200	630	420	315	420
72	158.4	4320	648	432	324	432
74	162.8	4440	666	444	333	444
76	167.2	4560	684	456	342	456
78	171.6	4680	702	468	351	468
80	176	4800	720	480	360	480

Based on determined species blood volume of: 60 ml/kg

RAT

Body Wt (grams)	Total Circulating Blood Volume (CBV) in mls	Maximum allowable survival bleed (mls) with fluid volume replacement (~15% of CBV)	Maximum allowable survival bleed (mls) without fluid volume replacement (~10% of CBV)	Total maximum volume (mls) allowable weekly (~7.5% CBV)	Total maximum volume (mls) allowable every other week (~10% CBV)
100	6.50	0.98	0.65	0.49	0.65
125	8.13	1.22	0.81	0.61	0.81
150	9.75	1.46	0.98	0.73	0.98
175	11.38	1.71	1.14	0.85	1.14
200	13.00	1.95	1.30	0.98	1.30
225	14.63	2.19	1.46	1.10	1.46
250	16.25	2.44	1.63	1.22	1.63
275	17.88	2.68	1.79	1.34	1.79
300	19.50	2.93	1.95	1.46	1.95
325	21.13	3.17	2.11	1.58	2.11
350	22.75	3.41	2.28	1.71	2.28
375	24.38	3.66	2.44	1.83	2.44
400	26.00	3.90	2.60	1.95	2.60
425	27.63	4.14	2.76	2.07	2.76
450	29.25	4.39	2.93	2.19	2.93
475	30.88	4.63	3.09	2.32	3.09
500	32.50	4.88	3.25	2.44	3.25

Based on determined species blood volume of: 65 ml/kg

MOUSE

Body Wt (grams)	Total Circulating Blood Volume (CBV) in mls	Maximum allowable survival bleed (mls) with fluid volume replacement (~15% of CBV)	Maximum allowable survival bleed (mls) without fluid volume replacement (~10% of CBV)	Total maximum volume (mls) allowable weekly (~7.5% CBV)	Total maximum volume (mls) allowable every other week (~10% CBV)
15	1.13	0.17	0.11	0.08	0.11
20	1.50	0.23	0.15	0.11	0.15
25	1.88	0.28	0.19	0.14	0.19
30	2.25	0.34	0.23	0.17	0.23
35	2.63	0.39	0.26	0.20	0.26
40	3.00	0.45	0.30	0.23	0.30
45	3.38	0.51	0.34	0.25	0.34

Based on determined species blood volume of: 75 ml/kg

RABBIT

MAXIMUM ALLOWABLE BLOOD WITHDRAWAL VOLUMES						
Body Wt (Kg)	**Body Wt (lbs)**	**Total Circulating Blood Volume (CBV) in mls**	**Maximum allowable survival bleed (mls) with fluid volume replacement (~15% of CBV)**	**Maximum allowable survival bleed (mls) without fluid volume replacement (~10% of CBV)**	**Total maximum volume (mls) allowable weekly (~7.5% CBV)**	**Total maximum volume (mls) allowable every other week (~10% CBV)**
0.5	1.1	27.5	4.125	2.75	2.0625	2.75
0.75	1.65	41.25	6.1875	4.125	3.09375	4.125
1	2.2	55	8.25	5.5	4.125	5.5
1.25	2.75	68.75	10.3125	6.875	5.15625	6.875
1.5	3.3	82.5	12.375	8.25	6.1875	8.25
1.75	3.85	96.25	14.4375	9.625	7.21875	9.625
2	4.4	110	16.5	11	8.25	11
2.25	4.95	123.75	18.5625	12.375	9.28125	12.375
2.5	5.5	137.5	20.625	13.75	10.3125	13.75
2.75	6.05	151.25	22.6875	15.125	11.34375	15.125
3	6.6	165	24.75	16.5	12.375	16.5
3.25	7.15	178.75	26.8125	17.875	13.40625	17.875
3.5	7.7	192.5	28.875	19.25	14.4375	19.25
3.75	8.25	206.25	30.9375	20.625	15.46875	20.625
4	8.8	220	33	22	16.5	22
4.25	9.35	233.75	35.0625	23.375	17.53125	23.375
4.5	9.9	247.5	37.125	24.75	18.5625	24.75
4.75	10.45	261.25	39.1875	26.125	19.59375	26.125
5	11	275	41.25	27.5	20.625	27.5

Based on determined species blood volume of: 55 ml/kg