

1. PURPOSE

This Standard Operating Procedure (SOP) describes recommended volumes and frequency for blood collection for commonly used laboratory animal species.

2. RESPONSIBILITY

Principal Investigators (PI) and their staff, veterinary care staff, Facility Animal Care Committee (FACC).

3. INTRODUCTION

- 3.1. The acceptable quantity and frequency of blood sampling in all species is dependent upon the total blood volume of the animal.
- 3.2. Considerations regarding the species to be bled, the size and health status of the animal, the quantity and type of sample needed (i.e. whole blood, serum, etc.), the frequency of sampling, and the training of the phlebotomist should all be taken into account.
- 3.3. It is recommended to take no more blood than is absolutely necessary. Remember to calculate beforehand the minimum amount of blood necessary to perform all tests and assays.

4. MATERIALS

- 4.1. Blood Withdrawal and Recovery Chart
- 4.2. Needles
- 4.3. Catheters
- 4.4. Syringes and/or lancets
- 4.5. Blood collection tubes (with or without anticoagulant)
- 4.6. Fluids for replacement (Lactated Ringer Solution, 0.9% Saline, or 5% dextrose)

5. PROCEDURES

- 5.1. Factors to Consider:
 - 5.1.1. Observe animals prior to sample collection for weakness, illness, dehydration, obesity, or anemia.
 - 5.1.2. Contact a veterinarian before sample collection if the animal is exhibiting any of these signs.
 - 5.1.3. Do not puncture a site presenting inflammation or a hematoma.
 - 5.1.4. Limit the number of punctures to four punctures per day with no more than two punctures per site.
 - 5.1.5. Use a catheter for multiple sampling in species in which it is possible.
 - 5.1.6. For recommendations of blood collection sites in multiple species, see Section 8.
- 5.2. Replacement of Fluids
 - 5.2.1. Replace isotonic fluids (i.e. fluids with the same tonicity as blood) if >10% of total blood volume is required.
 - 5.2.2. If >10% blood volume is required, it is recommended to replace collected blood volume by 3–4 times the volume of blood collected with isotonic fluids (i.e. fluids with same tonicity as blood, such as 0.9% saline, 5% dextrose or Lactated Ringer's solution).

6. RECOMMENDED BLOOD WITHDRAWAL VOLUMES AND RECOVERY PERIODS

6.1. Maximum volumes and recovery periods:

PERCENT OF BLOOD VOLUME COLLECTED IN A SINGLE SAMPLING	RECOVERY PERIOD (weeks)
7.5%	1
10%	2
15%	4

PERCENT OF BLOOD VOLUME COLLECTED OVER A 24-HOUR PERIOD (MULTIPLE SAMPLES)	RECOVERY PERIOD (weeks)
7.5%	1
10 - 15%	2
20%	4

6.2. Blood volume by species:

SPECIES	CIRCULATING BLOOD VOLUME (ml/kg BW)	7.5% (ml/kg BW)	10% (ml/kg BW)	15% (ml/kg BW)	20% (ml/kg BW)
Mouse	72	5.4	7.2	10.8	14.4
Rat	64	4.8	6.4	9.6	12.8
Rabbit	56	4.2	5.6	8.4	11.2
Non-human primate: Rhesus	56	4.2	5.6	8.4	11.2
Non-human primate: Cynomolgus	65	4.8	6.5	9.8	13.0
Non-human primate: Marmoset	71	5.3	7.1	10.6	14.2
Guinea pig	73	5.5	7.3	11.0	14.6
Hamster	78	5.8	7.8	11.7	15.6
Cat	56	4.2	5.6	8.4	11.2
Dog	85	6.4	8.5	12.8	17.0

7. MONITORING

- 7.1. If too much blood is withdrawn too rapidly or too frequently without replacement (approximately 2% of the animal's body weight at one time), the animal may go into hypovolemic shock.
- 7.2. Monitor the animal during and after blood sampling for signs of shock. (see section 7.4)
- 7.3. Contact the veterinary care staff if any signs of hypovolemic shock are observed.
- 7.4. Signs of shock include the following:
 - 7.4.1. Fast and thready pulse
 - 7.4.2. Pale dry mucous membranes
 - 7.4.3. Cold skin and extremities
 - 7.4.4. Restlessness
 - 7.4.5. Hyperventilation
 - 7.4.6. Sub-normal body temperature

8. COMMON SURVIVAL BLOOD COLLECTION SITES

SPECIES	SITE	GENERAL ANESTHESIA REQUIRED	REPEAT SAMPLING (DAILY)	OBTAINABLE VOLUME
Mouse	Saphenous vein	No	Yes	Medium to large
	Tail vein or artery	No	Yes	Small
	Submandibular puncture	No	Yes	Medium to large
	Tail tip ¹	No	Yes	1-2 drops
	Jugular vein	Yes	Yes	Large
Rat	Saphenous vein	No	Yes	Medium to large
	Tail vein or artery	No	Yes	Small to medium
	Jugular vein	Yes	Yes	Large
Gerbil and hamster	Lateral tarsal vein	No	Yes	Medium
	Jugular vein	Yes	Yes	Large
Guinea Pig	Saphenous vein	No	Yes	Medium
	Marginal ear vein	No	Yes	Small
	Jugular vein	Recommended	Yes	Large
Rabbit	Marginal ear vein or central ear artery	Local anesthesia	Yes	Large
	Jugular vein	Recommended	Yes	Large
	Femoral vein	No	Yes	Medium to large
	Cephalic vein	No	Yes	Medium to large

¹ The tail tip blood collection method should not be confused with the tail tip tissue collection for genotyping procedure.

SPECIES	SITE	GENERAL ANESTHESIA REQUIRED	REPEAT SAMPLING (DAILY)	OBTAINABLE VOLUME
Cat	Jugular vein	No	Yes	Large
	Medial saphenous vein	No	Yes	Large
	Femoral vein	No	Yes	Large
	Cephalic vein	No	Yes	Medium to large
Dog	Jugular vein	No	Yes	Large
	Lateral saphenous vein	No	Yes	Medium to large
	Femoral vein	No	Yes	Large
	Cephalic vein	No	Yes	Medium to large
Non-human primate	Femoral vein	Yes	Yes	Large
	Cephalic vein	Yes	Yes	Medium to large
	Saphenous vein	Yes	Yes	Medium to large
	Jugular vein	Yes	No	Medium to large
	Brachial vein	Yes	No	Medium to large
Swine	Ear vein	No	Yes	Medium to large
Cow	Jugular vein	No	Yes	Large
	Tail vein	No	Yes	Large
Sheep	Cephalic vein	No	Yes	Medium to large
	Jugular vein	No	Yes	Large
Bird	Brachial wing vein	No	Yes	Medium to large
	Right jugular vein	Yes	Yes	Medium to large
Fish	Caudal vein	Yes	-	-

9. REFERENCES

- 9.1. Diehl, K.-H. 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)
- 9.2. Wolfensohn, S., Lloyd, M. 2nd Edition, Blackwell Science Ltd. 1998.
- 9.3. Guidelines for survival bleeding of mice and rats; NIH:
http://oacu.od.nih.gov/ARAC/documents/Rodent_Bleeding.pdf
- 9.4. Guide to the Care and Use of Experimental Animals, Vol. 1 (2nd ed), Canadian Council on Animal Care, Canada, 1993: http://ccac.ca/en/CCAC_Programs/Guidelines_Policies/GUIDES/ENGLISH/V1_93/APPEN/APPVIII.HTM

SOP REVISION HISTORY

DATE	PREVIOUS VERSION	NEW VERSION
2016.09.22	9.3. Guidelines for survival bleeding of mice and rats; NIH: http://oacu.od.nih.gov/ARAC/Bleeding.pdf	9.3. Guidelines for survival bleeding of mice and rats; NIH: http://oacu.od.nih.gov/ARAC/Rodent_Bleeding.pdf
2017.08.30	(no text)	8. (footnote) The tail tip blood collection method should not be confused with the tail tip tissue collection for genotyping procedure.

Blood volumes - Mouse

Body weight (g)	Total circulating blood volume (mL)	Acceptable volume for collection (mL)			
		7.5%	10%	15%	20%
10	0.72	0.05	0.07	0.11	0.14
11	0.79	0.06	0.08	0.12	0.16
12	0.86	0.06	0.09	0.13	0.17
13	0.94	0.07	0.09	0.14	0.19
14	1.01	0.08	0.10	0.15	0.20
15	1.08	0.08	0.11	0.16	0.22
16	1.15	0.09	0.12	0.17	0.23
17	1.22	0.09	0.12	0.18	0.24
18	1.30	0.10	0.13	0.19	0.26
19	1.37	0.10	0.14	0.21	0.27
20	1.44	0.11	0.14	0.22	0.29
21	1.51	0.11	0.15	0.23	0.30
22	1.58	0.12	0.16	0.24	0.32
23	1.66	0.12	0.17	0.25	0.33
24	1.73	0.13	0.17	0.26	0.35
25	1.80	0.14	0.18	0.27	0.36
26	1.87	0.14	0.19	0.28	0.37
27	1.94	0.15	0.19	0.29	0.39
28	2.02	0.15	0.20	0.30	0.40
29	2.09	0.16	0.21	0.31	0.42
30	2.16	0.16	0.22	0.32	0.43
31	2.23	0.17	0.22	0.33	0.45
32	2.30	0.17	0.23	0.35	0.46
33	2.38	0.18	0.24	0.36	0.48
34	2.45	0.18	0.24	0.37	0.49
35	2.52	0.19	0.25	0.38	0.50
36	2.59	0.19	0.26	0.39	0.52
37	2.66	0.20	0.27	0.40	0.53
38	2.74	0.21	0.27	0.41	0.55
39	2.81	0.21	0.28	0.42	0.56
40	2.88	0.22	0.29	0.43	0.58

Blood volumes – Rat

Body weight (g)	Total circulating blood volume (mL)	Acceptable volume for collection (mL)			
		7.5%	10%	15%	20%
150	9.6	0.72	0.96	1.44	1.92
160	10.2	0.77	1.02	1.54	2.05
170	10.9	0.82	1.09	1.63	2.18
180	11.5	0.86	1.15	1.73	2.30
190	12.2	0.91	1.22	1.82	2.43
200	12.8	0.96	1.28	1.92	2.56
210	13.4	1.01	1.34	2.02	2.69
220	14.1	1.06	1.41	2.11	2.82
230	14.7	1.10	1.47	2.21	2.94
240	15.4	1.15	1.54	2.30	3.07
250	16.0	1.20	1.60	2.40	3.20
260	16.6	1.25	1.66	2.50	3.33
270	17.3	1.30	1.73	2.59	3.46
280	17.9	1.34	1.79	2.69	3.58
290	18.6	1.39	1.86	2.78	3.71
300	19.2	1.44	1.92	2.88	3.84
310	19.8	1.49	1.98	2.98	3.97
320	20.5	1.54	2.05	3.07	4.10
330	21.1	1.58	2.11	3.17	4.22
340	21.8	1.63	2.18	3.26	4.35
350	22.4	1.68	2.24	3.36	4.48
360	23.0	1.73	2.30	3.46	4.61
370	23.7	1.78	2.37	3.55	4.74
380	24.3	1.82	2.43	3.65	4.86
390	25.0	1.87	2.50	3.74	4.99
400	25.6	1.92	2.56	3.84	5.12
410	26.2	1.97	2.62	3.94	5.25
420	26.9	2.02	2.69	4.03	5.38
430	27.5	2.06	2.75	4.13	5.50
440	28.2	2.11	2.82	4.22	5.63
450	28.8	2.16	2.88	4.32	5.76
460	29.4	2.21	2.94	4.42	5.89
470	30.1	2.26	3.01	4.51	6.02
480	30.7	2.30	3.07	4.61	6.14
490	31.4	2.35	3.14	4.70	6.27
500	32.0	2.40	3.20	4.80	6.40