STANDARD OPERATING PROCEDURE #403

GUIDELINES FOR BLOOD COLLECTION VOLUMES AND FREQUENCY

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. 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 user should be taken into consideration.

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amount of blood necessary to perform all tests and assays as well as the maximum volume of blood that can be safely withdrawn.

3.4. Efforts should be made to refine techniques to reduce the blood sample volume and collection frequency required.

3.5. Blood sampling (except for terminal blood sampling) should not be performed on mice less than 14 days of age due to the risk of hypovolemic shock.

4. MATERIALS

4.1. Blood Withdrawal and Recovery Chart

4.2. Needles, syringes, lancets, catheters

4.3. Blood collection tubes (with or without anticoagulant)

4.4. Fluids for replacement (Lactated Ringer Solution, 0.9% Saline)

5. PROCEDURES

5.1. Observe animals prior to sample collection to assess general condition of the animal. Consult the veterinarian prior to collecting blood from animals presenting weakness, illness, dehydration, obesity, or anemia.

5.2. Do not puncture a site presenting inflammation or a hematoma.

5.3. Limit the number of punctures to three puncture trials per timepoint with no more than two venipunctures per site.

5.4. For multiple sampling, consider cannulation or the use of a fixed catheter in species in which it is possible.

5.5. For recommendations of blood collection sites in multiple species, see Section 8.

5.6. When possible, veins should be dilated by gentle obstruction or warming before sampling. If warming is used, the animal must be constantly observed to prevent hyperthermia.

5.7. Following collection of blood, and before the animal is returned to the cage, there must be assurance that bleeding has stopped

5.8. When the volume of blood collected exceeds 10% of the total blood volume, 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 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	RECOVERY PERIOD (weeks)
7.5%	1-2
10%	3-4
15% Only when taking multiple small samples over a 24-hour period	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)
Mouse	60	4.5	6.0	9.0
Rat	64	4.8	6.4	9.6
Hamster	78	5.8	7.8	11.7
Gerbil	67	5.0	6.7	10.0
Guinea pig	73	5.5	7.3	11.0
Rabbit	56	4.2	5.6	8.4
Cat	56	4.2	5.6	8.4
Dog	80	6.4	8.5	12.8
Ferret	60	4.5	6.0	9.0
Marmoset	60	4.5	6.0	9.0
Macaque	60	4.5	6.0	9.0
Bird	60	4.5	6.0	9.0
Fish	10	0.75	1.0	1.5
Swine	65	4.8	6.5	9.7
Cow	60	4.5	6.0	9.0
Sheep	60	4.5	6.0	9.0

7. MONITORING

7.1. If the volume of blood withdrawn exceeds the maximum recommended volume, or if blood is withdrawn more frequently than is recommended, 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	OBTAINABLE VOLUME
Mouse	Saphenous vein	No	Medium to large
	Tail vein or artery	No	Small
	Facial vein (Submandibular)	No	Medium to large
	Jugular vein	Yes	Large
Rat	Saphenous vein	No	Medium to large
	Tail vein or artery	No	Small to medium
	Jugular vein	No	Large
Hamster	Saphenous vein	No	Medium
	Jugular vein	Yes	Large
Gerbil	Lateral tarsal vein	No	Medium
	Jugular vein	Yes	Large
Guinea Pig	Saphenous vein	No	Medium
	Marginal ear vein	No	Small
	Jugular vein	Yes	Large
Rabbit	Ear vein or artery	Local anesthesia	Large
	Jugular vein	Recommended	Large
	Femoral vein	No	Medium to large
	Cephalic vein	No	Medium to large

SPECIES	SITE	GENERAL ANESTHESIA REQUIRED	OBTAINABLE VOLUME
Cat	Jugular vein	No	Large
	Medial saphenous vein	No	Large
	Femoral vein	No	Large
	Cephalic vein	No	Medium to large
Dog	Jugular vein	No	Large
	Lateral saphenous vein	No	Medium to large
	Femoral vein	No	Large
	Cephalic vein	No	Medium to large
Ferret	Saphenous vein	No	Medium to large
	Cranial Vena Cava	Yes	Large
Macaque	Femoral vein	Yes	Large
	Cephalic vein	Yes	Medium to large
	Saphenous vein	Yes	Medium to large
Marmoset	Femoral vein	Yes	Large
	Tail vein	Yes	Small
	Saphenous vein	Yes	Medium to large
Swine	Ear vein or artery	No	Medium to large
	Jugular vein	Yes	Large
Cow	Jugular vein	No	Large
	Tail vein	No	Large
Sheep	Cephalic vein	No	Medium to large
	Jugular vein	No	Large
Bird	Brachial vein	No	Medium to large
	Jugular vein	Yes	Medium to large
Fish	Caudal vein	Yes	-

9. TERMINAL BLOOD COLLECTION

9.1. Terminal blood sampling must only be carried out once the animal has been rendered unconscious using general anesthesia or euthanized by an acceptable method. When terminal blood collection is performed under anesthesia, the animal should be euthanized once blood collection is complete.

9.2. Terminal blood collection sites:

9.2.1. Inferior vena cava

9.2.2. Abdominal aorta

9.2.3. Cardiac puncture

9.2.4. Retro-orbital plexus (rodents only)

10. REFERENCES

10.1. 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)

10.2. Wolfensohn, S., Lloyd, M. 2nd Edition, Blackwell Science Ltd. 1998.

10.3. Guidelines for survival bleeding of mice and rats; NIH: http://oacu.od.nih.gov/ARAC/documents/Rodent_**Bleeding**.pdf

10.4. 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

10.5. Schultz-Darken NJ. Sample collection and restraint techniques used for common marmosets (Callithrix jacchus). Comp Med. 2003 Aug;53(4):360-3. PMID: 14524411.

10.6. CCAC guidelines: Experimental procedures (Part A – Administration of substances and biological sampling), Draft for Public Review – September 2021.

10.7. Lawrence MJ, Raby GD, Teffer AK, Jeffries KM, Danylchuk AJ, Eliason EJ, Hasler CT, Clark TD, Cooke SJ. Best practices for non-lethal blood sampling of fish via the caudal vasculature. J Fish Biol. 2020 Jul;97(1):4-15. doi: 10.1111/jfb.14339. Epub 2020 May 29. https://doi.org/10.1111/jfb.14339.

10.8. Canada Department of Fisheries and Oceans Animal User Training Template, 4.0 Blood Sampling of Finfish.

SOP REVISION HISTORY

DATE	NEW VERSION
2016.09.22	9.3. Guidelines for survival bleeding of mice and rats; NIH: http://oacu.od.nih.gov/ARAC/**Rodent_**Bleeding.pdf
2017.08.30	**8. (footnote) The tail tip blood collection method should not be confused with the tail tip tissue collection for genotyping procedure.**
2021.09.09	4.6. Fluids for replacement (Lactated Ringer Solution, 0.9% Saline~~, or 5% dextrose~~)
2021.09.09	5.1.1. Observe animals prior to sample collection to assess condition of the animal. **Consult the veterinarian prior to collecting blood from animals presenting weakness, illness, dehydration, obesity, or anemia.**
2021.09.09	~~5.1.2. Contact a veterinarian before sample collection if the animal is exhibiting any of these signs.~~
2021.09.09	5.1.4. ~~Use a catheter~~ For multiple sampling **consider cannulation or use a catheter** in species in which it is possible.
2021.09.09	~~5.2.1. Replace isotonic fluids (i.e. fluids with the same tonicity as blood) if >10% of total blood volume is required.~~
2021.09.09	5.2.1. 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
2021.09.09	6.2. CIRCULATING BLOOD VOLUME (ml/kg BW) Mice: ~~72~~ **60,** 7.5% ~~5.4~~ **4.5**, 10% ~~7.2~~ **6.0**, ~~10.8~~ **9.0**, ~~14.4~~ **12.0**
2021.11.16	4.2. Needles**, syringes, lancets, catheters** ~~4.3. Catheters~~ ~~4.4. Syringes and/or lancets~~
2021.11.16	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~~ **user** should ~~all~~ be taken into ~~account~~ **consideration**.

DATE	NEW VERSION
2021.11.16	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 **as well as the maximum volume of blood that can be safely withdrawn**.
2021.11.16	**3.4. Efforts should be made to refine techniques to reduce the blood sample volume and collection frequency required.** **3.6. Blood sampling (except for terminal blood sampling) should not be performed on mice less than 14 days of age due to the risk of hypovolemic shock.**
2021.11.16	~~5.1. Factors to consider:~~ 5.1. Observe animals prior to sample collection ~~for~~ **to assess general condition of the animal. Consult the veterinarian prior to collecting blood from animals presenting** weakness, illness, dehydration, obesity, or anemia. ~~5.1.2. Contact a veterinarian before sample collection if the animal is exhibiting any of these signs.~~ 5.3. Limit the number of punctures to ~~four~~ **three** ~~punctures~~ **puncture trials** per ~~day~~ **timepoint** with no more than two **veni**punctures per site. 5.3. Use **For multiple sampling, consider cannulation or the use of** a **fixed** catheter ~~for multiple sampling~~ in species in which it is possible. **5.6. When possible, veins should be dilated by gentle obstruction or warming before sampling. If warming is used, the animal must be constantly observed to prevent hyperthermia.** **5.7. Following collection of blood, and before the animal is returned to the cage, there must be assurance that bleeding has stopped.**
2021.11.16	~~5.2.1 Replace isotonic fluids (i.e. fluids with the same tonicity as blood) if >10% of total blood volume is required.~~ 5.8. ~~if >10% blood volume is required~~ **When the volume of blood collected exceeds 10% of the total blood volume**, 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 or Lactated Ringer's solution).
2021.11.16	6.2 Blood volume by species Dog: circulating blood volume (ml/kg BW) ~~85~~ **80** **Ferret: circulating blood volume (ml/kg BW) 60** Marmoset: circulating blood volume (ml/kg BW) ~~71~~ **60** ~~Non-human primate: Rhesus: circulating blood volume (ml/kg BW) 56~~ ~~Non-human primate: Cynomolgus: circulating blood volume (ml/kg BW) 65~~ **Macaque:** circulating blood volume (ml/kg BW) **60** **Bird: circulating blood volume (ml/kg BW) 60** **Fish: circulating blood volume (ml/kg BW) 10** **Swine: circulating blood volume (ml/kg BW) 65** **Cow: circulating blood volume (ml/kg BW) 60** **Sheep: circulating blood volume (ml/kg BW) 60**
2021.11.16	7.1. If ~~too much~~ **the volume of** blood ~~is~~ withdrawn ~~too rapidly or too~~ **exceeds the maximum recommended volume, or if blood is withdrawn more** frequently **than is recommended** ~~without replacement (approximately 2% of the animal's body weight at one time)~~, the animal may go into hypovolemic shock.
2021.11.16	8. COMMON SURVIVAL BLOOD COLLECTION SITES Mouse - Site: **Facial** vein (Submandibular) ~~Tail tip, [1] The tail tip blood collection method should not be confused with the tail tip tissue collection for genotyping procedure~~ Rat - Site: Jugular Vein, Anesthesia Required: ~~Yes~~ **No** ~~Gerbil and hamster - Site: Lateral tarsal vein, Jugular vein~~ **Hamster** - Site: **Saphenous vein, Jugular vein** **Gerbil** - Site:**Lateral tarsal vein** Guinea Pig – Site: Jugular Vein, Anesthesia Required: ~~Recommended~~ **Yes** Rabbit - Site: ~~Marginal~~ Ear vein or ~~central ear~~ artery **Ferret - Site: Saphenous vein, Jugular vein** ~~Non-human primate - Site: Femoral vein, Cephalic vein, Saphenous vein, Jugular vein, Brachial vein~~ **Macaque -Site: Femoral vein, Cephalic vein, Saphenous vein** **Marmoset -Site: Femoral vein, Tail vein, Saphenous vein** Swine -Site: Ear vein **or artery, Jugular vein**
2021.11.16	**9. TERMINAL BLOOD COLLECTION** **9.1. Terminal blood sampling must only be carried out once the animal has been rendered unconscious using general anesthesia or euthanized by an acceptable method. When terminal blood collection is performed under anesthesia, the animal should be euthanized once blood collection is complete.** **9.2. Terminal blood collection sites:** **9.2.1. Inferior vena cava** **9.2.2. Abdominal aorta** **9.2.3. Cardiac puncture** **9.2.4. Retro-orbital plexus (rodents)**
2021.11.16	**10.5. Schultz-Darken NJ. Sample collection and restraint techniques used for common marmosets (Callithrix jacchus). Comp Med. 2003 Aug;53(4):360-3. PMID: 14524411.** **10.6. CCAC guidelines: Experimental procedures (Part A – Administration of substances and biological sampling), Draft for Public Review – September 2021.** **10.7. Lawrence MJ, Raby GD, Teffer AK, Jeffries KM, Danylchuk AJ, Eliason EJ, Hasler CT, Clark TD, Cooke SJ. Best practices for non-lethal blood sampling of fish via the caudal vasculature. J Fish Biol. 2020 Jul;97(1):4-15. doi: 10.1111/jfb.14339. Epub 2020 May 29. https://doi.org/10.1111/jfb.14339.** **10.8. Canada Department of Fisheries and Oceans Animal User Training Template, 4.0 Blood Sampling of Finfish.**
2022.09.23	6.1. Percent of blood volume collected: 7.5%, Recovery period 1-**2** weeks

Blood volumes - Mouse

Body weight (g)	Total circulating blood volume (mL)	Acceptable volume for collection (mL)		
		7.5%	10%	15%
10	0.60	0.05	0.06	0.09
11	0.66	0.05	0.07	0.10
12	0.72	0.05	0.07	0.11
13	0.78	0.06	0.08	0.12
14	0.84	0.06	0.08	0.13
15	0.90	0.07	0.09	0.14
16	0.96	0.07	0.10	0.14
17	1.02	0.08	0.10	0.15
18	1.08	0.08	0.11	0.16
19	1.14	0.09	0.11	0.17
20	1.20	0.09	0.12	0.18
21	1.26	0.09	0.13	0.19
22	1.32	0.10	0.13	0.20
23	1.38	0.10	0.14	0.21
24	1.44	0.11	0.14	0.22
25	1.50	0.11	0.15	0.23
26	1.56	0.12	0.16	0.23
27	1.62	0.12	0.16	0.24
28	1.68	0.13	0.17	0.25
29	1.74	0.13	0.17	0.26
30	1.80	0.14	0.18	0.27
31	1.86	0.14	0.19	0.28
32	1.92	0.14	0.19	0.29
33	1.98	0.15	0.20	0.30
34	2.04	0.15	0.20	0.31
35	2.10	0.16	0.21	0.32
36	2.16	0.16	0.22	0.32
37	2.22	0.17	0.22	0.33
38	2.28	0.17	0.23	0.34
39	2.34	0.18	0.23	0.35
40	2.40	0.18	0.24	0.36

Blood volumes – Rat

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