



STANDARD OPERATING PROCEDURE 303 BLOOD COLLECTION VOLUMES AND FREQUENCY

1. PURPOSE

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

2. RESPONSIBILITY

Principal investigator (PI) and their research staff.

3. INTRODUCTION

- 3.1. The acceptable quantity and frequency of blood sampling in all species depend upon the animal's total blood volume.
 - 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 user's training should be considered.
 - 3.3. Do not take more blood than necessary calculate beforehand the minimum amount of blood required to perform all tests and assays, as well as the maximum volume of blood that you can safely withdraw.
 - 3.4. Avoid Blood sampling (except for terminal blood sampling) on mice **under 14 days** of age due to the risk of hypovolemic shock.
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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).
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5. PROCEDURES

- 5.1 Observe animals before sample collection to assess the animal's general condition. Consult the veterinarian before 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 using a fixed catheter.
- 5.5. For recommendations of blood collection sites in multiple species, see Section 8.
- 5.6. Veins should be dilated by gentle obstruction or warming before sampling. The animal must be constantly observed if warming is used to prevent hyperthermia.
- 5.7. Following blood collection and before the animal is returned to the cage, there must be an 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 the collected blood volume with 3 - 4 times the volume of blood collected with isotonic fluids, i.e., fluids with the 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
10%	3-4
15%	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 (1.5ml/25g)	4.5 (~100ul/25g)	6.0 (~150ul/25g)	9.0 (0.220ul/25g)
Rat	64 (16/250g)	4.8 (1.2ml/250g)	6.4 (1.6ml/250g)	9.6 (2.4ml/250g)
Guinea pig	73	5.5	7.3	11.0
Rabbit	56	4.2	5.6	8.4
Bird	60	4.5	6.0	9.0
Fish	10	0.75	1.0	1.5
Sheep	60	4.5	6.0	9.0

6.3 For additional information, refer to **File A. SOP303 Blood Volume Annex**.

7. MONITORING

- 7.1. If the volume of blood withdrawn exceeds the maximum recommended volume or is drawn more frequently, 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 signs of hypovolemic shock are observed.
- 7.4. Signs of hypovolemic 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	Tail vein or artery	No	Small
	Facial vein (Submandibular)	No	Medium to large
Rat	Tail vein or artery	No	Small to medium
Gerbil	Retro-orbital sinus	Yes	Medium
Guinea Pig	Retro-orbital sinus	yes	Medium
	Marginal ear vein	No	Small
	Jugular vein	Yes	Large
Rabbit	Ear vein or artery	Under sedation	Large
	Femoral vein	Yes	Medium to large
	Cephalic vein	Yes	Medium to large
Sheep	Cephalic vein	No	Medium to large
	Jugular vein	No	Large
	Saphenous vein	No	Medium to large
Fish	Caudal vein	Yes	-
Bird	Brachial vein	No	Medium to large
	Jugular vein	Yes	Medium to large
	Saphenous vein	No	Small to Medium
	Claw clip	No	Small

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.
- 9.2. When terminal blood collection is performed under anesthesia, the animal should be euthanized once the blood collection is complete.
- 9.3. Terminal blood collection sites:
 - 9.3.1. Inferior vena cava
 - 9.3.2. Abdominal aorta
 - 9.3.3. Cardiac puncture
 - 9.3.4. Retro-orbital plexus (rodents only)

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Approved by the BGU Animal Policy and Welfare Oversight Committee