	UCSD INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE POLICY MANUAL	POLICY # 5.04 Originally Issued: 1/18/95
	Blood Collection	Revised: 7/13/01 Revised: 11/17/04 Revised: 4/19/17

I. Background and Purpose

This policy has been prepared to assist researchers and staff with the appropriate choice and application of bleeding techniques for many species of laboratory animals. These guidelines are based on several peer-reviewed publications, including those from ILAR and NIH, as well as regulatory guidance from the USDA. Proper training is critical for these procedures. The comfort and skill level of the phlebotomist and other factors such as sample volume, frequency of sampling, and species should be considered when selecting a method. It is the responsibility of the researcher and the IACUC to ensure the techniques and procedures chosen result in the least pain and distress to the animal, while adequately addressing the needs of the experiment. Any exceptions to this policy must be scientifically justified and approved in the animal care and use protocol.

II. Who Should Read This Policy

All personnel with assigned blood collection responsibilities on an approved protocol.

III. Definitions

Term	Definition
Mean Blood Volume	Total circulating blood volume - calculation based on 6% of body weight
Tail Tip Amputation	Removal (snip) of 1-2mm of the tip of the tail.
Tail Nick	Small superficial incision (nick) of the tail vein for low volume blood collection where the tail tip is not removed

IV. Policy

1. Training is the responsibility of the PI. Only those personnel assigned blood collection in an approved protocol may perform these procedures. All personnel must have training and proficiency documented. Training is also available through ACP.
2. For survival bleeds, the total circulating blood volume of an animal is approximately 6% of body weight. While lost fluid volume of blood may be restored in 24 hours, it takes longer to regenerate and replenish erythrocytes, platelets and other circulating factors. This is the rationale for recovery periods following blood draws. Frequency and blood volume collected must not exceed clinically established limits. These limits are described in the table in section VI.2. Any deviations must be described and justified in the animal use protocol.
3. No animal may be left unattended until hemostasis is achieved and, if applicable, it has recovered appropriately from anesthesia.

4. Exsanguination may only be performed as a terminal blood collection procedure, and the animal must be anesthetized, followed by humane euthanasia.
5. Retro-orbital bleeding in rodents may not be performed without anesthesia and scientific justification must be provided in the protocol.
6. Multiple/serial tail snip collection must be scientifically justified and approved in the animal use protocol.

V. Related Documents

UCSD Documents	Policy 13 Euthanasia Anesthetic and Analgesic Dosages for Rodents and Rabbits Safe Use of Anesthetic Gases in the Research Environment Brochure

VI. Additional information

7. General guidelines and factors to consider when selecting the blood collection technique include:
    - Species to be bled
    - Size of the animal and approximate total blood volume
    - Type of sample required (serum, whole cells, etc.)
    - Quality of sample required (sterility, tissue fluid contamination, etc.)
    - Quantity of blood required
    - Frequency of sampling
        - The use of cannulae or vascular access ports is recommended when serial samples are required over a period of days or weeks.
    - Health status of animal being bled
    - Training and experience of phlebotomist

8. Approximate blood volume samples by species based on body weight and recovery period *(USDA Animal Welfare Inspection Guide)*:

SPECIES	*Mean blood volume	AVERAGE WEIGHT	+MAXIMUM VOLUME IN MILLILITERS FOR SINGLE SAMPLING Based on Recovery Period		
			Weekly (7.5% of blood volume removed)	Every 14 days (10.0% of blood volume removed)	Every 30 days (15.0% of blood volume removed)
Mouse/Dormice (Based on mean blood volume)	72 ml/kg	20 g	0.10	0.15	0.20
		30 g	0.16	0.23	0.32
		40 g	0.20	0.30	0.45
Rat/Cotton Rat (Based on mean blood volume)	64 ml/kg	250 g	1.20	1.60	2.40
		500 g	2.40	3.20	4.80

SPECIES	*Mean blood volume	AVERAGE WEIGHT	+MAXIMUM VOLUME IN MILLILITERS FOR SINGLE SAMPLING Based on Recovery Period		
			Weekly (7.5% of blood volume removed)	Every 14 days (10.0% of blood volume removed)	Every 30 days (15.0% of blood volume removed)
Rabbit (Based on mean blood volume)	62 ml/kg	3 kg	13.0	18.0	27.0
		4 kg	18.0	24.0	37.0
		5 kg	23.0	31.0	46.0
Hamster (Based on mean blood volume)	78 ml/kg	100 g	0.50	0.70	1.10
		125 g	0.70	0.90	1.40
		140 g	0.80	1.00	1.60
Guinea Pig (Based on mean blood volume)	75 ml/kg	300 g	1.70	2.20	3.30
		500 g	2.80	3.70	5.60
		800 g	4.50	6.00	9.00
Gerbil (based on mean blood volume)	67 ml/kg	40 g	0.20	0.25	0.40
		50 g	0.25	0.30	0.50
		60 g	0.30	0.40	0.60
Rhesus/Pigtail monkey (based on mean blood volume)	54 ml/kg	3 kg	12.0	16.0	24.0
		5 kg	20.0	27.0	40.0
		8 kg	32.0	43.0	64.0
Cynomologus monkey (Based on mean blood volume)	65 ml/kg	2 kg	9.0	13.0	19.0
		4 kg	19.0	26.0	39.0
		6 kg	29.0	39.0	58.0
Squirrel/Owl monkey (Based on mean blood volume)*	70 ml/kg	0.5 kg	2.0	3.0	5.0
		1.0 kg	5.0	7.0	10.0
		1.5 kg	7.0	10.0	15.0
Common Marmoset Tamarin (based on mean blood volume)	58 ml/kg	350 g	1.50	2.00	3.00
		450 g	1.90	2.60	3.90
		550 g	2.40	3.20	4.80
Ferret (based on mean blood volume)	75 ml/kg	0.5 kg	2.8	3.7	5.6
		1.0 kg	5.6	7.4	11.2
		1.5 kg	8.4	11.1	16.8
Dog (based on mean blood volume)	86 ml/kg	7 kg	45.0	60.0	90.0
		10 kg	64.5	86.0	129.0
		13 kg	83.0	111.0	168.0
Cat (based on mean blood volume)	66 ml/kg	1 kg	4.0	6.0	9.0
		3 kg	14.0	19.0	29.0
		5 kg	24.0	33.0	49.0
Goat (based on mean blood volume)	70 ml/kg	40 kg	210.0	280.0	420.0
		80 kg	420.0	560.0	840.0
		100 kg	525.0	700.0	1050.0
Sheep (based on mean blood volume)	66.4 ml/kg	50 kg	249.0	332.0	498.0
		100 kg	498.0	664.0	996.0
		150 kg	747.0	996.0	1494.0

Calculations:

Mean blood volume x Average weight x weekly volume removed = Maximum volume for single sampling.

Examples:
Gerbil: 67 ml/kg x 0.050 kg x .075 = 0.25 ml in a single sample with 7 day recovery
Goat: 70 ml/kg x 40.0 kg x 0.10 = 280.0 ml in a single sample with 14 day recovery
Dog: 86 ml/kg x 10.0 kg x 0.15 = 129.0 ml in a single sample with a 30 day recovery

+ Calculated amounts may have been rounded to the most convenient withdrawal volume
+ Rats and mice are used as comparable species for similar exotic or wild rodents

9. Collection Sites in Mice and Rats:

   A. Lateral Tail Vein or Ventral/Dorsal Artery

      - Can be used in both mice and rats by cannulating the blood vessel or by nicking it superficially perpendicular to the tail.
      - Sample collection by nicking the vessel is easily performed in both species, but produces a sample of variable quality that may be contaminated with tissue and skin products.
      - Sample quality decreases with prolonged bleeding times and tail stroking.
      - Sample collection using a needle (cannulation) minimizes contamination of the sample, but is more difficult to perform in the mouse.
      - Repeated collections possible.
      - Relatively non-traumatic.
      - In most cases warming the tail with the aid of a warm compresses or warm water will increase obtainable blood volume.
      - Routinely done without anesthesia, although effective restraint is required.
      - Arterial sampling produces larger volumes and is faster, but special care must be taken to ensure adequate hemostasis.

   B. Tail Tip Amputation

      - A **single** tail tip amputation may be performed in both rats and mice
      - Only 1-2mm length per single amputation allowed
      - Restraint is required
      - Anesthesia is recommended
      - Yields only a few drops of blood
      - May not be suitable for older animals
      - Care must be taken to ensure proper hemostasis post-collection
      - Serial blood sampling is possible via scab removal
      - Serial amputations may not exceed total 5mm shortening of the tail and must have been scientifically justified in an approved protocol

C. Submandibular (Facial vein/artery) (*limited to adult mice*)

- Can be used in mice by piercing the submandibular vein or artery with a needle (20 gauge) or lancet (4 mm).
- Obtainable blood volumes: medium to large (100-200 ul mouse).
- Repeated sampling is possible.
- Sample quality is good
- Procedure generally performed on awake animals, but effective restraint is required.
- Ensure that gentle pressure is applied for approximately 30 seconds post-collection for proper hemostasis.
- Can be performed rapidly and with a minimal amount of equipment.

D. Saphenous Vein (medial or lateral)

- Can be used in both mice and rats by piercing the saphenous vein with a needle (23-25g mouse; 21-23g rat).
- Obtainable blood volumes: small to medium (100 ul mouse; 0.4 ml rat)
- Repeat sampling is possible
- Variable sample quality
- Procedure generally performed on awake animals, but effective restraint is required.
- Requires more hands-on training than tail vein method to reliably withdraw more than a minimal amount of blood.
- Can be more time-consuming due to time required for site preparation.
- Prolonged restraint and site preparation time can increase animal distress when handling an awake animal.
- Temporary favoring of limb may be noted following procedure
- Care must be taken to ensure proper hemostasis post-collection.

E. Retro-orbital Sinus/Plexus

- ** Due to the increased risk of complications associated with this procedure, other routes of blood collection should be considered first, such as the submandibular method.
- General anesthesia is required, including topical ophthalmic anesthetic, such as proparacaine.
- Individuals performing the procedure must be skilled and their training documented.
- Can be used in both mice and rats by penetrating the retro-orbital sinus in mice, or the retro-orbital plexus in rats, with a sterile capillary tube or pipette.
- Rapid method – many animals can be bled within a short period of time.
- Obtainable volume: medium to large.
- Sample quality is good – possible contamination with the topical anesthetic should be considered.
- A minimum of 10 days before repeat sampling of the same eye, to allow tissue repair.
- Care must be taken to ensure proper hemostasis post-collection.

- Potential complications:

    - Hematoma and excessive pressure on the eye resulting from retro-orbital hemorrhage
    - Corneal ulceration, keratitis, rupture of the eyeball or micro-ophthalmia caused by pressing on the eye to stop bleeding or from a hematoma
    - Damage to the optic nerve or other structures leading to visual deficits or blindness
    - Fracture of the orbital bones and neural damage by the capillary tube/pipette
    - Loss of vitreous humor due to penetration of the eyeball

F. Terminal Blood Collection

- Cardiac Puncture
    - Only under deep anesthesia
    - Requires proper training
    - Can be used in mice and rats by penetrating the heart
    - Obtainable volume: medium to large
    - Animal must be euthanized by a secondary physical method immediately after collection

- Pre-mortem Collection from the Aorta or Vena Cava
    - Only under deep anesthesia
    - Requires proper training
    - Can be used in mice and rats with a needle and syringe
    - Obtainable volume: medium to large
    - Animal must be euthanized by a secondary physical method immediately after collection

10. Collection Sites in Rabbits:

A. Ear Arteries and Veins

- Preferred blood collection method
- Anesthesia is not required, however, topical anesthesia is recommended.
- Both artery and vein are easily accessible from the dorsal ear
- Animal must be securely restrained
- Requires proper training
- Sterile sample collection is possible
- Catheterization is possible

B. Saphenous Vein

- Useful if aural vessels are not large enough – small rabbits
- Anesthesia not required, but recommended if animal is not conditioned to handling and/or procedure
- Animal must be in lateral recumbency to access vein

- Requires proper training
- Moderate blood volumes
- Sterile sample possible
- Catheterization possible, but hematomas may occur due to fragile vessel

C. Jugular Vein

- Anesthesia recommended to facilitate procedure
- Requires proper training – and 2 people to perform
- Large volume of blood can be collected
- Sample quality good
- Sterile sample possible
- Should not be used for frequent or serial sampling

D. Terminal Blood Collection

- Cardiac Puncture
    - ➢ Only under deep anesthesia
    - ➢ Requires proper training
    - ➢ Can obtain large volume of blood
    - ➢ Rapid collection
    - ➢ Sterile sample possible
    - ➢ Animal must be humanely euthanized following blood collection

11. Collection Sites in Guinea Pigs:

A. Lateral Saphenous Vein

- Anesthesia not required
- Repeated sampling possible by removal of scab
- Small volume obtainable
- Requires proper restraint – may need 2 people

B. Femoral vein

- Requires anesthesia
- Requires proper training
- Larger volume of blood obtainable

C. Terminal Blood Collection

- Cardiac Puncture
    - ➢ Only under deep anesthesia
    - ➢ Requires proper training
    - ➢ Can obtain large volume of blood
    - ➢ Rapid collection
    - ➢ Sterile sample possible
    - ➢ Animal must be humanely euthanized following blood collection

12. Collection Sites in Hamsters:

   A. Lateral Saphenous Vein

      - Anesthesia not required
      - Repeated sampling possible by removal of scab
      - Small volume obtainable
      - Requires proper restraint – may need 2 people

   B. Retro-orbital sinus

      - ** Due to the increased risk of complications associated with this procedure, other routes of blood collection should be considered first.
      - ** See Mice and Rats above for specifics

   C. Terminal Blood Collection

      - Cardiac Puncture
        - Only under deep anesthesia
        - Requires proper training
        - Can obtain large volume of blood
        - Rapid collection
        - Sterile sample possible
        - Animal must be humanely euthanized following blood collection

For all other species such as pigs, dogs, birds and non-human primates or additional sampling sites for the species described above, contact ACP Veterinary Services for the appropriate method(s) to use, supplies and equipment needed, specifics of restraint and anesthesia, acclimation/habituation requirements and training.

References:

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