

AIM: INTRODUCTION OF COLLECTION OF BLOOD SAMPLE FROM EXPERIMENTAL ANIMALS

Blood sample from experimental animals are frequently required for study of effects of drugs on biochemical parameters and for the study of pharmacokinetics of drugs in the experimental animals.

The sampling procedures for blood collections are of two types:

A) Non-terminal Blood collection: In this type, blood is collected from the conscious or unconscious experimental animals through a single or multiple withdrawals. Animal are not sacrificed after non-terminal blood collection.

a) Lateral Tail Vein or Ventral/Dorsal Artery:

- Can be used in both rats and mice by cannulating the blood vessel or by nicking it superficially perpendicular to the tail.
- Obtainable volume: Mouse - small to medium [50-100 ul]
: Rat – medium [0.2-0.4 ml]
- Procedure is carried out in the conscious mice or rat. The tail is dipped in warm water (about 50-60°C) or xylol is applied to the tail to increase circulation through tail vein. The needle (25-27 gauge, 0.5 to 1 length) is inserted, bevel up in the distal portion of tail vein. The blood is slowly aspirated avoiding the collapse of vein.
- Sample collection using a needle minimizes contamination of the sample, but is more difficult to perform in the mouse.
- 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.
- Repeated collection possible.
- Relatively non-traumatic.
- Routinely done without anesthesia, although effective restraint is required.
- In most cases warming the tail with the aid of a heat lamp or warm compresses will increase obtainable blood volume.
- Arterial sampling produces larger volumes and is faster, but special care must be taken to ensure adequate hemostasis.

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- Piercing the tail vein with a needle is also a way to collect a very small blood sample.

b) Mandibular Vein/Artery:

- Can be used in both rats and mice by piercing the mandibular vein or artery with a needle [20G] or stylet.
- Obtainable volume: medium to large [100-200 ul, mouse; 0.4-0.5 ml rat]
- Sample quality is good.
- The procedure is customarily done on an unanesthetized animal, but effective restraint is required.
- Arterial sampling produces large volumes very rapidly.
- Venous sampling produces medium volumes more slowly.
- Ensure that gentle pressure is applied for approximately 30 seconds post-collection to ensure hemostasis.

c) Saphenous/Lateral Tarsal:

- Can be used in both rats and mice by piercing the saphenous vein with a needle [23-25G: mouse, 21-23G: rat].
- Obtainable blood volumes: small to medium [mouse: 100 ul; rat: 0.4 ml]
- Repeat sampling is possible.
- Variable sample quality.
- The procedure is customarily done on an unanesthetized animal, but effective restraint is required.
- Can be more time-consuming than other methods due to time required for site preparation.
- After training, it requires more practice than tail or retro-orbital sampling to reliably withdraw more than a minimal amount of blood. Prolonged restraint and site preparation time can result in increased animal distress when handling an unanesthetized animal.
- Temporary favoring of the limb may be noted following the procedure.
- Care must be taken to ensure adequate hemostasis following the procedure.

d) Retro-orbital:

*Note: Due to the increased risk of complications associated with this procedure, the CPCSEA recommends that other routes of blood collection be considered prior

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to use of this method. The mandibular technique permits an equivalent volume of blood to be collected in a rapid manner with less risk or complications.

- Individuals performing the procedure must be certified by Animal Ethical Committee (AEC).
- Can be used in mice by penetrating the retro-orbital sinus with a glass capillary tube [0.5 mm in diameters] or via the retro-orbital plexus in rats with a capillary tube.
- Must be performed by a skilled operator.
- Follow-up required 24-48 hours after blood collection. If complications such as squinting or bulging of the eye are noted, an animal health report must be completed.
- Obtainable volume: medium to large
- Collection is limited to once per eye.
- In the hands of an unskilled operator, retro-orbital sampling has a greater potential than other blood collection routes to result in the following 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 stem persistent bleeding or from a hematoma
 - Damage to the optic nerve and other intra-orbital structures leading to vision deficits or blindness
 - Fracture of the bones of the orbit and neural damage by the pipette; loss of vitreous humour due to penetration of the eyeball
- Skilled personnel can conduct retro-orbital bleeding in unanesthetized mice. Anesthesia is recommended for retro-orbital blood collection in mice and is required during the training of personnel.
- In rats, the presence of a venous plexus rather than a sinus can lead to greater orbital tissue damage than in the mouse. General anesthesia must be used unless scientific justification is provided and approved by the CPCSEA. In addition, a topical ophthalmic anesthetic, e.g. proparacaine or tetracaine, is recommended prior to the procedure. Retro-orbital bleeding performed in rats by a trained practitioner represents more than “minimal or transient pain or distress” and therefore should be considered a Category 2 procedure.
- Care must be taken to ensure adequate hemostasis following the procedure.

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B) Terminal/Post-Mortem blood collection: In this type, large volume of blood is collected in single or multiple withdrawals from the anesthetized experimental animals. Animal is generally sacrificed during or after such blood collection.

Blood withdrawal by cardiac puncture or axillary cut down are considered terminal procedures and must be performed only after ensuring that the animal is under surgical anesthesia. The post-mortem collection from the aorta is performed immediately after euthanasia.

a) Cardiac Puncture

- Can be used in both rats and mice by penetrating the heart.
- Must be performed by a skilled operator.
- Obtainable volume: medium to large.
- Animal must be euthanized immediately after blood collection.

b) Axillary cut down

- Can be used in both rats and mice.
- Axillary vessels are cut with a scalpel blade or scissors and the pooled blood is collected via capillary tube.
- Obtainable volume: medium to large.
- Animal must be euthanized immediately after blood collection prior to recovery from anesthesia.

c) Pre-mortem collection from the aorta or vena cava

- Can be used in both rats and mice as a pre-mortem procedure on anesthetized animals.
- Blood is collected using a needle.
- Animal must be euthanized immediately after blood collection prior to recovery from anesthesia.
- Obtainable volume: medium to large.

d) Post-mortem collection from the aorta

- Can be used in both rats and mice as a post-mortem procedure in a euthanized animal.
- Must be done rapidly after euthanasia to ensure blood flow.
- Aorta is cut and the blood pools in the pleural cavity.
- Blood is collected in a mini capillary tube. The tube must be held continuously in a horizontal position during the blood draw.

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- Obtainable volume: medium to large

Summary of Blood Sampling Techniques

Route	Anesthesia Required		Speed		Sample Quality		Repeat Samples		Relative Obtainable Volume (approximations)		Potential for Complications	
	Mouse	Rat	Mouse	Rat	Mouse	Rat	Mouse	Rat	Mouse	Rat	Mouse	Rat
Tail Vein	No	No	Med	Med	Fair	Good	Yes	Yes	Small (50 ul)	Small (.2 mls)	Low	Low
Tail Artery	No	No	Fast	Fast	Good	Very Good	Yes	Very Good	Medium (100 ul)	Medium (.4 mls)	Low	Low
Retro-orbital	No	Yes	Fast	Med	Very Good	Good	Alternate eyes	Alternate eyes	Med.-Large (200 ul)	Med.-Large (.5 mls)	Moderate-High	Moderate-High
Saphenous	No	No	Med.	Med	Good	Good	Yes	Yes	Small-Med. (100 ul)	Small-Med. (.4 mls)	Low	Low
Mandibular Vein	No	No	Slow-Med.	Slow-Med.	Fair-Good	Fair-Good	Yes	Yes	Small-Med. (100 ul)	Small-Med. (.4 mls)	Moderate	Moderate
Mandibular Artery	No	No	Very Fast	Very Fast	Very Good	Very Good	Yes	Yes	Large (200 ul)	Large (.5 mls)	Moderate	Moderate

Blood Collection Limits

The AEC limits one time survival blood collection to 15% of an animal's blood volume in most circumstances. Serial blood sampling limit vary by species, strain, and frequency of blood collection as outlined in Tables 1 and 2. The AEC may require monitoring for anemia (using assays such as hematocrit and/or serum protein levels) when repeated collections or collection of larger volumes are required. Blood collected for diagnostics or other veterinary procedures must be considered when evaluating total volume available for experimental use. In all cases blood collection volumes should be limited to the minimum volume that will allow for successful experimentation or diagnostics.

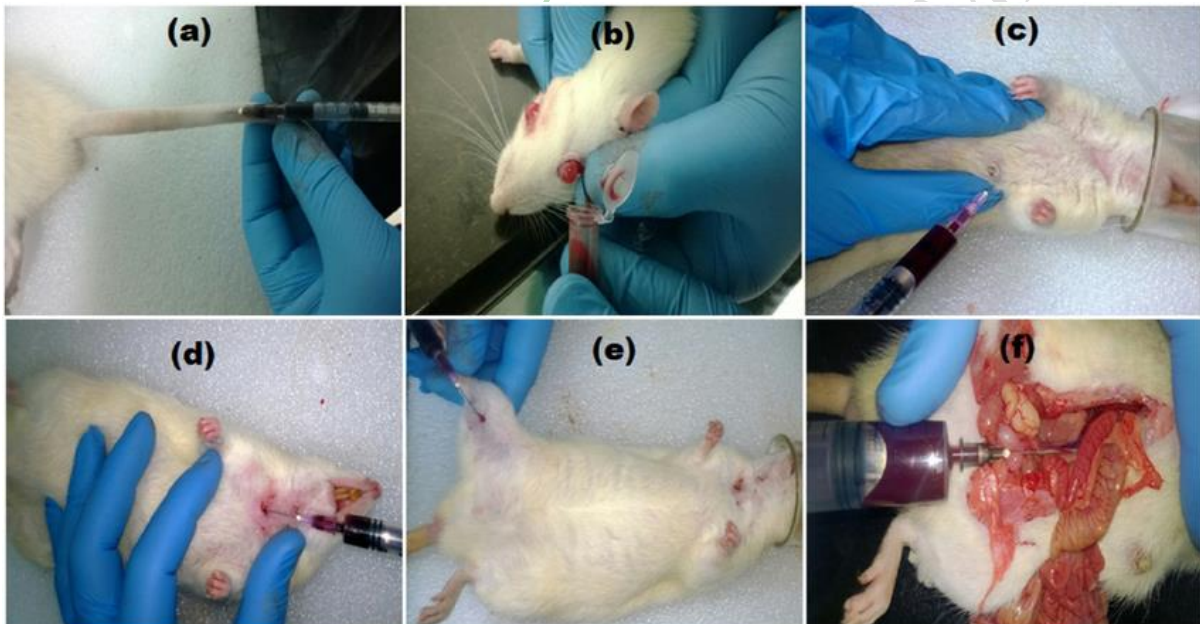
Table 1:

Species	Blood Volume Mean (ml/kg)	Blood Volume Range (ml/kg)	Blood Volume (average)		
			7.5%	10%	15%
Mouse (25 g average Wt.)	58.6	55-80	7.5%	10%	15%
Rat (250 g)	64	58-70	1.2 ml	1.6 ml	2.4 ml
Rabbit (4 kg)	56	44-70	17 ml	22 ml	34 ml
Nonhuman primate (NHP; 8 kg)	56	55-75	34 ml	45 ml	67 ml

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Table 2:

Single sampling		Multiple sampling	
% Circulatory blood volume removed	Approximate recovery period	% Circulatory blood volume removed (cumulative volume)	Approximate recovery Period
7.5%	1 week	7.5%	1 week
10%	2 weeks	10-15%	2 weeks
10-15%	4 weeks	20%	3 weeks



Rat blood sampling sites: (a) Lateral tail vein, (b) Retro-orbital sinus, (c) Cardiac puncture, (d) Jugular vein, (e) Saphenous (lateral tarsal) vein, and (f) Inferior vena cava.

TEACHER'S SIGNATURE