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A Review on Techniques of Blood Withdrawal in Experimental Small Animals

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ABSTRACT

In preclinical research, experimental studies in small animals like pharmacokinetics/ toxicokinetics, serum biochemistry and hematology necessitate the biological matrix sampling to estimate drug level, serum enzyme, and blood parameters respectively. The biological matrix is withdrawn from experimental animals by employing the recommended blood collection technique and must be conducted in compliance with the ethical guidelines. It has demanded a minimal painful and least stressful procedures, which can be achieved using inhalant/parenteral anesthesia and/or proper restraint technique. This leads to better experimental outcome. The study personnel conducting blood withdrawal must be well-versed. All these procedures must be approved by IAEC protocol and should include scientist and study personnel names who are involved in animal handling and blood withdrawal. In this review, the different techniques of blood withdrawal from small animals, including collection guidelines, limit volumes and recovery periods, and advantages and disadvantages of sites used for blood collection are demonstrated and discussed.

Keywords: *Blood withdrawal; Pain; Animal Studies; Rodents; Pharmacokinetics.*

Introduction

The blood collection procedures from animals must be approved by an IAEC protocol. The procedures used for blood collection must be less painful and less stressful. Blood can be collected with the help of anesthesia or without anesthesia. The study personnels who are involved in blood collection must have adequate skills and animal handling training. The study protocol must include procedures related to techniques used for collection of blood, appropriate time interval of blood collection, required volume, and anesthesia used. Approximately 6 % of normal body weight is considered to be total blood volume. The single blood sample collection without replenishment of fluid is limited to 15 % of total circulating blood volume for non-terminal blood collection from healthy adult animals. Four weeks of recovery period shall be maintained between the collection. The serial blood sampling for study like toxico-pharmacokinetics demanded higher volumes, which can be collected into multiple small samples. For such studies, 15% blood collection of total circulating blood volume is suggested and recovery period to be provided 2 weeks. Ideally, for multiple blood draws, a maximum of 1% of the animal's body weight can be removed, separated by a period of two weeks.

Materials

Species: Albino Mouse/Albino Rat/NZW Rabbit
Sex: Male and/or Female
Adult weight range: Mice- 19 g to 28 g , Rats – 160 g to 280 g, Rabbits- 1.5 to 3.5 kg

Vehicle: Distilled water or WFI
General Anesthesia: Isoflurane
Equipment: Syringe, needle, mice, and rat restrainer

Procedures

Restraint (Refer Restraint Procedure)

The restraint and awake animal can be used effectively for blood collection. The restraint procedure prevents animal movement that may result into laceration of a blood vessel or other organ. The restraint procedure, which involves chemical or physical can be selected based on the species and the site being used.

Collection Guidelines: Volumes

The criteria stated in table 1-3 are provided to select the maximum safe amounts of volumes for blood withdrawal. Usually, the serum fraction obtained is approximately half of the total blood volume. The circulating blood volume if it is based on the body weight then the body weight in kg will be converted to blood volume in litre.

Blood Withdrawal Techniques & Sampling Sites

The common laboratory animals employed for blood collection from various sites are demonstrated and discussed. Sites are listed from most common/ most desirable to least common/ least desirable based on ease of collection.

Key considerations

- Collection from each site in smaller species, an approximate volume of blood attainable is collected.
- In terminal procedure, the cardiac puncture may be used to collect a single, large volume from deeply anesthetized animals.
- Gauze with direct pressure may be used to stop the bleeding or to achieve hemostasis.

Lateral Tarsal (Saphenous) Vein and Tail Poke Techniques

Lateral tarsal (Saphenous) Vein

This technique can be used in small animals and large animals.
This procedure can be conducted with the help of two person or alone under light anaesthesia. The following technique is demonstrated in mouse and rat.
Requirements: Animal, rodent handling gloves, towel, cotton, sample collection tubes and 25G needle.

1. The animal can be restrained by one hand and the other hand is used to extend one hind leg or alternatively, light anaesthesia can be used.
2. This technique usually allows a volume of 5% of the circulating volume and is useful for multiple samples in PK studies.
3. The shaved foot should be used for blood collection, if necessary. Alcohol can be applied on the shaved part of skin to dilate the blood vessel.
4. The blood can be occluded by applying the gentle pressure against the flow of blood from the site of collection on leg vein, The occlusion can dilate the blood vessel.
5. 25G needle can be used to pierce the vein and allow blood to flow out of the nick. The Flow can be elevated by stroking the leg gently on pad.
6. Blood drops can be collected into a tube (refer Fig.1).
7. Immediately after collection, gentle pressure can be applied with gauze for 15-30 Sec to cease the flow of blood.
8. The animal can be kept to its cage or if anesthetized, then monitor the animal till it fully awake.

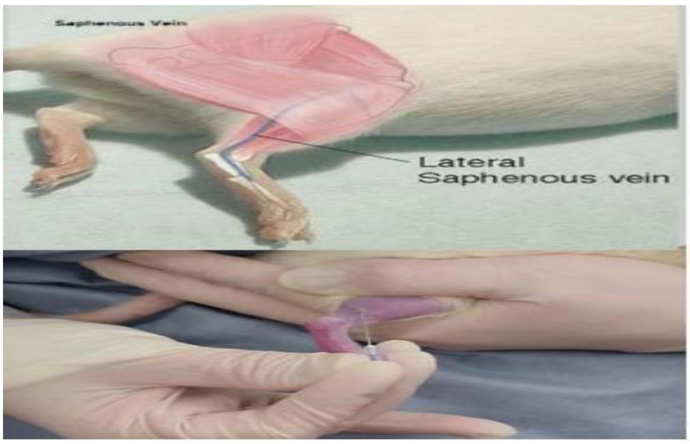

Figure 1: Saphenous vein in rat.

Tail Poke Method – This method is demonstrated in mouse:

1. In this method heat lamp can be kept over the animal's cage for 3-4 minute to warm the mice and dilate tail vessel.
2. After this, the animal placed into restraint device with the tail extended out. Alternatively, the gauze soaked in warm water can be used to rub the tail for several seconds to dilate the vessels.
3. Then, wipe the tail with 70% alcohol and allow it to dry.
4. The lateral tail vein can be exposed, located on either side of the tail.
5. The needle can be poked in lateral tail vein and approximately 2-3 cm from the tip of the tail. Then, blood can be dropped out from the nick and immediately collected into the collecting tube either by capillary tube touching to the bead of blood or by allowing the blood to drop into a collecting tube. The flow of blood can be elevated by stroking the tail from the base towards the tail end.
6. After collection, a gentle pressure can be applied to the nick with a clean cotton gauze for 15-30 seconds to cease the flow.
7. The drop of tissue glue can be applied to the nick to stop the flow of blood.
8. By this method, volume upto 0.2 ml can be collected.

Marginal Ear Vein/Central Ear Artery Technique

Requirements: Animal, anesthetic agent, cotton, 23G butterfly needles, 70% v/v isopropyl alcohol, xylene, surgical blade, and blood sample collection tube.

1. Rabbit and Guinea Pig are commonly used for blood withdrawal. The procedure is demonstrated in rabbits.
2. The animal can be placed in restrainer and 70% alcohol can be used to clean the ear site as well as dilate the marginal ear vein or xylene (topical vasodilator) can be applied.
3. A 23G butterfly needle or 24-gauge needle can be used to collect the blood from the animal's marginal vein.
4. After collection, cotton gauze can be applied on site and put gentle pressure with finger to stop the bleeding [Fig.2 and 3].

Figure 2: Central artery withdrawal

Figure 3: Marginal ear vein withdrawal

Lateral Tail Vein Technique

Blood collection method is demonstrated in rat/mouse:

1. In this technique, animals can be held in a restraint device with the tail end freely extended out of device or light anesthesia (isoflurane) can be used before restraint.
2. This route allows smaller blood volume of 0.1-0.15 ml for mice and ~ 2 mL for warmed rats.
3. A tail can be exposed to heat (heat lamp) for 2-3 min, or 70% alcohol can be used to wipe the tail. This process can dilate the lateral vessels.
4. Blood can be collected with the help of 25G needle by inserting into one of the lateral tail veins, it should be inserted an approximately 2-3 cm from the tip of the tail. (Refer Fig.4).
5. After collection of desired amount, the needle can be removed and gentle pressure can be applied with cotton gauze for 15-30 seconds to attain hemostasis.
6. The animal can be removed from the device and kept in its cage. In case of anesthetized animal, monitor the animal till it fully awake.

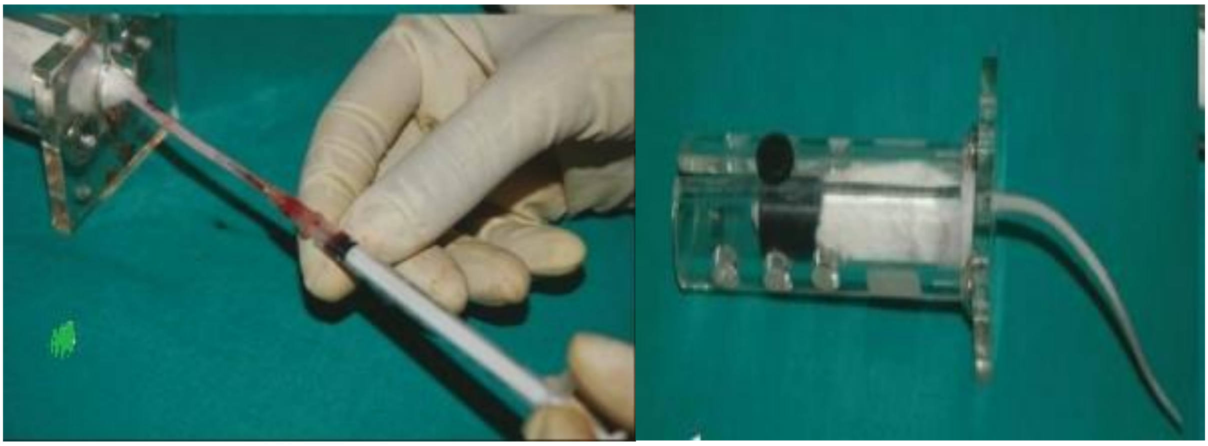

Figure 4: Blood sample collection from mouse tail vein.

Retrobulbar Plexus Technique

This technique is conducted in mouse and rat under light anesthesia:

1. The animal can be held by left hand and by other hand one drop of topical ophthalmic anesthetic can be instilled into the eye that to be bled.

2. An anesthesia method must be described in an IAEC protocol.
3. The scruff around the neck and head can be secured gently. This process can cause eyeball to protrude slightly but it makes sure that animal breathing should not be obstructed.
4. A sterile capillary tube can be inserted into the inner corner of the eye into the orbital sinus and gently pushed towards the midline, which directed towards the back of the head. Care should be taken that capillary tip should not break.
5. The blood flows down by capillary action once it is inserted into orbital sinus. (refer fig. 5 and 6).
6. After collection, capillary tube can be removed, and gentle pressure can be applied to the eye with cotton gauze for 15-30 sec to attain hemostasis.
7. To prevent the risk of infection, anti-infective eye preparation like ointment can be applied to the eye.
8. The animal can be kept into its cage and monitor till it fully awake.

Caution

- This method demands skill personnel to collect the blood.
- Two weeks of recovery must be given for next collection.
- Adverse effects like hematoma, corneal ulceration, keratitis, pannus formation, rupture of the globe, damage of the optic nerve, and necrotic dacryoadenitis of the harderian gland.

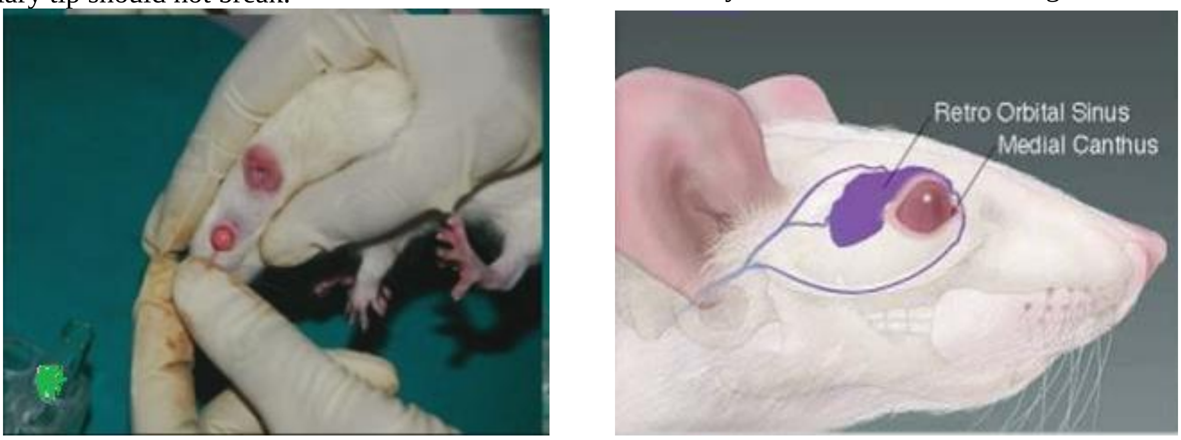

Figure 5: Blood sample collection from rat orbital sinus and Retro orbital sinus and medial canthus.

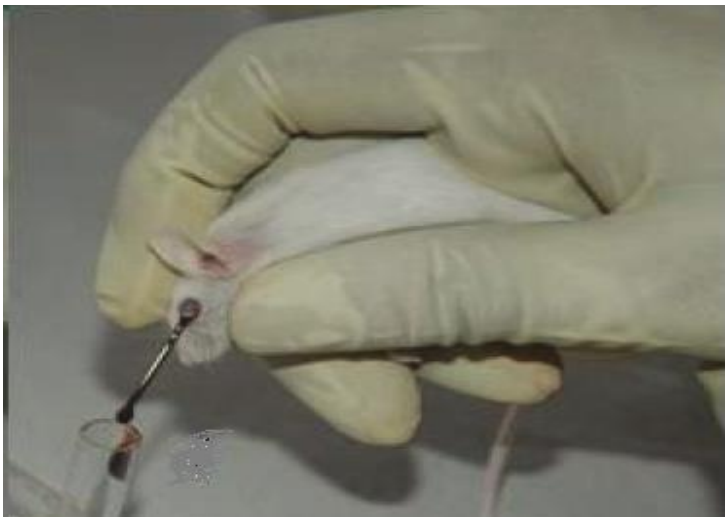

Figure 6: Blood sample collection from mouse orbital sinus.

Cardiac Puncture Technique

This technique is used to collect a large volume in terminal animal (mouse and rat) under deep anesthesia:

1. The animal can be anesthetized or euthanized; the procedure can be approved by an IAEC.
2. The animal can be turned on its back and cleaned the chest and abdomen with 70% alcohol.
3. A 25 G ½ inch needle attached to a syringe can be inserted at a 30° angle just below the xyphoid process and directed the needle slightly towards the left shoulder. A 25G ½ inch needle can be sufficient for mouse. A 23G 1 inch or longer needle can be used for rat and Hamster.
4. A vacuum in syringe can be created by slightly aspirating the plunger, then slightly pushing the needle until blood appears in the hub of the needle (refer fig.7).
5. The blood can be collected in a syringe by slowly retracting the plunger.
6. After blood collection, the needle can be removed and euthanized for the animal. Further cervical dislocation or CO_2 can be recommended for a euthanasia procedure.

Figure 7: Blood sample collection through cardiac puncture in Rat.

Cannulation Technique

The method is described to collect multiple samples in rat:

Requirements: Animal, anesthetic agent, cotton, 25G needle, i.v. cannula, surgical blade, heparin (or any anticoagulant) and blood sample collection tubes.

1. This method allows the continuous and multiple blood collection from an experimental animal.
2. This method demands close monitoring of the animal.
3. Cannulation in blood vessels, such as femoral artery, femoral vein, carotid artery, jugular vein, vena cava, and dorsal aorta can be performed.
4. To perform cannulation surgery in animals, anesthesia and analgesia can be given prior to surgery to minimize the pain. Procedure should be described in an IAEC protocol.
5. After surgery, animal can be housed singly in a spacious cage.
6. 24 h recovery can be provided, and blood samples can be collected over 24 h at the volume of 0.1 to 0.2 ml/sample.
7. After each withdrawal, the cannula can be flushed, and withdrawn volume must be replaced with an anticoagulant saline. Cannula should be closed tightly [Fig. 8].

Figure.8: Blood Vessel Cannulation of rat femoral vein.

Circulation Blood Volume in laboratory animals

Table 1 reports circulating blood volumes in different species, which are commonly considered in drug safety or kinetic evaluation.

Table 1: Reports circulating blood volumes in different species.

Species	Blood Volume (ml/Kg)	
	Recommended mean*	Range of means
Mouse	72	63-80
Rat	64	58-70
Guinea Pig	75	65-90
Rabbit	56	44-70
Dog(Beagle)	85	79-90

Macaque (Rhesus)	56	44-67
Macaque(Cynomolgus)	65	55-75
*The recommended mean corresponds to the mid-point of the range of means		

Table 2 addresses both single and multiple sampling regimes as well as time required for recovery.

Table 2: Limit volumes and recovery periods.

Single Sampling (e.g. Toxicity study)		**Multiple Sampling e.g. PK/TK study**	
% Circulatory blood volume removed	**Approximate recovery period**	**% Circulatory blood volume removed in 24h**	**Approximate recovery period**
7.5%	1 week	7.5%	1 week
10%	2 weeks	10-15%	2 weeks
15%	4 weeks	20%	3 weeks

Table 3 addresses the reference guide for the blood volumes that can be removed without significant affecting the animal's normal physiology.

Table 3: Total blood volumes and recommended maximum blood sample volumes for species of given body weight.

Species (weight)	**Blood (mL)**	**7.5% (mL)**	**10% (mL)**	**15% (mL)**	**20% (mL)**
Mouse (25 g)	1.8	0.1	0.2	0.3	0.4
Rat (250g)	16	1.2	1.6	2.4	3.2
Rabbit (4 kg)	224	17	22	34	45
Dog (10 Kg)	850	64	85	127	170
Macaque(rhesus) (5 kg)	280	21	28	42	56
Macaque(Cynomolgus) (5kg)	325	24	32	49	65

Table 4 enlists the advantage and disadvantages for blood withdrawal from different sites.

Table 4: Advantages and disadvantages for Blood withdrawal from different sites.

Route/Vein	**General Anesthesia**	**Tissue damage[a]**	**Repeat bleeds**	**Volume**	**Species**
Jugular	(Yes),No	Low	Yes	+++	(R)[#], D,Rb
Cephalic	No	Low	Yes	+++	Mac,D
[#]Saphenous/lateral tarsal	No	Low	Yes	(++),+++	(Mo),R,D, Mac
Marginal ear	No(local)	Low	Yes	++	Rb
Femoral	No	Low	Yes	+++	Mac
Lateral tail	No	Low	Yes	(+),++	(Mo), R
Central ear artery	No(local)	Low	Yes	+++	Rb
Tail tip amputation	Yes	Mod	Limited	+	Mo, R

***Retrobulbar plexus**	Yes	Mod/high	Yes	+++	Mo, R
#Cardiac[b]	yes	Mod	No	+++	Mo/R/Rb
Continued					

R-Rat, D-Dog, Rb-Rabbit, Mac-Macaque, Mo-Mouse, Mod-Moderate

[a] The tissue damage liability is depend on the incidence likely occurs and the severity of any injury results into inflammatory reaction or hematoma.

[b] Performed only for terminal procedure under deep general anesthesia.

+-small,++- moderate,+++-large

*Performed by train person as the untrained procedure may result in to ophthalmic damage, further post bleed monitoring also required.

Training needed

Table 5 enlists recommended sites for repeated blood sampling.

Table 5: Recommended sites for repeated blood sampling.

Species	Recommended site
Mouse	Saphenous, lateral tail
Rat	Saphenous, lateral tail
Rabbit	Marginal ear, central ear artery, jugular
Dog	Cephalic, jugular, saphenous
Macaque	Cephalic, saphenous, femoral

Observations

Blood collection is commonly performed while an animal is awakened using appropriate restraint technique. Restraint could be chemical or physical in nature. Chemical e.g. isoflurane and physical e.g. acrylic restrainer. When the blood samples are collected at frequent intervals, then a recommended maximum blood collection to be 1 % of the animal's body weight, with a recovery period of two weeks.

Different sampling sites used for blood collection are as follows**:**

1. Saphenous vein is mostly used to collect blood from mouse and rat. This technique is excellent for serial sampling with moderate volume of blood can be collected.
2. A tail poke technique is used to collect blood from mouse. By this technique serial sampling with limited to small sample sizes can be collected. After collection, tissue glue is applied to the site.
3. Marginal ear vein or central artery is used to collect blood from rabbit. Rabbit holder is used for blood collection and local anesthesia can be applied on the collection site 10 min prior to sampling.
4. Lateral tail vein technique is used to collect blood from rat/mouse. Restrainer devices and light anesthesia can be used while blood collection. This technique yields smaller blood volumes.
5. Orbital sinus or plexus is used for large samples to be collected under light anesthesia from mouse and rat. The IAEC- approved protocol is essential for blood collection. This technique requires training as it may cause ophthalmic complications.
6. Cardiac puncture (cardiocentesis) is only used for terminal procedures under deep general anesthesia in mouse and rat. By this technique maximum blood can be collected.
7. Cannulation is the most commonly used technique in multiple sampling studies (PK) in rat. This technique is used for continuous and multiple sampling usually from femoral or jugular vein. The surgical procedure of cannulation must be described in IAEC- approved protocol.

Inference & Conclusion

1. Different techniques of blood collection for mice, rats and rabbits are demonstrated. In rodents like mice and rats, commonly used techniques are saphenous vein, tail poke, lateral tail vein and retro bulbar plexus, which could be used depending on the sample size. Cannulation (femoral or jugular vein) in rats is the most common technique for multiple sampling (e.g. PK/TK). Further, rabbit marginal ear vein is the most common site for blood collection. Cardiac puncture in mice or rats is used only for non-survival procedure.

2. Procedures of surgical and general anesthesia must be described in an IAEC approved protocol e.g. blood collection by cannulation technique.
3. For various species, maximum blood sampling volumes are recommended.
4. Advantages and disadvantages of various methods of blood collection and recommended sampling volumes for various species are demonstrated.
5. Single sample and multiple samples collected in TK/PK studies from different species with recovery periods are discussed.

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