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Review Article

Blood collection guidelines

Blood Collection Guidelines and Techniques in Small Laboratory Animals for Biomedical Research: A Systematic Review

Leena Rajathy Port Louis[1], Prithiviraj Nagarajan[2*] and Asokan Balakrishnan Ramajayam[3]

[1]Animal Welfare Officer, Central Animal House, Department of Pharmacology, Aarupadai Veedu Medical College & Hospital, Vinayaka Mission's Research Foundation (Deemed to be University), Kirumampakkam, Puducherry-607403, India,

[2]Assistant Professor, Multi-Disciplinary Centre for Biomedical Research, Aarupadai Veedu Medical College & Hospital, Vinayaka Mission's Research Foundation (Deemed to be University), Kirumampakkam, Puducherry-607403, India .*

[3]Professor and Head, Department of Pharmacology, Aarupadai Veedu Medical College & Hospital, Vinayaka Mission's Research Foundation (Deemed to be University), Kirumampakkam, Puducherry-607403, India.

Abstract: Venipuncture, phlebotomy, or blood collection is a specific and important procedure for collecting samples from research animals. Many research techniques require multiple blood samples to analyze the data. In truth, laboratory rodents are smaller in size and have a relatively low blood volume compared to humans. Any major reduction in blood volume would have an extreme consequence on the animal's biology. Consequently, the safe amount of blood samples that can be obtained from an animal and the frequency of safe blood collection are the two extremely important factors to consider for protecting the health and welfare of the animals. Because stress to the study animal affects the research outcome, the techniques used should allow blood sampling while diminishing the potential for pain, suffering, avoidable stress, or unexpected results in the study animal. Several regulatory bodies have issued guidelines for collecting blood from study animals. Information on general blood collection guidelines and the different blood collection sites in laboratory animal species are scattered across various literature. This review presents a consolidated data on all the guidelines and precautions involved in blood sampling from small laboratory animals. Using suitable keywords, reference documents were collected from online databases like PubMed, Embase, Scopus, Medline and Google Search. However, researchers are the better judges in choosing the right blood collection method to use in their study, keeping in mind the welfare and health of their research animals.

Keywords: Blood collection, blood sample, small laboratory animals, sampling techniques, rodents

***Corresponding Author**

Prithiviraj Nagarajan , Assistant Professor, Multi-Disciplinary Centre for Biomedical Research, Aarupadai Veedu Medical College & Hospital, Vinayaka Mission's Research Foundation (Deemed to be University), Kirumampakkam, Puducherry-607403, India

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1. INTRODUCTION

1.1. The need for blood sample collection

Blood is often collected from laboratory animals for a variety of experimental purposes, including:

- For the analysis of biochemical, toxicological, metabolic or immunological parameters.
- For screening or culturing of microorganisms.
- For the production of antibodies.
- For determination of pharmacokinetics.
- For clinical pathology evaluation.
- For hormone analysis, etc.

This review is focused on presenting consolidated guidelines on blood sampling and authorized sampling techniques that can be effectively used on small experimental animals like rodents (mice, rats, gerbils, and hamsters), lagomorphs (rabbits), and non-rodents (guinea pigs) with the objective that it will function as a single reference document for animal researchers. We collected research and review articles using databases like PubMed, Scopus, MEDLINE, and Embase and Google web search using suitable keywords like laboratory animal, blood collection, blood sample, rodents, rabbit blood collection, etc.

1.2. Classification of blood collection

Blood collection can be divided into two categories: survival and nonsurvival or terminal blood collection. "Survival blood collection" is called so when blood is obtained from animals that are expected to survive the procedure, and in "nonsurvival" or "terminal blood collection", collection of blood is the last event before animals are sacrificed. Both methods may or may not require anaesthesia/sedation (Table 1), to reduce stress in animals and help in comfortable blood collection. While the amount of blood obtained from an animal to be sacrificed is unrestricted, the amount of blood collected from animals that survive collection is restricted to avoid complications like anaemia and hypovolemic shock. As a general rule, during survival blood collection, no more than 10% of the total blood volume of an animal should be obtained in any 14-day time. A maximal amount of 0.6 ml per kg per day, or 1.0 per cent of the animal's body weight, shall be drawn every 24 hours when repeated blood samples at short intervals are needed.[1] While the lost fluid volume of blood can be recovered in about 24 hours, the regenerative replenishment of erythrocytes, platelets, and other circulating components takes longer. This is why there is a recovery interval (i.e., the interval between blood collection events) after the blood is drawn.

1.3. Blood collection techniques or methods

Collecting blood from laboratory animals can be done using a variety of methods. For example, blood could be collected either from an artery or a vein (from live animals) or from heart chambers (an anaesthetized or recently euthanized animal).

1.3.1. Survival blood collection techniques that do not require anaesthesia

a) Lateral Saphenous Vein (mice, rat, gerbil, hamster, rabbit, guinea pig)
b) Lateral Tail Vein (mice, rat, gerbil)
c) Marginal Ear Vein (guinea pigs, rabbit) and Ear Artery (rabbit)
d) Tarsal Veins (guinea pig)
e) Facial or Submandibular Vein (mice)
f) Ventral Tail Artery (mice, rat)
g) Dorsal Pedal Vein (mice, rat)

1.3.2. Survival blood collection techniques that require anaesthesia

a) Retro-orbital Sinus (mice, rat, gerbil, hamster)
b) Jugular Vein (mice, rat, gerbil, hamster, rabbit, guinea pig)
c) Cranial Vena Cava (hamster, rat, guinea pig)
d) Femoral Vein (guinea pig)
e) Sublingual Vein (rat, hamster)
f) Submandibular Vein (rat)
g) Gingival Vein (mice, rat, hamsters, guinea pigs)
h) Tail Tip Amputation (mice, rat)
i) Penile Vein (rat, guinea pig)

1.3.3. Nonsurvival blood collection techniques that require anaesthesia

a) Cardiac Puncture or Cardiocentesis (mice, rat, gerbil, hamster, rabbit, guinea pig)
b) Aorta or Caudal Vena Cava (mice, rat, gerbil, guinea pig, hamster)
c) Decapitation (mice, rat, guinea pig)
d) Axillary Plexus Dissection (mice, rat)
e) Carotid Artery (rabbit)

1.4. Requirements for blood collection

Analgesics, anaesthetics, syringes, needles, blood collection containers, lancets, tourniquets, butterfly syringes, etc. Blood collection in rodents requires special micro-collection tubes treated with anticoagulants for plasma or untreated for serum. Most tests work on both plasma and serum. If the blood volume is limited, choose plasma, as it gives a higher yield per sample than serum. For example, 0.4 ml of blood provides 0.15 ml of serum and 0.2 ml of plasma. Anticoagulants of choice for plasma collection include heparin and ethylenediaminetetraacetic acid (EDTA).[2]

1.5. Choice of technique

Before starting any blood collection technique, be sure it aligns with your experimental goals. Several factors will influence the technique used, including:

- The animal's species, age, size, health status, and total blood volume
- Quantity of blood sample required (in micro or millilitres)
- Sample type required (serum, blood cells, etc.)
- Sample quality needed (sterility, tissue or body fluid contamination, etc.)
- Frequency of sample collection (when serial samples are needed for about days or weeks, cannulae or vascular access ports are suggested.)
- The purpose for which the sample is collected (biochemical analysis, DNA extraction, etc.)

- The necessity for repeat or terminal operations
- The competence and expertise of the individuals involved in sample collection
- Influence of the blood collection site, type of restraint used, or anaesthesia on the blood parameter estimated.[3]

Table 1: Common anaesthetic agents used in laboratory animals [1,28]

Species	Drug	Dose	Route	Duration of Anaesthesia	Comments
Mice	Ketamine + xylazine	80-100 mg/kg Ket + 10-12.5mg/kg Xyl	IP	20-30 minutes	Thermal support and eye lubricant are important. If more anaesthetic is required, supplement with 1/3 dose of ketamine only. Xylazine is reversed with 1 - 2 mg/kg yohimbine IP
	Ketamine + Xylazine + Acepromazine	60-100 mg/kg Ket + 10-15 mg/kg Xyl + 2-5 mg/kg Ace	IP	60-90 minutes	
	Pentobarbital	40-70 mg/kg	IP	20-40 minutes	Nil
	Isoflurane	1-3% for maintenance; up to 5% for induction.	Inhalation	30 minutes	Concurrent preemptive analgesia is needed for survival surgery.
Rat	Ketamine + xylazine	40-100 mg/kg Ket + 5-13 mg/kg Xyl	IP	60-80 minutes	Thermal support and eye lubricant are important. If more anaesthetic is required, supplement with 1/3 dose of ketamine only. Xylazine is reversed with 1 - 2 mg/kg yohimbine IP.
	Ketamine + xylazine + acepromazine	20-50 mg/kg Ket + 2-10 mg/kg xyl + 0.5-1.5 mg/kg Ace	IP	60-120 minutes	
	Pentobarbital	30-50 mg/kg	IP	90-120 minutes	Nil
	Isofluorane	4% (v/v) for induction; 2-3% (v/v) for maintenance	Inhalation	30 minutes	Survival surgery needs concurrent preemptive analgesia.
Rabbit	Ketamine + xylazine	10-40 mg/kg Ket + 3-5 mg/kg xyl Recommended starting point is 10 mg/kg Ketamine and 3 mg/kg Xylazine.	IM	NA	Thermal support and eye lubricant are important. Supplement with 1/3 dose of ketamine only to prolong anaesthesia. Xylazine is reversed with an equal volume of Atipamazole or Yohimbine 0.2-1.0 mg/kg IV
	Ketamine + Xylazine + Acepromazine	10 mg/kg Ket +3 mg/kg Xyl +0.25- 0.75 mg/kg Ace	IM	NA	
	Pentobarbital	20-60 mg/kg	IV, IP	NA	Nil
	Isoflurane	3-5% 1-3% maintenance, up to 5% for acquisition of surgical plane of anaesthesia	Inhalation	NA	Using isoflurane as the sole agent of anaesthesia induction can be associated with breath-holding and distress.
Guinea pig	Ketamine + Xylazine	40-50mg/Kg Ket + 5 mg/kg Xyl	SC, IP, IM	60 - 90 minutes	Xylazine is reversed with yohimbine 1-2 mg/kg IP or with an equivalent volume of atipamezole 0.5 mg/kg IM or IP as the dose of xylazine.
	Ketamine + Xylazine + Acepromazine	50mg/kg Ket + 5mg/kg Xyl + 1mg/kg Ace	IP, SC, IM	30 minutes	The ophthalmic ointment is vital. Atipamezole is the antidote for xylazine @ 0.2ml/100g body weight SC or IV.
	Isoflurane	3-5%	Inhalation	NA	Guinea pigs salivate profusely when induced anaesthesia with gases. So, premedicated with atropine (0.05mg/kg SC), then induce with isoflurane in a chamber.
	Pentobarbital	40 mg/kg	IP	60 minutes	Poor analgesic effect. AVOID

		30 mg/Kg	IV		buprenorphine co-administration, which will result in cardiorespiratory depression.
Hamster	Ketamine + Xylazine	200 mg/kg Ket + 10 mg/Kg Xyl	IP	NA	Nil

Atropine @ 0.02 - 0.05 mg/kg (SC, IM, or IV) is to be used as a preanesthetic in all species to reduce bronchial secretion and salivation and protect the heart from vagal inhibition. NA – data not available

2. COLLECTION OF BLOOD FROM LABORATORY ANIMALS: GENERAL PRINCIPLES OR GUIDELINES

The procedure used and the volume of blood drawn are determined by the research animal species, the frequency of blood sample collection, and the experiment's objectives. Researchers should plan and execute each sampling method considering the stress involved with blood loss to reduce animal suffering to the greatest extent possible. Good organization and control of blood collection and all the associated variables of the experiment increase the well-being of the animals and the quality of the research findings. [4]

2.1. Total blood volume of animals

- In general, an animal's blood volume is 6 to 8 per cent of its total body weight[5,6], with some species having a higher or lower amount depending on the species of origin.
- It is generally accepted that 60 to 80 ml/kg of blood flows throughout the circulatory system. Table 2 lists the total and average blood volumes of several laboratory animals.
- The total blood volume in overweight and aged animals will be 15 per cent lower than in young animals; thus, use caution when calculating the blood collection volume in such animals.

2.2. Approved blood collection volume

- Blood collection is calculated based on the animal's body weight or total blood volume.
- The maximum amount of blood that can be collected for over 14 days without the need for additional fluid supplements is 1% of body weight or 10% of the total blood volume of the animal. This applies to both total and repeated blood sampling. Calculate the total volume required over 14 days for irregular blood collection schedules.
- Blood samples of 0.07 % of body weight can be taken daily without the need for additional fluids.
- Exsanguination can collect 4 to 5 per cent of the body weight in blood.
- It is safe to collect about 10% of the total circulating blood volume every 2 to 4 weeks, 7.5% every 7 days and 1% every 24 hours[4,6,7] (Table 2).

Table 2: Laboratory animal's total blood volume, safe single bleed volume, and exsanguination blood volume

Species	Body weight Range (g)	Total Blood Volume in Range (ml/Kg)	Average Blood Volume (ml/Kg)	Total blood volume (TBV), adult (ml)		Safe volume for single bleed @ 10% TBV (ml)		Bleed-out volume or exsanguination (ml)	
				Male	Female	Male	Female	Male	Female
Mice	18-40	62-80	71	1.5 -2.4	1.0 - 2.4	0.1 - 0.2		0.8 - 1.4	0.6 - 1.4
Rat	250 - 500	58-70	64	29 - 33	16 - 19	2.9 - 3.3	1.6 - 1.9	13 - 15	7.5 - 9
Hamster	85-150	64-80	72	6.3 -9.7	7.1 - 11.2	0.6 - 0.9	0.7 - 1.1	2.9 - 4.5	3.3 - 5.2
Gerbil	55 - 100	59-85	72	4.5 - 7	3.8 - 6	0.4 - 0.7	0.4 - 0.6	2.2 - 3.5	1.9 - 2.9
Guinea Pig	700 - 1200	64-90	77	59 - 84	48 - 63	6 - 8	5 - 6	29 - 42	24 - 31
Rabbit	1000 - 6000	44-70	56	58.5 - 585		5 - 50		31 - 310	

When more frequent sampling is required, the blood of around 0.5% of the body weight may be collected once because it will require two weeks or more for all the components of the blood to come back to normal, even though the blood volume will be returning to normal within 48 hours after collection. In unhealthy animals, this process takes considerably longer. [7] Keeping in mind the species and age of the animals, there are variations in total blood volume. To be safe, draw blood only once every 14 days, or utilize published charts that demonstrate the blood volume that can be obtained when estimated based on 0.08 per cent, 1 per cent, or 10 per cent of the body weight of the animal. [8] Remember to collect the required blood volume only and that the serum output is only around half of the entire blood volume.

Examples of blood volume that can be collected when 1% of an animal's body weight is considered are:

a) It is safe to collect 0.2 ml of blood from a 20-gram mouse over two weeks, whereas 0.4 ml taken from a 20 g mouse for over 2 weeks needs fluid replacement with 0.4 ml saline by subcutaneous (SC) route.

b) Blood samples up to 3 ml from a 300-gram rat over 2 weeks is acceptable, whereas up to 6 ml from a 300-gram rat over 2 weeks needs replacement with 6 ml saline by SC route.

c) Blood sample up to 30 ml from a 3-kg rabbit over 2 weeks is acceptable, whereas up to 60 ml from a 3-kg rabbit over 2 weeks needs replacement with 60 ml saline by SC or IV route.

- There are many publications and regulatory guidance from USDA on recovery time between blood collections (Table 3).

Table 3: Recovery time and replacement fluids for blood collection based on circulating blood volume

Circulating Blood Volume	Body Weight Equivalent	Volume per unit Body Weight	Recovery Time	Replacement Fluid
7.5%	0.6%	6 ml/kg	1 week	Optional
10%	0.8%	8 ml/kg	2 weeks	Recommended
15%	1.2%	12 ml/kg	4 weeks	Required

2.3. Formula for calculating blood sample volume

Blood sample volume = body weight in g [or kg] x mean blood volume (ml/g) [or (ml/kg)] x per cent of blood volume to be removed (as a decimal, e.g. 10% = 0.10)
For example, 25 g mouse x 0.071 ml/g = 1.78 ml TBVx 0.10 = 0.18 ml
Based on the above formula, it is allowed to collect 0.18 ml of a blood sample from a 25 g mouse without any fluid replacements. The animal should be given a recovery period of 2 weeks before the next blood collection. [3]

- If repeated blood samples are needed, cannulation should be considered as a low-stress alternative to frequent venepuncture.
- When calculating blood volume based on body weight, body weight in kilograms gives blood volume in litres and grams yield blood volume in millilitres (ml). One drop of blood equals 0.05 ml (50µl) for calculations.

2.4. Exsanguination

At terminal exsanguination, it is possible to collect about 50 to 75 % of total blood volume (that is 4-5% of its body weight) as a blood sample. Exsanguination must be performed on deeply anaesthetized or freshly euthanized animals. In deeply anaesthetized animals, as the heart is still beating, more blood samples can be collected compared to dead animals.

3. BLOOD COLLECTION SITES AND PROCEDURES

There are different sites for collecting blood from various laboratory animals. Table 4 represents the various blood collection sites, sample volumes in different species, equipment needed, and adverse effects.

3.1. The saphenous Vein, lateral

Blood collection from the lateral saphenous Vein is rapid but requires additional preparation time in a conscious animal. The Vein runs over the tarsal joint at first dorsally and then laterally. Unanesthetized animals must be manually restrained or use a mechanical restraint device. For blood collection, immobilize the hind leg by gently pressing down just above the knee joint, so it becomes easy to clip the hair and stabilize the saphenous Vein. Hair removal using a scalpel blade is not suggested because of the removal of the epidermal layer of skin. Following aseptic precautions, it is advisable to use a hair trimmer or a hair removal cream. Applying sterile petroleum jelly to the puncture location helps form blood droplets, increasing the amount of blood collected. Anaesthesia is not required but can be used for hard-to-contain animals. Sedatives which cause peripheral vasodilatation should be used in low doses to reduce extended bleeding from the perforation site. Blood samples should be taken with fewer attempts (keep the needle sticks to less than three in one attempt). The Vein can be pierced with a needle and blood can be collected passively into a tube or by capillary action into a haematocrit tube (Figure 1). Stop the blood flow by applying gentle finger pressure on the punctured site or by simply releasing the grip on the leg of the animal. This method allows a maximum of four blood samples in 24 hours. Temporary or surgical cannulation is recommended if more samples are needed. Several samples can be collected from the same puncture site by removing the scab or blood clot. [8]

3.2. Lateral tail vein

More blood (about 2 ml in rats) can be collected by cannulating the lateral tail vein or making a superficial nick perpendicular to the vessel. This method works for all mouse strains except for black or pigmented mice, whose vasculature is difficult to view through their skin. Warming the animal before collecting the sample dilates the blood vessels. We can utilize a warming cabinet no hotter than the animal's body temperature. To avoid fighting, warm the male mice individually. But warming the animal causes stress, and salivation causes dehydration and increases metabolic rate, affecting the experimental data. To avoid leukocytosis, do not rub the tail from the base to the tip. [8] To dilate the Vein, immerse the tail in 40°C warm water for a few minutes. Then insert a needle into the blood vessel and collect blood in a capillary tube or in a syringe and needle (Figure 2). The tail can be cleansed and washed with an antiseptic solution to increase visibility and dilate the blood vessel. This is beneficial in pigmented mice. If necessary, illumination devices can be used to visualize the tail vein. Only two blood samples should be taken every 24 hours to avoid bruising and tail damage. When multiple sampling is required on the same day (e.g., glucose tolerance test), the scab or clot may be gently soaked and removed for blood collection. A temporary or surgical cannula should be employed for more than four samples. No more than three needle pokes should be used for a single blood sample. For serial samples, alternate the sides of the tail and poke about one-third of the way down the tail's length from tip to base. Generally, cannulation and tail nicking is routinely done without anaesthesia, but with effective restraint. After sampling, stop the blood flow by pressing on the soft tissue for a few seconds before releasing the animal.

3.3. Marginal ear vein

Blood sampling from a rabbit's marginal ear vein or artery is one of the most common and least invasive procedures. This approach applies to all rabbit strains for both single and multiple sample collections. The ear artery is most commonly used to collect more volume or arterial blood samples, although there is a higher risk of hematoma and bruising. The rabbit must be carefully restrained or wrapped in a thick cloth or towel to prevent sudden movements. Since the restraint creates stress, its duration must be limited. A local anaesthetic cream may be administered to the site approximately 30 minutes before the blood sampling. Begin the blood sample at the tip of the ear, away from the base. Serial blood collection can be done by switching ears or by moving to the bottom of the ear in the same Vein. Gently massaging the ear helps dilate the vessel, and a heat lamp can be used if necessary. For animals that are difficult to hold, anaesthesia may be used for welfare reasons. The ear vein can be cut or pierced with a needle for blood collection. A surgical blade of size 11 can be utilized to cut the marginal ear vein and collect blood into a collection tube or into a needle with a syringe (Figure 3). Before inserting the needle, the Vein is usually blocked distally. Eight samples can be taken in 24 hours via the marginal ear vein. To avoid injury to the ear, keep needle sticks to not more than three in any attempt. Apply finger pressure to the site of the blood sample for approximately two minutes to stop blood flow before returning the animal to its cage or enclosure. [1]

3.4. Tarsal Vein

This procedure can be performed on all guinea pig strains, although it is difficult to operate, and the risk of subcutaneous bleeding is high. Two persons are needed, the first person to restrain the animal and the second to collect the blood. The tarsal Vein is readily approachable when the guinea pig is confined. Gently massage the area (closer to the animal) where the blood sample will be obtained to dilate the blood vessel. Each hind leg can be used to collect three samples, each containing 0.1 to 0.3 ml of blood. Samples should be obtained from both the hind legs, starting distal (between the toes) and moving proximal (towards the ankle) to the animal. Surgical cannulation should be explored to collect more sample volume. [1]

Caution

- A maximum of only two-needle sticks is allowed per attempt.
- At most, six samples are to be taken from both hindlegs.

3.5. Superficial temporal or submandibular or facial Vein

Taking blood samples from a superficial temporal or submandibular vein is a secure and simple procedure in mice. Perfecting this technique requires training. In this procedure, serial sampling is possible by taking turns in pricking both sides of the face. This procedure generates a sample of mixed venous and arterial blood. The size of the needle or lancet used to pierce the site helps in sample volume control. The use of lancets helps manage the depth of vein penetration and prevent further complications. [10] In this technique, restrain the animal with the skin of its back, neck, and tail to ensure venous congestion and immobility of the head and body. Puncture using a lancet in an area on the right cheek 3 mm caudal and 1 mm dorsal to the lateral tactile hairs. Collect the blood droplets from the punctured Vein into a suitable container (Figure 4). Release the restraint to stop bleeding. Gently press the punctured area using a cotton swab for 30 seconds after collection to ensure good haemostasis. [11]

3.6. Ventral tail artery

The ventral tail artery facilitates the collection of a large sample volume with minimal animal strain. A skilled person can perform this on a unaesthetized restrained animal. The animal must be confined or placed in a dorsal recumbent position. Vasodilation and vascular access can be accomplished by either heating the entire animal or the tail. Start puncturing the artery from the mid-point of the tail (repeated attempts should be made at the tail slightly proximal and with a fresh needle). A 25 to 28G needle is inserted at approximately 20-degree angle from the skin, with its slope facing away from the artery. The needle may be inserted parallel to the tail, right below the skin, by bending the tail. Slowly insert the needle into the artery to visualize it. Reduce the needle angle and route it 2 mm cranially after piercing the artery wall. Aspirate a small quantity of blood into the syringe. When very small quantities of blood are required, use a needle without a syringe, allow the needle hub to fill up with blood, and collect it into a microhaematocrit tube. Remove the needle after collecting the blood and gently apply pressure to the puncture site to halt the blood flow. Check for bleeding before reintroducing the animal to its cage.

3.7. Dorsal pedal vein

Restrain the animal using a restrainer. Find the medial dorsal pedal vein at the top of the foot. Sanitize the foot with absolute alcohol and puncture the Vein using a 23G or 27G needle. Collect the blood droplets from the skin's surface into a capillary tube and apply finger pressure to ensure proper haemostasis. [1]

3.8. Retro-orbital Sinus or plexus sampling

The Sinus around the eyeball helps obtain large amounts of blood in mice, but it is not advised in rats. To get blood from this site, general anaesthesia is required. Grasp the anaesthetized mouse so that the palm of your left hand supports the back of the mouse (right hand in left-handed persons) with its head facing your thumb. Place your thumb on the lateral side of the mouse's trachea to compress the jugular vein from the same eye from which you are collecting blood. As a result, the animal's eye slightly bulges. Take care not to impede the trachea! Then insert a 50 µL sterile microhaematocrit or capillary tube into the medial canthus of the eye, rotating slightly as well as directing the tube behind the eyeball. Apply sufficient pressure to sever the fibrous layer that surrounds the Sinus. Blood begins to flow in and around the tube after the sinus ruptures (Figure 5). If no blood appears quickly, carefully withdraw the capillary to

allow blood flow. For mice and hamsters, capillaries with an outer diameter of 0.8 mm are recommended, whereas rats require capillaries with an outer diameter of 0.9 mm. Doubling capillary diameter leads to a fourfold increase in eye damage. Before withdrawing the capillary tube, loosen the grip on the animal's scruff to reduce bleeding into the tissue. [12]After blood collection, remove the tube, close the eyelids, and gently dab the eye with a dry cotton pad to prevent retro-orbital haemorrhage. Avoid collecting blood from the same eye more than twice and space collections by at least two weeks. Following bleeding, antibiotic ophthalmic ointment is applied to the eye. Although researchers have been using the orbital venous sinus technique, various undesirable side effects have been reported. [13] Concerns over animal welfare have grown in recent years as a result of these harmful repercussions. Hence, other techniques are developed to further scientific goals while also improving animal care.

Caution

- Not useful for repeated blood sampling.
- Sampling is possible only for skilled personnel.
- More chances of eye damage.
- Minimum 2 weeks should be given for the next sampling.

3.9. Jugular sampling

This technique is followed when a large volume of survival blood collection is required. This procedure produces a high-quality sample. Although anaesthesia is not necessary for jugular sampling in rats, it significantly assists the method and decreases the chance of injury to the animal or person (e.g., bites, needle sticks). However, it is not suitable for repeated or serial sampling. Shave the animal's neck and maintain it in a hyperextended posture. Apply a topical anaesthetic cream 30 minutes before sampling. [14] The jugular vein is visible as a blue-coloured structure 2 to 4 mm on the lateral side of the sternoclavicular junction. Insert a 25 G needle in the caudocephalic direction, not entering more than 3 to 4 mm into the blood vessel and withdraw blood slowly to prevent the collapse of blood vessels (Figure 6). After blood collection, arrest the bleeding using finger pressure. If the blood collection attempt fails, withdraw the needle gently and monitor the site for haemorrhage. If there is no haemorrhage, repeat the procedure. Keep collection attempts to a maximum of three.

3.10. Cranial vena cava

The cranial vena cava is the most prominent and accessible blood vessel in rodents[15,16]. Still, it may result in accidental penetration of the pericardial sac and haemorrhage into the thoracic cavity if wrongly performed. [17] Under dorsal recumbency, the piercing point is just cranial to the first rib, 0.3 to 0.8 cm lateral to the manubrium. Insert a needle with a syringe at a 30° to 45° angle in the direction of the opposite femoral head. [18, 19] The needle should be inserted cranial to the manubrium and the first rib of guinea pigs and chinchillas, as they have an underdeveloped clavicle. In rodents with a developed clavicle, like rat, mouse, hamster, and gerbil, the needle should be inserted between the clavicle and the first rib. [17] This technique involves placing the rat in a dorsally recumbent position, with the forearms drawn back in line with the chest. Insert a 23 to 25 G needle at a 3-8 mm lateral side to the manubrium and just cranial to the first rib. Then direct the needle toward the opposite femoral head at an angle of 30° down towards the table on which the animal is positioned. Advance the needle 2 to 10 mm to obtain the blood sample. This area may yield about 0.8 to 2.5 ml of blood. After removing the needle, apply pressure for 30 seconds to the collection site to establish haemostasis. [18]

3.11. Femoral Vein

A trained person can collect blood samples from the femoral Vein of an anaesthetized guinea pig. Collecting up to 3 ml of blood from this Vein is possible using a 23 G needle. The femoral artery is parallel to the Vein, so the sampling may be from an artery to some extent. If blood is gathered in a bright red colour, apply pressure for two minutes to halt the bleeding. [8]

3.12. Sublingual Vein

Blood can be drawn from a sedated mouse, rat, hamster or guinea pig via the sublingual Vein. [19] Each side of the frenulum of the tongue has deep sublingual veins. For sublingual blood collection, one person should hold the animal by its scruff to ensure animal restraint and consequently cause partial venous congestion. Then bring the animal into a supine position. The other person should extend and hold the animal's tongue between their thumb and a cotton bud and puncture the right vena sublingualis's thick caudal portion using a 23 to 26 G needle. Turn and hold the animal horizontally above a micro collection tube to collect the blood. Leave the skin on the animal's neck to halt the bleeding. Clean any blood from the mouse's mouth using dry cotton. [11] Blood collection from this Vein can result in tongue bruising and swelling, which might result in anorexia. According to Zeller and colleagues [20], white blood cell counts declined marginally from baseline two hours after blood was drawn from the sublingual Vein. Although the white blood cell count declined significantly two hours after sublingual vein collection, Scipioni R [21] reported that the red blood cell count remained steady.

3.13. Gingival Vein

Anaesthetize the rat, position it dorsally over a table and stretch the lower lip caudally. Then, holding a 26 G needle at a 30 to 60-degree angle, insert it 2 to 4 mm deep into the mucosa just below the edge of the gingival incisor, toward the caudal side, following the centre line between the lower incisors. The needle is inserted 3 to 5 mm deep in guinea pigs at a 35 to 60-degree angle. When drawing blood, always use disposable needles and syringes. The gingival Vein can yield about 200 microlitres (µl) of blood in two minutes. [22]

3.14. Tail clipping or snipping

Tail clipping or snipping is a crude method of collecting blood that should be avoided due to irreversible injury to the tail and misery to the animal. This process yields a small volume of blood. Cut or amputate about 1 mm (mice) or 2 mm (rats) of the distal tail under local anaesthesia. The tail snip technique is not recommended in other animal species.

Typically, this process results in blood contaminated with tissue and skin products. Repeated sampling is possible, but the quality of the samples degrades as bleeding duration and tail "milking" increase. Serial sampling (for glucose monitoring) is possible by carefully detaching the clot or scab. The sample volume is typically increased by warming the tail using a heat lamp or warm compress. After collecting the sample, dabbing the tail tip stops the blood flow. As the tail is re-warmed, topical hypothermic anaesthetics stimulate blood flow. Hence, allow sufficient time for the local anaesthetic to take effect. [23]

3.15. Penile Vein

In rats, guinea pigs, and chinchillas, the penile Vein is big and readily approachable. The only concern is that the procedure will cause harm to the penis if performed poorly. [2]

3.16. Cardiac puncture

Cardiac puncture allows a significant quantity of sample collection. This procedure should be performed only as a last option and under general anaesthesia. This technique ensures the collection of a single, high-quality blood sample. A cardiac puncture can be done with or without thoracotomy using an appropriate needle. A blood sample without thoracotomy is performed using a 1cc syringe and a 25G needle. Insert a needle at a 35 to 40° angle right below and to the left of the xiphoid process. Slowly aspirate until blood starts to flow (Figure 7). Following inhalant euthanasia, ensure the animal's death by opening the thoracic cavity or decapitating. Following a thoracotomy, blood can be aspirated directly from the heart's ventricles using a syringe and needle. Following euthanasia, a laboratory animal's heart can be punctured to get a considerably large quantity of blood.

Caution
Sometimes sampling may be unsuccessful as a result of dextrocardia.

3.17. *Abdominal aorta and caudal vena cava*

After laparotomy, a blood sample can be collected from the abdominal aorta or caudal vena cava in a deeply anaesthetized animal. This is a terminal blood sampling procedure. After opening the abdomen, gently remove the intestines, push the liver forward, and identify the caudal vena cava between the kidneys (Figure 8). Then insert a 21 to 25 G needle at a shallow angle to extract blood from the caudal vena cava.

3.18. Decapitation

Only in exceptional circumstances should the head be decapitated using a guillotine or sharp scissors and a blood sample collected from the severed location. The sample that has been collected has a significant risk of contamination with other physiological fluids and tissues. Furthermore, decapitation is a difficult task requiring a high level of competence. [24] Therefore, before performing a beheading on a living animal, practice on dead ones. Blood samples can be taken from the rats and guinea pigs in required quantities of up to 10 ml and from mice up to 1 ml.

3.19. Axillary or Brachial Vessels

This is also a terminal sampling technique from a deeply anaesthetized animal. Cut through the skin in the axillary region to expose the axillary blood vessels. Cut the axillary blood vessels and let the blood collect in the skin pocket. Then collect the blood sample (mixture of arterial and venous blood) in a suitable container. This technique yields about 0.8 ml of blood from mice. Following blood collection, euthanize the animal. [8]

3.20. Carotid artery

This is one of the terminal blood collection procedures that should be performed under sedation. Trim the hair from the neck region and cut the neck's major vessels as deep as the carotid using sharp scissors or a scalpel. After that, immediately obtain the blood sample using a pipette and euthanize the animal. [23] This technique yields a relatively huge sample volume.The quantity of blood that can be collected at exsanguinations is given for a few animals in Table 2. Roughly it is around:

- 0.8-1.0 ml blood from a 20 g mouse.
- 12-15 ml blood from a 300 g rat.
- 120-150 ml blood from a 3 kg rabbit

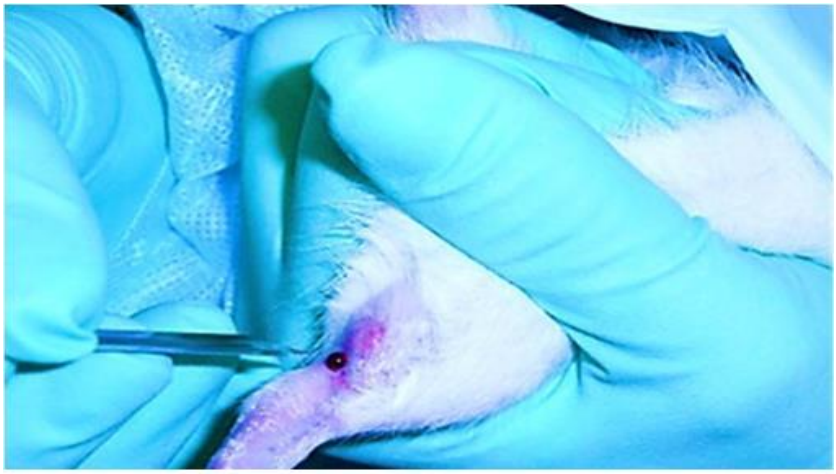

Fig.1 Lateral Saphenous vein blood collection

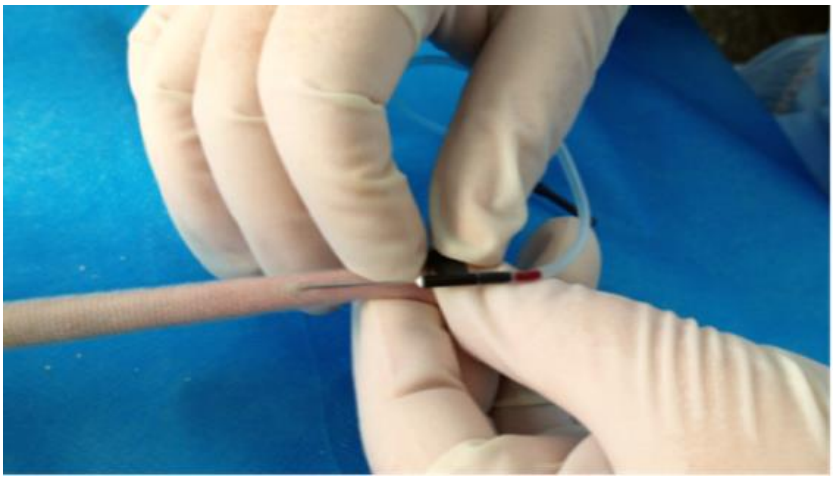

Fig 2. Lateral tail vein blood collection

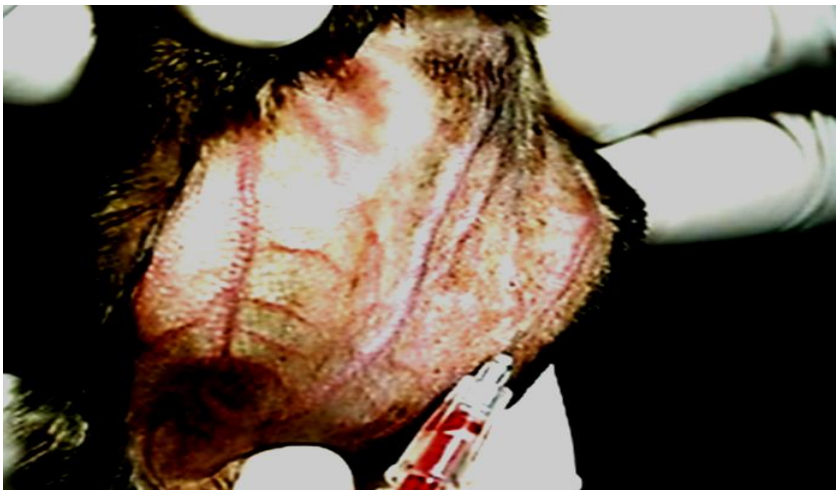

Fig 3. Marginal ear vein blood collection in rabbit

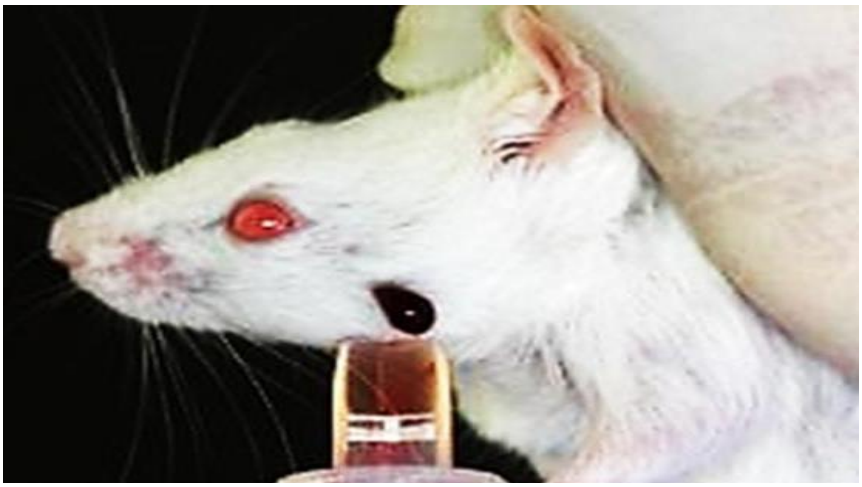

Fig 4. Facial or submandibular vein blood collection in mice

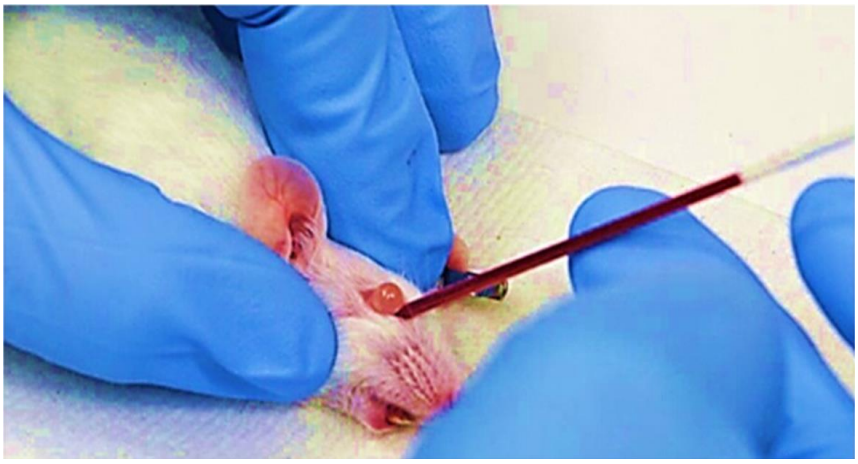

Fig 5. Retro-orbital bleeding in mice

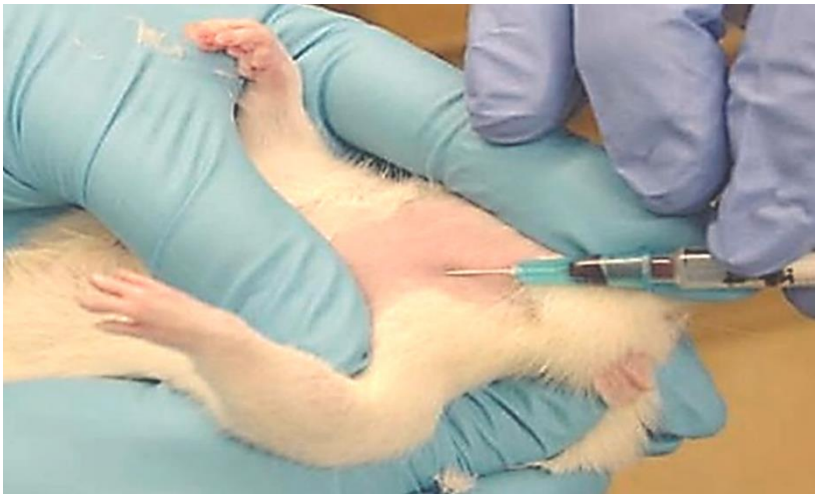

Fig.6 Jugular vein blood sampling in rat

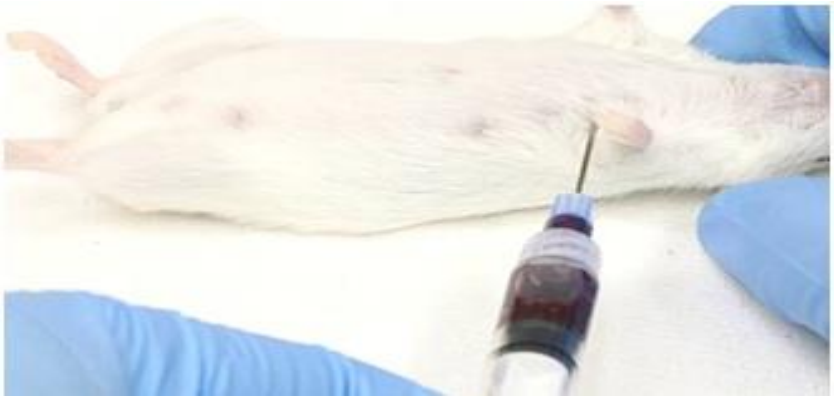

Fig7. Cardiac puncture in mice

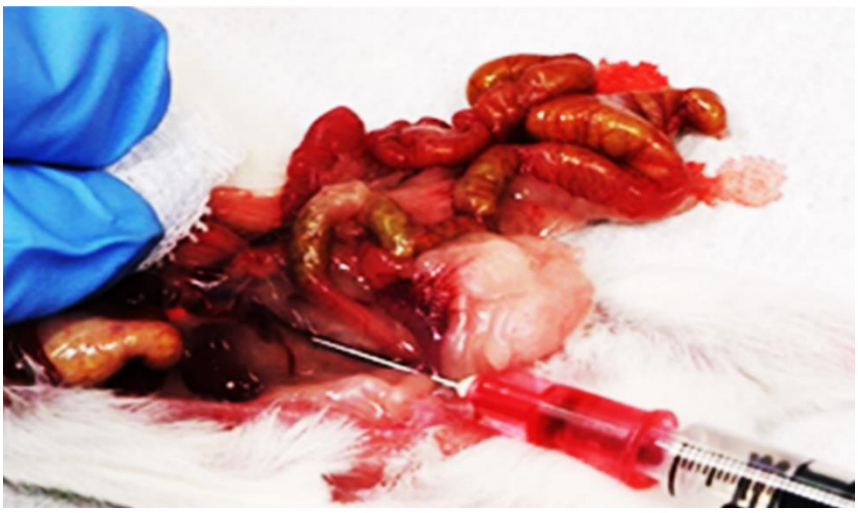

Fig 8. Caudal vena cava blood collection

Table 4: Blood collection sites and sample volumes of different laboratory animals

Blood collection site	Sample volume	Equipment needed	Adverse effects
Lateral Saphenous Vein	**Single sample**: Up to 0.2 ml (rat, mice); up to 0.15 ml (hamster); 0.5 % of animal's body weight (guinea pig) **Multiple smaller samples**: 0.01 ml daily (mice, hamster), 0.02 ml daily (rat); 0.05% of body weight (guinea pig)	27G or 25G needle or lance (hamster, mice); 23 G (rat,)	Bruising, haemorrhage, infection, temporary favouring of the opposite limb
Lateral Tail Vein	50 µl to 0.2 ml (mice); 0.1-2 ml (rat)	25G needle or lance (mice); 21G - 23G needle or butterfly needle or lance (rat)	Infection, haemorrhage
Marginal Ear Vein	Up to 0.5-10 ml, depending on the size and strain of the rabbit.	19G - 23G butterfly needle, depending on the strain and size of the rabbit.	Bruising, haemorrhage, infection
Tarsal Vein	0.1 to 0.3 ml (guinea pig)	23 G needle or lance	Skin abrasion from the shaving, bruising, infection, haemorrhage
Submandibular Vein	Up to 200µl (mice)	Needle (less than or equal to 20 G) or lancet (4 mm)	Skin abrasion from the shaving, bruising, infection, haemorrhage
Ventral tail artery	Up to 0.5-0.75 ml (mice); 0.2-3.0 ml (rat)	Needle (19-27G)	Blood loss, haematoma (rare)
Retro-orbital Sinus	0.2-0.3 ml (mice)	Microhematocrit tube or Pasteur pipette	Eye hematoma, corneal ulceration, pannus, globe rupture, keratitis, optic nerve damage, blindness etc.
Jugular Vein	Up to 2ml (rat); 2.5 ml (Guinea pig)	23G needle (rat); 24 G Needle (Guinea pig)	Haemorrhage resulting in a haematoma

Cardiac puncture	0.8-1 ml (mice); 5-15 ml (rat)	22G needle (mice); 23 G Needle (rat)	Haemopericardium, Terminal procedure
Caudal Vena cava and abdominal aorta	0.8 ml (mice); 5-15 ml (rat)	23-25G needle (mice); 19-20 G Needle (rat)	Terminal procedure

The technique of blood collection is chosen based on several factors such as the number of blood samples required, the frequency of sample collection, the blood parameters to be studied in the sample, the skill level of the person collecting blood[7], the scientific approach used, the research animal's total blood volume, etc. Whittaker AL and Barker TH[26] have concluded in their systematic review that an optimal blood sampling route for laboratory animals cannot be determined as there is a need for more high-quality evidence. However, a study by Meyer N and colleagues [27], which assessed the impact of three commonly used blood collection techniques (tail vein, facial Vein, retrobulbar) on the welfare of laboratory mice, concluded that tail vein blood collection is the most animal welfare-friendly method. Hence, further studies are needed to find the best blood collection techniques with the least stress to the research animals.

4. BLOOD COLLECTION VIA SURGICALLY IMPLANTED CANNULA

The surgically inserted cannula helps get blood samples with fewer disturbances to the animal. Blood from the cannula can be obtained either manually or mechanically. It is important to protect the exteriorized cannula to keep it safe from the animal or its cage mates. Table 5 details the needle size for cannulation and the maximum blood collection volume in different species.

Table 5: Needle size for blood vessel cannulation in small laboratory animals[1]

Species	Needle size	Maximum blood collection volume
Mice	23 to 25 G	1 ml
Rat	19 to 21 G	10 to 15 ml
Guinea pig	20 to 21 G	1 to 25 ml
Rabbit	19 to 21 G	60 to 200 ml

5. BLOOD COLLECTION VIA VASCULAR ACCESS PORT (VAP)

Vascular access ports are implanted subcutaneously, and it is necessary to control the frequency and the total number of piercings done on the implanted access port over time. Repeated puncturing of the skin above the access port can result in necrosis, which exposes the access port and increases the risk of infection. To ensure the VAP's integrity and usable life, it is critical to maintain an aseptic condition of the skin over the port.

6. MONITORING

The animal might suffer from hypovolemic shock and anaemia when excessive blood is drawn very fast or very often without any fluid replacements. The symptoms of shock comprise fast and thready pulse, pale and dry mucous membranes, cold skin and extremities, restlessness, hyperventilation and reduced body temperature. The physiologic consequences of excessive blood loss[4] include the following:

- By reducing 10% of the circulating blood volume, homeostatic cholinergic mechanisms are triggered.
- Reducing the total blood volume by 15 to 20% decreases cardiac output and blood pressure.
- Hemorrhagic shock occurs when 30 to 40% of the total blood volume is removed.
- If more than 40% of the total blood volume is lost, 50% of the animals will perish.
- Drawing fewer amounts of blood too frequently may result in anaemia.

Regularly examine the red blood cell pack volume (PCV) to identify when blood collection should be halted to allow the animal to get better from probable anaemia. Although healthy adult animals may regain their blood volume in one day, specific blood components (cells, proteins) can take up to two weeks to regenerate. Haematocrit (HCT or PCV) and haemoglobin levels can be determined to find out whether the animal has recovered from blood loss. As a general rule, if the animal is anaemic (having less PCV than the normal range for the species, Table 6) or if the haemoglobin concentration is less than 10 g/dl, it is not recommended to collect blood.

Table 6: Normal Packed Cell Volume (PCV) for some lab animals (%) [25]

	Species	Range of Packed Cell Volume (%)
1.	Mice	39-49
2.	Rat	36-54
3.	Rabbit	30-50
4.	Guinea pig	37-48
5.	Hamster	40-61
6.	Gerbil	43-60

7. FLUID REPLACEMENT

A warm (30 to 35°C) isotonic solution (for example, 0.9 per cent saline (normal, physiological), Ringer's lactate solution) is used to replace the blood that is taken from an animal that exceeds the maximum permitted blood collection volume. Blood should be extracted slowly and steadily, and the replacement solution should be supplied similarly. Fluid replacement therapy involves the administration of 3 ml of crystalloid fluids (by subcutaneous or slow intravenous route) for every 1 ml of blood removed. In addition, 1.0 ml of warm isotonic solution can be administered intraperitoneally or subcutaneously for adult mice. For adult rats, 5 to 10 ml warm solution is recommended (one-half of the total volume via intraperitoneal and the other half via subcutaneous route). [23]Fluid replacement therapy should not be seen as an excuse to collect blood more frequently than the established guidelines.

8. CONCLUSION

In this review, data from several published papers, books and internet sources are compiled into a consolidated overview of blood sample collection from common small laboratory animal species. This review aims to help researchers choose the most appropriate technique for blood sample collection based on their study requirements in a humane and efficient manner. It should be remembered that in any healthy animal species, not more than 10% of its total circulating blood volume in 2 weeks and not more than 1% of its body weight in 24 hrs can be collected as blood samples. It is critical to minimize animal suffering, distress, and discomfort by employing appropriate approaches for the target species. For scientific, ethical, and legal reasons, adverse effects should be avoided since they may result in biological changes that impair the blood sample itself, affecting the validity and repeatability of the results. Even though blood collection is stressful for laboratory animals due to handling, restraint, anaesthesia or discomfort caused by the techniques employed, stress to the research animal should be reduced significantly by using humane handling or analgesia or anaesthesia for good science and animal welfare. In conclusion, the researchers should choose the appropriate blood collection method for their study animals based on several guidelines presented in this review.

9. AUTHORS CONTRIBUTION STATEMENT

Dr. Leena Rajathy Port Louis conceptualized, curated and prepared the original draft. Dr. Prithiviraj Nagarajan and Dr. B.R. Asokan provided constructive inputs and reviewed and edited the manuscript. All the authors read and approved the final version of this manuscript.

10. CONFLICT OF INTERESTS

Conflict of interest declared none.

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