

Different blood collection methods from rats: A review

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ABSTRACT It is essential for collection of blood samples from laboratory animals employed in wide range of scientific research. There is a no. of techniques available for blood sample collection from Rats/mice. The stress produced in animals during the blood collection may compromise with the research data which may invalidate the results. It is necessary that the blood sample collection is done by trained individuals so as to minimize the stress and other physiological reactions in the animals. This article reviews some of the common approved blood collection techniques for rats/mice.

Keywords: stress, mice, rat, blood collection techniques.

1. INTRODUCTION

In experiments and research works where impact of some substances on different parameters are to be tested, a mammalian model is required. Often mice/rats are used for this. The maintenance of rats, rearing, blood sample collection, disposal of deads and waste is a tedious and responsible task. For rearing and maintenance of rats the researchers should follow standard guidelines. Primarily an Animal Ethical Committee (AEC) should be formed, which sets and formulates standard guidelines for maintenance, rearing etc of models used in experiments. It is important to note that, if handled carelessly the blood collection procedures may stress the animal which may have impacts on the results and parameters under study. It is even more important that the personnel handling the mice/rat should learn the skills of handling and collection of blood so as to minimise the stress on rats during blood collection (Hoff, 2000). In this review we have tried to put light on various methods of blood collection techniques used around the globe; for this we have intensively reviewed the available literatures. The purpose of this review is to educate the young researchers who require

handling mice/rats during their works which involves the collection of blood sample.

2. BLOOD SAMPLE COLLECTION TECHNIQUES

A. SAPHENOUS VEIN BLOOD COLLECTION

Blood sample collection from the lateral saphenous vein is relatively quick method of blood collection from all strains of rats. This technique does not require the rat to be anaesthetized (Table 1). Blood sample is taken from the saphenous vein which runs dorsally and laterally over the tarsal joint. The site is first shaved (hairs removed). The rat should be restrained (Hoff, 2000). This may stress the rat therefore the duration of restraint should be as minimum as possible. The hind limb should be immobilised in the extended position prior to blood collect. If the animal is difficult to handle, a mild anaesthesia should be given to animal (Van *et al*, 2001). A puncture is made with help of fine needle to collect the blood sample. The number of attempt to puncture the vein should not exceed three in one attempt. Blood flow can be stopped by pressing gently with finger above the puncture site and the animal should only be returned to the cage after the blood flow has stopped completely (LAREF, 2004).

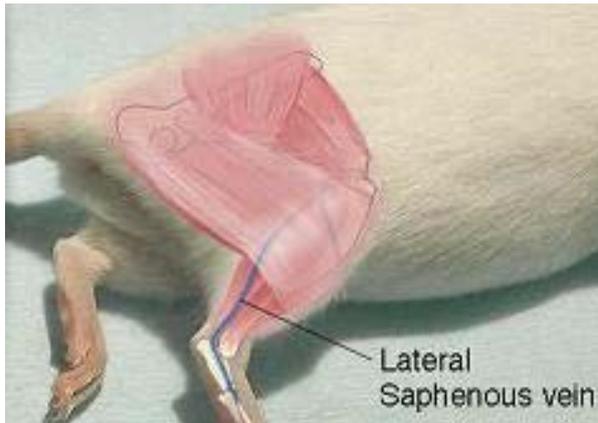


Figure 1: The lateral Saphenous Vein in Albino Rat (OLAC, 2014)

B. TAIL VEIN BLOOD COLLECTION

The tail vein blood collection is suitable for all strains, it is quick and simple to perform. However prior to this technique the rats need to be warmed so as to dilate the blood vessel. This method is stressful and can deviate the results. Therefore the saphenous vein sampling method should be implemented for blood collection wherever possible. Wash the tail with dilute Hibiscrub in order to see the blood vessel. The number of sample collection should be minimised and sufficient time should be given for the tail to recover. The lateral tail vein is usually accessed one-third along the length of the tail from the tail tip, moving towards the base of the tail for multiple sample. The blood sample should be collected from the distal end first and then

moving towards the proximal end; since taking the first sample(s) from the proximal end of the tail can cause perivascular clot and inflammation than can significantly reduce the blood flow to the distal portion of the blood vessel (NCRRRAR, 2017; Johns Hopkins University, 2017)



Figure 2: Tail vein blood collection method (OLAC, 2014)

C. DORSAL PEDAL VEIN BLOOD SAMPLE COLLECTION

The rat is restrained, and the hind foot around ankle is held and medial dorsal pedal vessel is located. The foot is cleaned with absolute alcohol and the vein is punctured with fine needle. The blood will start dropping out. The blood drops are collected in a capillary tube.



Figure 3: blood sample collection from the dorsal pedal vein (Parasuraman *et al.*, 2010)

D. ORBITAL SINUS BLOOD SAMPLE COLLECTION

For this technique, the rat needs to be anaesthetized. Standard heparinised micro-hematocrit capillary tubes are used for blood collection. The animal is held by the back of the neck and the loose skin of the head is tightened with the thumb and middle finger. The tip of the capillary tube is placed at the medial canthus of the eye under the nictitating membrane. A short thrust past the eyeball is applied to enter the slightly resistant sinus membrane. The eyeball remains uninjured. As soon as the sinus is punctured, the blood enters the capillary tube and comes out. After collection of the allowable amount of blood (Table 2) the capillary tube is drawn out. To prevent excess bleeding a slight pressure on the eyeball is applied (Johns Hopkins University, 2017).

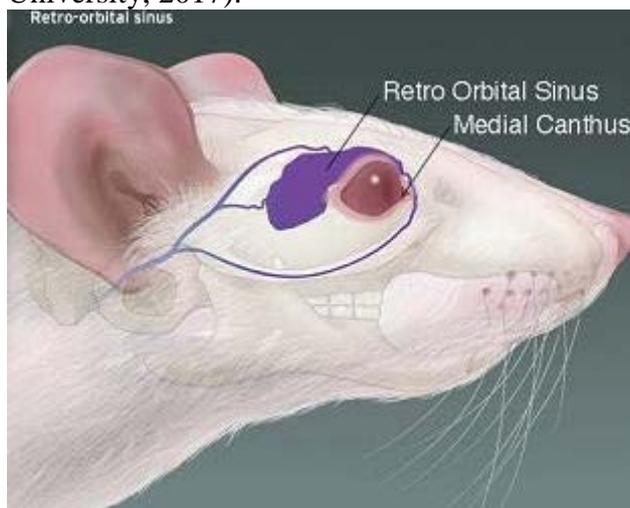


Figure 4: position of Retro Orbital Sinus (OLAC, 2014)

E. CARDIAC PUNCTURE

The cardiac puncture blood collection technique is recommended for terminal stage of the study to collect a single, good quality volume of blood from the animals. The blood sample is taken from the heart, from the ventricle slowly to avoid collapsing of the heart [7-8]. A 23G1 needle may be used. The rat is deeply anaesthetized. To ensure prolonged anaesthesia, a paper towel soaked in volatile anaesthetic is placed on rat's nose during the procedure. The rat is placed on its back. Place your finger at the level of lowest ribs without applying any pressure. The heart

is roughly 1 cm above this point, slightly right. Now holding the syringe at 45 degree angle, the needle is inserted between the two ribs and watched for a drop of blood which ascertains that the needle is inside the heart. Without moving the syringe, the syringe plunger is pulled to fill the syringe with blood. Once the syringe is full, it is carefully disconnected from needle and emptied into a tube. The syringe can then be reattached to collect more blood, it is possible to draw 5-10 ml of blood from 150-200 gm rat. The rat should be immediately euthanized post blood collection [9-10].



Figure 5: blood sample collection by cardiac puncture (Parasuraman *et al.*, 2010)

3. AESTHETIC AGENTS

The commonly used/recommended (Parasuraman *et al.*, 2010; Paulose, 1987) aesthetic agents for use on rats/mice in laboratory experiments are as follows

Table 1: Commonly recommended aesthetic agents for Rats/Mice (Parasuraman *et al.*, 2010)

Anaesthetic agents	Short anaesthesia	Medium anaesthesia	Long anaesthesia
Isoflurane	Inhalation	—	—
Halothane	Inhalation	—	—
Xylazine + Ketamine	—	5 mg + 100 mg i.m.	16 mg + 60 mg i.m./i.p.
Urethane	—	—	1200 mg/kg i.p.

4. PRINCIPLES OF BLOOD COLLECTION

- The method(s) should be described and approved by the Institutes animal ethics committee (IAEC) (Parasuraman *et al.*, 2010).
- The procedure should be least painful and stressful and the personnel handling the mice/rats should have had adequate training for blood collection (Parasuraman *et al.*, 2010).
- A needle size of 23-25G (mice) can be used upto 1 ml blood collection and 19-21G (rats) can be used for blood collection upto 10-15 ml (Parasuraman *et al.*, 2010; Beeton *et al.* 2007; Luzzi *et al.* 2005).

5. EFFECTS OF BLOOD SAMPLING ON RESEARCH DATA

The stress and other physiological reactions in the rats/mice during blood collection may have adverse impacts on the variables under study which may result in invalidation of results. The blood collection may have following impacts on research data (UNc – ACEC, 2012).

- All blood sampling techniques are invasive and presumable all cause at least some discomfort if used without anaesthesia. Unless the animal is unusually well adjusted to having a blood sample taken, it will be stressed by the procedures involved.
- There may be contamination of sample with skin bacteria, secretions and debris or by subcutaneous tissue components (Beeton *et al.* 2007; Luzzi *et al.* 2005).
- Smith *et al.* (1988) reported that peripheral haematology parameters vary with a number of sample sites (right ventricle, abdominal aorta, abdominal vena cava, retro-orbital sinus and tail). Bickhardt *et al.* (1983) concluded that variations of haematological and metabolic blood constituents are influenced by the conditions of housing as well as the circumstances of handling for blood sampling. They suggested that in order to avoid "significant" differences between groups which are purely an artefact arising from the condition of bleeding, sampling conditions should be standardised, and animals of different experimental treatment groups should be bled in a strictly random

order according to a formal experimental design

6. PERMISSIBLE COLLECTION VOLUMES

Both the quantity and frequency of blood sampling is dependent on the circulating blood volume of the animal. The approximate blood volume of a mouse is 80 ml/kg and 70ml/kg for the rat. The table 2 shows the permissible blood volume removal amounts (ASRC – IACUC, 2016).

Table 2: permissible volume of blood sample collection

Model	Body weight	Permissible sample volume
MOUSE	20-25 g	0.11 – 0.14 ml
	30 – 35 g	0.17 –0.21 ml
	40 – 45 g	0.22 – 0.28 ml
RAT	100 – 150 g	0.60 – 0.91 ml
	200 – 250 g	1.1 – 1.5 ml
	300 – 350 g	1.7 – 2.1 ml

7. CONCLUSION

The use of rats/mice in laboratory should follow the standard procedures so as to minimise the stress, pain and other physiological reactions in the animal. Prior to use of animals in the laboratory, an IAEC should be setup which formulates and defines the standard procedures of blood sample removal from animals. It is also evident that other factors such as housing conditions can also lead to stress and infections in the animals, thus for obtaining proper valid data from the blood samples of animals, not only the blood collection techniques should be refined, but also the housing conditions and other factors should be improved. Precautions must be always taken to only remove the permissible amount of blood sample.

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