1. Overview

This document provides additional information to support the Animal Care and Ethics Committee (ACEC) approved Standard Operating Procedures (SOPs) for blood collection in research animals.

The document is intended to provide information on the expectations of the Animal Care and Ethics Committee when researchers collect blood from animals as part of an approved research protocol. While the information provided in these guidelines is specific for rodents and rabbits, much of the information can be extrapolated to other species.

2. Definitions

In the context of this document:

- **anaemia** means a decrease in the number of red blood cells, or amount of haemoglobin in the blood;

- **asepsis** means prevention of microbial contamination by excluding, removing or killing microorganisms;

- **haemostasis** means cessation of bleeding;

- **haemorrhage** means excessive loss of blood;

- **hypovolaemia** means a decrease in the volume of circulating blood in the body.

3. Methodology

3.1 INTRODUCTION

Blood collection is a common laboratory procedure that potentially can affect both the welfare of the animal, and the quality of the scientific results. Techniques that cause the
least pain and distress to the animal while addressing the research needs must be used. The following factors should be considered:

(i) The effects of blood loss and blood sampling on the animal.
(ii) The total blood volume of the animal.
(iii) The volume of blood that can be withdrawn, either in a single or multiple samplings, without causing adverse impact on the welfare of the animal.
(iv) How frequently blood is collected.
(v) The period over which blood will be collected.
(vi) The site of blood collection.
(vii) Whether anaesthesia and / or analgesia should be used, and its effect on the parameters being measured.
(viii) The type of restraint required and its effect on the blood parameters.
(ix) The health status of the animal to be sampled.
(x) How the animal should be monitored following blood collection and, for repeated sampling, during the period of blood collection.
(xi) The competence and experience of the researcher in relation to the technique for blood collection, and the species of animal to be used.
(xii) The effects of blood loss and blood sampling on research data.
(xiii) The type of sample required (serum, whole blood, plasma)
(xiv) The quality of the sample collected (sterility, contamination with tissue fluid)
(xv) The quantity of the sample required for the analytic process.
(xvi) Handling of the blood samples after collection.

3.2 THE EFFECTS OF BLOOD LOSS AND BLOOD SAMPLING ON THE ANIMAL.

Haemorrhage and blood-sampling procedures may have significant negative impacts on the health and well-being of laboratory animals. Significant changes may be seen in cardiovascular, clinicopathological, and histological parameters that are referrable to hypovolaemia and/or anaemia.

The sympathetic autonomic nervous system is especially reactive to stressful environmental stimuli, and its activation during the blood sampling procedure can rapidly modify cardiovascular dynamics and also influence metabolic functions of other tissues and organ systems.

The quantity and frequency of blood that can be safely collected depends on the circulating blood volume and the red blood cell life span eg in mice the RBC lifespan is 38-47 days and in rats, 42-65 days.

Variables which may affect an animal's response to blood sampling may include:
- rate of blood loss
- site and technique of withdrawal
- skill of the operator
- the use and type of sedation, analgesia and/or anaesthesia
- the age and sex of the animal
- the animal's nutritional and health status.
- Effectiveness of haemostasis after blood collection.

McGuill and Rowan (1989) provide an overview of the physiological responses to acute haemorrhage:

**Minor blood loss ie. losses of 10% or less of total blood volume:**
- Animal may be asymptomatic.
- Increase in sympathetic nerve activity resulting in increase in heart rate, constriction of arteriolar beds in muscle and skin, and constriction of veins and venous reserve
- Arterial pressure, venous return and cardiac output are maintained or minimally affected
- Secretion of antidiuretic hormone, and activation of renin-angiotensin aldosterone system, act to help replace lost volume.

**Moderate blood loss ie. losses of 10-15% of total blood volume:**
- Animal will suffer drops in arterial pressure and cardiac output despite compensatory mechanisms.
- Massive cholinergic release results in increase in heart rate, intense arteriolar constriction, and distribution of blood away from gut and skin.
- Venous constriction partially sustains venous return.
- Mobilisation of fluid to the intravascular compartment restores some of the lost fluid volume.
- Anaerobic glycolysis occurs due to lack of oxygen. Increased plasma lactate causes metabolic acidosis and compensatory tachypnoea.

**Severe blood loss ie. losses of greater than 15%:**
- Decreases in cardiac output, blood pressure and tissue perfusion become life threatening.
- Tissue anoxia, hypercapnia and acidosis can result in widespread cell injury and irreversible tissue damage, organ compromise and death.
- Cardiac function is limited by decreased cardiac perfusion.
- Late in shock, decreased perfusion of the medullary vasomotor centre causes diminished compensatory reflexes.
If blood sampling is continued for several months, a sustained reduction in red cell count, packed cell volume (haematocrit), and haemoglobin levels will be seen. Signs of anaemia include pale mucous membranes of the conjunctiva or inside the mouth, pale tongue, gums, ears or footpads (if non-pigmented), exercise intolerance, and at the extreme level an increased respiratory rate when at rest.

3.3. THE EFFECTS OF BLOOD LOSS AND BLOOD SAMPLING ON RESEARCH DATA.

The substantial haematological and physiological reactions in the experimental animal to blood collection may comprise yet another variable in the research protocol, and may invalidate results. The importance of such confounding variables, especially if they remain unrecognised and uncontrolled, can hardly be overemphasised. For example:

(i) A single blood sampling procedure can elicit a stress response comparable to many other laboratory procedures like handling, restraint or exposure to a novel environment (eg. raised catecholamine, glucocorticoid and β-endorphin levels in the blood). Haemisch et al (1999) demonstrated that such responses occurred although all rats were well accustomed to the procedure and did not exhibit any behavioural signs of stress during the whole sampling procedure.

(ii) All blood-sampling techniques are invasive, and all presumably 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.

(iii) Some commonly used anaesthetic agents can affect red cell values.

(iv) Collection of blood via an indwelling catheter may significantly affect basal levels of hormones (such as prolactin, cortisol, corticosterone and glucose) and counts for red and white cells, and platelets, and packed cell volume. The potential for influence from the surgical intervention and the reaction to the implanted catheter cannot be excluded (Zellar et al, 1998).

(v) There may be sample contamination with skin bacteria, secretions and debris (through inadequate site preparation) or by subcutaneous tissue components.

(vi) There is extensive literature on the influence of sampling sites on a range of haematological and biochemical measurements. For example, Smith et al (1986) 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.

3.4 BLOOD COLLECTION - VOLUME AND FREQUENCY

Recommendations for blood withdrawal limitations vary depending on factors such as the period over which the blood collection extends, and rest intervals prior to a subsequent blood collection.

At present there are few clear guidelines regarding the amount of blood which can be withdrawn, either in a single or multiple samplings, without raising concerns for the welfare of the animal. Some investigators believe that current recommendations lack adequate scientific data to clearly demonstrate that the limits identified are truly safe maximums. Many early papers recorded absolute values of the volume of blood removed from the test animals rather than mls/kg. Therefore reported results cannot be correlated with the % of blood volume removed. This issue is confounded by the significant difference in blood volume between strains and breeds. Red blood cell indices and other haematological values, circulating corticosteroid concentration, and body weight and body condition have been used to monitor the effects of blood withdrawal (eg. Cardy and Warner, 1979; Scipioni et al, 1997; Zellar, 1998). However, there has been little documentation of a clear correlation of these parameters with the health and well-being of rabbits and rodents. In addition, accurate indicators of well-being and distress in these species are continually being defined and refined. For example, for prey species such as rodents and rabbits, expression of overt pain and stress related behaviour would simply alert predators. Hence such behaviour is often suppressed or masked.

When determining limits of non-stressful blood collection regimes, both sampling volumes and frequency, the researcher must consider the acute physiological effects of the blood collection on both the welfare of the animal in the short-term, and the implications of these effects for the research itself. These factors must be considered even in situations where it is likely that the animal will be able to compensate for the blood loss over time.

In addition, it is recommended that researchers should err on the side of caution in their calculations. As an example - the mean total blood volume of cross bred dogs was reported to be 79 mls/kg, whereas the mean total blood volume in greyhounds was reported to be 114 mls/kg. If researchers working with cross bred dogs based estimates of blood volume on values obtained from greyhounds, they may assume that 20% of the animal's blood volume was being removed, when in fact they would be removing closer to
30% of the cross bred dog’s blood volume.

3.5 LITERATURE REVIEW

McGuill and Rowan (1989) published a comprehensive review of this subject. A UK working party (BVA/FRAME/RSPCA/UFAW, 1993) examined the most humane and efficient ways to collect blood from laboratory mammals and birds. These publications recommended that blood volumes should be estimated on body weight, and that 10% to 15% would be the maximum taken on any one occasion without undue effect to the animal. More recently, Zellar et al (1998) reported on the effect in rats of blood collection over a 24 hour period (7 samples collected under anaesthesia). Blood volume removed was closely related to the body weight of the rats. The removal of a total of between 10-15% did not reduce the haematocrit below 40% (normal range 36-52). Removal of approximately 23% resulted in unacceptably low haematocrit values.

Removal of larger volumes can result in demonstrable stressful effects, for example, a sustained elevation of corticosterone levels after removal of more than 3 mls from rats (Wiersma and Kastelign, 1985). In this study, there was no direct correlation between volume of blood removed and the weight of the animals. However, the rats ranged in weight form 180-430 gms. Hence 3 mls could represent between 11% and 27% of blood volume. They stated that if the animal survives removal of larger volumes of blood (eg. more than 20-25% of blood volume), it is unlikely to be of value as an animal model.

Walsh et al (1980) examined the effects of removal of a total of 2 mls from rats over a period of 5 hours (samples of 0.2 mls for a total of 10 samples). In the 200-300 gm rats, this represented approximately 14.3 to 9.5% of blood volume. Parameters measured included mean arterial pressure (MAP), cardiac index (CI), total peripheral resistance index (TPRI), oxygen consumption (Vo2), heart rate (HR), arterial oxygen content (aO2), arteriovenous blood oxygen content difference [(a-v)O2], and haematocrit (Hct). They also examined the effect of replacement of shed blood, and the effect of haemorrhage on arterial blood gases, pH and electrolytes using a 25 ml/kg haemorrhage (ie.35.7% of blood volume). The studies showed that Hct declined significantly after a total blood removal of 1.6 mls or greater (11.4 to 7.6% of blood volume), resulting in a decreased aO2. The gradual loss of 2mls of blood did not significantly lower CI, whereas a rapid single loss of 2 mls might be expected to reduce CI by 33%. In the rat, the rapid shift in interstitial and/or cellular water into the plasma compartment in response to haemorrhage may result in haemodilution. Haemorrhage of approximately 40% produced profound decreases in mean arterial pressure, oncotic pressure, and haematocrit. The loss of blood due to sampling can produce metabolic alterations which should be taken into account in any biochemical study. Although metabolic alterations can be prevented by replacing lost blood, pulmonary function may be adversely affected by injecting donor blood.
Berger (1982) evaluated the changes in blood morphology to examine the recovery of rats to a single acute haemorrhage of either 10 mls/kg or 25 mls/kg. If the total blood volume of a rat is taken as 70 mls/kg, such losses represent 14.3% and 35.7% of blood volume respectively. A broad range of haematological parameters were examined. Within 30 minutes, blood loss of 25 mls/kg produced statistically significant decreases in red cell count, haemoglobin concentration and haematocrit, and loss of 10 mls/kg produced significant decrease in haemoglobin concentration only. For both groups, all major characteristics of the red blood picture were markedly affected after 3 days. Apart from reticulocyte count, all red and white cell indices were normal within 7 days. The increase in erythropoiesis rate was reflected in the increase in reticulocyte count for 2 weeks following loss of 25 mls/kg.

Various studies on repeated sampling demonstrate the development of hypochromic anaemia. Wiersma and Kastelign (1986) reported that removal of 17-30% of blood volume in rats at weekly interval produced a hypochromic anaemia after 3 weeks. Repeated collection of the same volume at 2 weekly intervals did not affect blood composition. Scipioni et al (1997) examined withdrawal of between 10 and 40% of blood volume (calculated on 60 mls/kg body weight) with volume of blood removed being directly correlated with the weight of the subject animal. Significant blood withdrawal responses were found in several of the haematological parameters evaluated. For example, following removal of 40% blood volume, erythrocyte count decreased from approximately 8.5x10^6 to 5.5x10^6. The changes were positively related to the amount of blood withdrawn, and were most severe in the first 3 days following blood collection. Return to control values was observed in most parameters by day 14, with rats in the 30% and 40% groups tended to recover more slowly. Recovery rate was slower following repetition of the procedure 14 days after the first episode of blood collection. The effect of blood collection beyond two episodes was not studied. These authors measured standard haematological parameters, and used body weight as an indication of well-being. However, they acknowledged that future investigations should include cardiac output, blood pressure, organ perfusion and tissue oxygenation.

Pfeil (1988) reported on removal of approximately 3% of total blood volume in mice at intervals of either twice or three times weekly for a total of 4 weeks. Blood was removed via tail incision under light ether anaesthesia. Sampling twice weekly resulted in some depression of the red cell count. Sampling 3 times weekly caused significant depression of red cell count, haemoglobin and packed cell volume. The effects of blood sampling on spontaneous behaviour increased with the number and the frequency of blood sampling. In general, and particularly during the first 12 hours after bleeding, the mice moved less than control animals. Even in mice which did not show a clear depression of the erythrocyte values, the repeated blood collections altered the spontaneous behaviour of the animals.
Cardy and Warner (1979) reported that, in rats, withdrawal of not more than 1 ml of blood every 2 weeks produced no noticeable effects on common haematological parameters. However, it did cause a decrease in the rate of body weight gain. The difference in body weight between rats bled and not bled was evident 3 weeks after the study began, and persisted throughout the 23-week study. The blood volume removed represented approximately 14% of blood volume in 100 gms rats at the commencement of the study, and 4% of blood volume in 350 gm rats at its conclusion.

Kurata et al (1997) studied the effects of blood collection on erythrocytic parameters (red cell count, haematocrit, haemoglobin, MCV, MCH, MCHC) white cell count, platelet count, reticulocyte count and body weight in rats. Repeated blood samplings were carried out on days 0 and 28, with one point sampling done every week between these days. Each sample was 0.3 mls. On days 0 and 28, samples were taken on either 3 (Group I), 5 (Group II) or 10 (Group III) occasions. Over the 29 days, RBC, Ht, Hb and MCHC gradually increased in all groups, with the exception of decreases after repeated blood sampling. In contrast, both MCV and MCH decreased. During the repeated blood collection periods, Group I showed no changes in RBC and Hb. In Groups II and III, significant decreases in haematological parameters were observed after the 4th sampling (ie. after the removal of 1.2 mls). They concluded that 0.9 mls of blood over 24 hours caused few changes in haematological parameters. This volume represented approximately 6.5% of blood volume in 200 gm rat. They also found that in animals where 2 or 3 times that amount (13% or 20%) was removed, recovery occurred within 7 days. Body weight temporarily decreased after repeated blood collection.

Svendsen et al (1998) reported on the effects of the removal of 0.5 mls of blood at weekly intervals for seven weeks in mice. For a body weight range of 20-27 gms, this volume represented approximately 35% - 26% of total blood volume. One day following the final blood collection, haemoglobin concentration, red blood cell count and haematocrit were 15-18% lower than control level (for example, Hb was 7.9 ± 1.0 compared to 9.5± 0.5). Nine days after the final blood collection, these parameters were 18-22% higher than control level (for example, Hb was 11.2 ± 0.9 compared to 9.5± 0.5). They concluded that the animals were able to compensate for the blood loss of this kind. Body weight and behaviour were not examined.

3.6 RECOMMENDATIONS

Prior to any blood collection, the animal must be weighed and an estimate of the safe removable blood volume must be made. In determining the volume of blood to be removed safely, the researcher must establish:

- The minimum volume of blood required for the experimental protocol;
- The likely short-term effects of removal of this volume of blood from the
animal; (clinically, organ perfusion and oxygenation);

- Whether the effects of the blood collection (both immediate and long-term) will interfere with the aim of the research protocol.

Protocols should also be developed for the monitoring of the animals for the effects of blood sampling, both acute and chronic.

3.6.1 Blood Volume of an Animal

Blood volume of an animal can generally be taken as approximately 7% of the animal's body weight or 70 mls/kg. This estimate does not reflect the differences among individual breeds or strains, or variations due to age, sex or illness (see Table 1).

3.6.2 Volume of Blood that can be safely withdrawn

(i) Single Bleed

Maximum volume removed from a live animal at one time should be no more than 10% of the animal's blood volume (examples – Table 2).

(ii) Multiple samplings

Maximum removable volume on a daily basis should be no more than 1% of the animal’s blood volume.

3.6.3 Frequency of Collection

Repeated blood samples of 10% of total blood volume can usually be obtained at two- to three- week intervals without normally causing any obvious ill-effects.

3.6.4 Maximum Number of Collections

It is difficult to make recommendations on this aspect of blood collection given that each bleeding protocol will differ in the amount of blood withdrawn on each occasion, and the interval between collections. Again, the primary consideration is the welfare of the animal, eg:

- the clinical condition of the animal;
- the effect of blood sampling on its haematological parameters;
- the reaction of the animal to the research environment and to the blood sampling procedure.

Each protocol should be assessed on an individual basis, with monitoring of the animal's clinical condition and haematological parameters, and establishment of suitable end-points, being an essential part of the protocol.
3.6.5 Site of Sampling

Blood collection invariably damages the tissues punctured. Recommended criteria for determining the collection site are:

- minimal effects on experimental parameters being examined (from literature search);
- easy access;
- minimal effects on the welfare of the animal.

The recommended sites for blood collection in the species currently used at the University of Newcastle are indicated below and summarised in Table 3.

(i) Lateral saphenous vein (mouse, rat, guinea pig, dunnart)

This procedure involves the puncture of the superficial vein which runs dorsally and then laterally over the tarsal joint (lateral saphenous vein) (Hem et al, 1998). Sedation is not necessary for this procedure. The method is a humane and practical alternative to cardiac and retro-orbital puncture, in species where venipuncture has traditionally been regarded as problematic. An ACEC approved SOP for this technique is available.

Step-wise illustrations of the procedure can be viewed at the website address:
http://film.oslovet.veths.no/saphena/

(ii) Medial Metatarsal Vein (bird)

This procedure involves either puncture of, or insertion of a needle into, the superficial medial metatarsal vein which runs down the inner aspect of the lower leg in birds. An ACEC approved SOP for this technique is available.

(iii) Coccygeal or tail vein (mouse, rat, tammar wallaby)

The animals should be warmed to dilate the vessels, and should be either anaesthetised or placed into a suitable restraint device. A needle is introduced into the lateral tail vein. Blood can then be withdrawn by syringe, or capillary tube or dripped directly into a sample tube from the needle hub. An ACEC approved SOP for this technique is available.

(iv) Wing (Brachial) Vein (bird)

This technique involves venepuncture of the brachial vein where it crosses the inner aspect of the ‘elbow’ of the wing. The vessel is easily visualised. In most birds the vessel is very fragile and large haemotoma’s may be a consequence of blood collection from this site.
(v) **Ear vein (rabbit)**

The vein is easily located on the caudal margin of the ear of the rabbit. The animal should be restrained by wrapping in a towel. If the animal is warm and relaxed, vasodilation of the vessel will occur, and the blood sampling will be considerably easier. The ear is held between the thumb and forefinger, with the forefinger providing firm support for the edge of the ear. A 23-21 gauge needle can be introduced into the vein and blood gently aspirated. An alternative technique is to make a small (2-3mm longitudinal incision over the vein and collect the blood as it drips from the incision. Vaseline placed onto the skin prior to the incision will prevent clotting of the blood during the procedure. Following completion of the procedure, manual pressure on the site with a clean swab will provide haemostasis.

(vi) **Jugular vein (rat, guinea pig, tammar wallaby, bird)**

This technique requires some skill and is best carried out in the anaesthetised animal. Samples are withdrawn with a syringe after venepuncture of the jugular vein.

(vii) **Mandibular Vein (Mouse)**

Collection of a blood sample by puncture of the mandibular vein in a restrained, awake animal. The technique is relatively simple and allows the rapid collection of a moderate blood volume. The blood can be allowed to drip into a collection tube or collected with a capillary (microhaematocrit) tube.

(viii) **Sublingual Vein (rat)**

The animal must be anaesthetised for this technique. The animal is placed in dorsal recumbency and the tongue pulled forward until the sublingual vein is exposed. Venepuncture is carried out with a 23G needle. A cotton bud is used to apply pressure to the site for haemostasis.

(vii) **Retro-orbital sinus**

This technique involves puncturing of the venous sinus behind the globe of the eye and has been used for blood collection in rats, mice and guinea pigs. However, it is a technique which can have severe consequences for the animal; for example, haemorrhage along the puncture track, intra-orbital haemorrhage and inflammation, damage to the optic nerve and other intra-orbital structures, infection, corneal ulceration (Van Herck et al, 1992; Van Herck et al, 1998). Mahl et al 2000, reports higher levels of corticosterone in blood samples obtained by retro orbital sinus collection that sublingual vein collection in rats indicating a greater level of stress in rats sampled by orbital sinus. In addition, a suitable alternative exists in the lateral saphenous vein (Hem et al, 1998). Therefore, the use of retro-orbital sinus bleeding is not recommended for any situation other than non-recovery in the anaesthetised animal.
(viii) Cardiac puncture

For this procedure, the left ventricle is punctured with the animal laid on its right-hand side. Alternatively, the animal is laid on its back and a long needle is introduced just beneath the sternum into the heart. Severe complications from this technique include haemopericardium and haemothorax. Consequently, cardiac puncture is permitted only as a terminal procedure in the deeply anaesthetised animal. Death after exsanguination should be ensured by the administration of an overdose of an anaesthetic agent or by incising the heart.

3.6.6 Site Preparation

It is important to maintain asepsis throughout sampling. Hair and superficial skin debris over the vein should first be removed. The method for hair removal will depend on the site of the vein and the species of animal, with plucking, clipping or shaving commonly used. The clipped or plucked area should be cleaned with warm water with the addition of an antiseptic such as chlorhexidine in 70% alcohol in water.

Topical application of anaesthetic creams will significantly alleviate any discomfort associated with venepuncture. Flecknell et al (1990) found that application of a local anaesthetic cream 30-60 minutes prior to blood collection appeared beneficial in dogs, cats and rabbits, but did not influence the response of rats to a tail vein injection.

3.6.7 Haemostasis

Haemostasis after blood collection is important to prevent further loss of blood and consequent adverse effects on the animal. The animal should not be returned to its cage or pen until complete haemostasis is achieved and then should be monitored over the next hour to ensure that bleeding does not recommence.

Haemostasis can be effected through direct pressure with a piece of surgical gauze. Pressure should be applied continuously for at least 60 seconds in venous puncture (more if the vein has been lacerated rather than just punctured with a needle) and for several minutes if arterial puncture has occurred.

3.6.8 Monitoring

The investigator must be aware of the signs of adverse effects of blood collection, and must monitor for these signs.

(I) Acute blood loss

If the volume of blood removed is such that the animal’s physiological
compensatory mechanisms are unable to adjust to the loss of blood volume, hypovolaemic shock will occur. This presents as fast and thready pulse, pale dry mucous membranes, cold skin and extremities, restlessness, hyperventilation, and a subnormal body temperature.

(ii) Chronic blood loss
Clinical signs of anaemia from chronic blood loss include pale mucous membranes of the conjunctiva or inside the mouth, pale tongue, gums, ears or footpads (if non-pigmented), exercise intolerance, and at the extreme level an increased respiratory rate when at rest. Monitoring of the individual animal using its own baseline is essential. Monitoring should include packed cell volume, haemoglobin level, red cell count and reticulocyte count. Peripheral blood smears can be examined in order to detect early changes associated with anaemia, for example, polychromasia of the red cells.

3.6.9 Animal Treatment and Support after blood collection
In animals that have more than 10% of their blood volume removed on a single occasion, consideration must be given to the replacement of blood with equivalent volume of warm 0.9% saline or dextrose. A maximum of 5% of the animal’s body weight can be given as subcutaneous fluid, eg. 1-1.5 mls for a 30 gm mouse.

Wiersma and Kastelign (1986) reported that the reduction of blood volume by repeated blood sampling could be avoided by giving the rats a blood transfusion. Transfusion between F1 rats with fresh or preserved donor blood did not affect normal blood composition or induce immunological responses or interfere with health, behaviour, cyclicity or pseudopregnancy. Transfusion with preserved blood may interfere with normal hormone secretion whereas transfusions with fresh blood do not. However, Walsh et al (1980) reported that, although metabolic alterations can be prevented by replacing lost blood, pulmonary function may be adversely affected by injecting donor blood.

3.6.10 Training
Training in blood collection techniques is absolutely essential in order to ensure minimal distress to the animal, to decrease the possibility of introducing unwanted variables into the experimental protocol, and thus achieve scientifically valid data.
Table 1: HAEMATOLOGICAL DATA for common laboratory animal species

(Lumley et al, 1990,)

These values do not reflect the differences among individual breeds or strains, or variations due to age, sex or illness

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>RBC (x 10^6/mm³)</th>
<th>PCV (%)</th>
<th>Haemoglobin (g/100ml)</th>
<th>Whole Blood Volume (ml/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>9.2 (6.7-12.5)</td>
<td>41.8 (32-54)</td>
<td>11.1 (10.2-16.6)</td>
<td>70-80</td>
</tr>
<tr>
<td>Rat</td>
<td>8.5 (5.0-12.0)</td>
<td>45.9 (36-52)</td>
<td>14.2 (11-18)</td>
<td>50-65</td>
</tr>
<tr>
<td>Rabbit</td>
<td>6.5 (4.0-8.6)</td>
<td>40.8 (30-53)</td>
<td>13.5 (9.9-19.3)</td>
<td>45-70</td>
</tr>
<tr>
<td>Guinea Pig</td>
<td>5.2 (3-7)</td>
<td>43.6 (37-51)</td>
<td>14.3 (11-18)</td>
<td>65-90</td>
</tr>
</tbody>
</table>

Table 2: Examples of Volumes collected

<table>
<thead>
<tr>
<th>Body Weight</th>
<th>Mouse</th>
<th>Rat</th>
<th>Guinea pig</th>
<th>Rabbit</th>
</tr>
</thead>
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<tr>
<td>30 gms</td>
<td>250 gms</td>
<td>300 gms</td>
<td>3 kg</td>
<td></td>
</tr>
<tr>
<td>Estimated whole blood volume (mls/kg)</td>
<td>80</td>
<td>70</td>
<td>70</td>
<td>75</td>
</tr>
<tr>
<td>Blood volume (mls)</td>
<td>2.4</td>
<td>17.5</td>
<td>21.0</td>
<td>225</td>
</tr>
<tr>
<td>Volume to be removed- single collection</td>
<td>0.24 mls</td>
<td>1.75 mls</td>
<td>2.1 mls</td>
<td>22.5 mls</td>
</tr>
<tr>
<td>Volume to be removed- single collection- daily</td>
<td>24ul</td>
<td>175ul</td>
<td>210ul</td>
<td>2.25mls</td>
</tr>
<tr>
<td>Method</td>
<td>Mouse</td>
<td>Rat</td>
<td>Guinea Pig</td>
<td>Rabbit</td>
</tr>
<tr>
<td>-------------------</td>
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</tr>
<tr>
<td>Saphenous Vein</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Tail Vein</td>
<td>++</td>
<td>+++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ear Vein</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Mandibular Vein</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sublingual vein</td>
<td>-</td>
<td>++ a</td>
<td>-</td>
<td>-</td>
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<td>Jugular vein</td>
<td>-</td>
<td>++ a</td>
<td>++ a</td>
<td>++ a</td>
</tr>
<tr>
<td>Medial Metatarsal vein</td>
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<td>-</td>
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<td>Brachial vein</td>
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+++ = Preferred route  
++ = Acceptable route  
+ = Possible alternative  
- = Not recommended/Not applicable  
A = Under anaesthesia
4. Essential Supporting Documents

Animal Research Policy

Animal Care and Ethics Committee Procedures

5. Related Documents

ACEC approved Standard Operating Procedures (SOPs) related to blood collection.

REFERENCES AND FURTHER READING:


region of rats after orbital puncture. *Laboratory Animals* **26**: 53-58


