



Curtin University Standard Operating Procedure

BLOOD SAMPLING OF RODENTS

Number: TEC 02

Version: 1.1

Date: 01/10/2008

Aim/Purpose: To describe the method used for collection and identification of blood samples for clinical biochemical and haematological analyses. Methods employed should cause the least stress and discomfort to the animal.

1. Labelling of tubes

- a. Check protocol and relevant laboratory assay procedures for type and method of sampling required (serum, whole blood or plasma) for biochemistry, haematology, or blood drug level determinations.
- b. The specific type of anticoagulant may be crucial for the study. A wide variety of blood collection tubes are available for specific needs. Use heparinized tubes for coagulation studies/biochemistry. Use EDTA tubes for routine haematology.
- c. Label the tube with enough information to trace back to the animal/study in the case of mishandling. At a minimum this is likely to include date, protocol number and animal/cage number.

2. Blood Volume

As a general rule most mammals can be assumed to have approximately 70 mls circulating blood volume (CBV) per kg of body weight. **10%** of this volume (**7mls/kg**) can be taken from healthy animals at any one time without deleterious effects (hypovolaemic shock). It is however advised that no more than 15% of CBV should be taken in any 28 day period. These are recommended maximum guidelines and as a rule the amount taken should be the smallest volume that can provide the required results.

3. Collection of blood samples

- a. If fasting samples are required, ensure that animals are deprived of food for at least 8 hours prior to blood collection (note coprophagy occurs in rodents so it is difficult to get true fasted samples).
- b. Verify the identity of each animal at the time of sampling and verify that the corresponding, correctly labelled sample tubes are used for collection.



- c. Shave the site of venipuncture (unless hairless) and apply an alcohol based disinfectant to the skin prior to bleeding. When obtaining blood, a quick and accurate venipuncture is essential for a proper specimen. Do not draw back too forcefully on the syringe plunger as this may lead to haemolysis. Apply digital pressure to the area to cause haemostasis-ensure that bleeding has stopped before leaving the animal.
- d. When a syringe and hypodermic needle are used to collect blood samples, remove the needle before transferring the blood into the collection tube to prevent haemolysis.
- e. Ensure that collection tubes (especially ones with anticoagulant) are filled to the marked level to obtain the optimum blood-anticoagulant ratio.
- f. Gently invert the tube 2 or 3 times after obtaining a specimen, to evenly distribute the anticoagulant. Do not shake vigorously since haemolysis may result. **DO NOT INVERT** plain tubes (i.e. when collecting blood for separation of serum).
- g. Transfer samples to the laboratory for processing as soon as possible
- h. Centrifuge samples at 2000 to 3000 rpm for 10 minutes if plasma or serum is required. Remove supernatant with a Pasteur pipette and transfer to properly labelled storage tubes. Discard the pellet properly. Syringes and needles should be handled and discarded appropriately (see PC2-05). If analysis of the sera will not be done immediately freezing is generally the best method of storage. Plasma samples for use in coagulation studies should be frozen until analyzed. Do not freeze whole blood.

4. Venipuncture site

- a. Rodents can be bled from the jugular vein, tail vein/artery by needle puncture/nick, and sublingual vein. Retro-orbital sinus bleeding is carried out but has major welfare implications if done incorrectly (ocular damage).

This route should only be used with AEC approval after justification of necessity for use.

- b. Some of these techniques will require anaesthesia e.g. jugular vein/sublingual.
- c. Removal of a small (less than 3 mm section of the end of the tail) should be reserved for mice only and should not be practiced more than a couple of times to avoid removing bony tissue. To get repeat samples from this site the scab can be disturbed to restart bleeding.



- d. When using the tail vein, dilation of the vein is usually necessary- this can be achieved by BRIEF (1-2 second) dipping of the tail into hot water at 40 degrees C or putting the animal into a warming box. The warming box should be kept at **no more** than 37 degrees C and mice should **not be kept in there for longer than 5 minutes**, rats for **no longer than 15 minutes** (pregnant animals should not be put into a warming box). When using warming chambers animals should be monitored continuously and removed if any signs of distress are shown.
- e. More recent techniques using the saphenous and submandibular vessels have been described and are also likely to be humane. Cardiac puncture should **only** be used in terminally anaesthetised animals- this technique is likely to be painful and can cause a fatal cardiac tamponade if used in animals that are allowed to recover

5. Anaesthesia

- a. Investigators using a different species/technique for the first time can use anaesthesia with all bleeding techniques in order to make the blood collection less painful and stressful for the animal. Cardiac puncture must **only** be performed on anesthetized animals regardless of the experience level of the investigator.
- b. EMLA cream (Lidocaine 25mg/ml and Prilocaine 25mg/ml) is a topically applied local anaesthetic that is very useful in removing sensation from the venipuncture site and can be used to augment many restraint and anaesthetic protocols. Remember to apply the cream 30-45 minutes before phlebotomy.
- c. Many other anaesthetic drugs are available (Xylazine, Ketamine, Isoflurane, Halothane) -see TEC-01. Inhalational agents are ideal for blood sampling due to the fast recovery times seen with such agents. Choice of drug should be made on its suitability in a given species to provide safe and adequate anaesthesia in the hands of the investigator.

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