Saphenous vein puncture for blood sampling of the mouse, rat, hamster, gerbil, guineapig, ferret and mink

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Summary

A method is described for blood collection from the lateral saphenous vein. This enables rapid sampling, which if necessary can be repeated from the same site without a need for new puncture wounds. The method is a humane and practical alternative to cardiac and retro-orbital puncture, in species where venepuncture has traditionally been regarded as problematic.

Keywords Saphenous vein; blood sampling; mouse; rat; hamster; gerbil; guineapig; rodent; ferret; mink

Blood removal is one of the most common procedures performed on laboratory animals, and yet there is still a need to refine available techniques both from a welfare point of view (Russell & Burch 1959) and because stressful blood sampling techniques may profoundly affect physiological variables (O'Neill & Kaufmann 1990, Sarlis 1991).

Methods for blood removal from laboratory mammals and birds have been reviewed by the BVA/FRAME/RSPCA/UFAW Joint Working Group on Refinement (1993). This Group concluded that amputation of the tail tip under analgesia is the preferred method for blood sampling in the mouse, and recommended puncture of the tail vein in the rat. The Group was unable to recommend a preferred route for venepuncture in the gerbil and hamster. Cardiac puncture was quoted as the preferred site for the guineapig, but 'anaesthesia/analgesia should be given'. Retro-orbital puncture is also a widely used method for blood collection in rodents (van Herck *et al.* 1991a). Puncture of the sublingual vein has also now been reported (Zeller *et al.* 1998), but this method requires general anaesthesia and may result in reduced feed consumption.

In our opinion all these methods, with the exception of the use of the tail veins in the mouse and rat (Wolfensohn & Lloyd 1994), are sub-optimal. In Norway, cardiac puncture, whether it is for blood removal or injection purposes, may only be performed under terminal general anaesthesia unless special permission has been granted from the National Animal Research Authority (Norwegian Ministry of Agriculture 1996).

Rusher and Birch (1975) have published a brief communication describing saphenous venepuncture in the rat. Their technique, however, necessitated two operators and employed the vein on the inner aspect of the thigh. Other workers have concluded that the technique is technically difficult and instead advocate tail sectioning (Liu *et al.* 1996). Nau

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and Schunck (1993) describe cannulation of the saphenous vein in anaesthetized guinea-pigs.

We describe what we consider to be a simple method for venepuncture that may be employed in a range of species down to, and including, the mouse, without the need for anaesthesia. Some details have already been reported briefly (Solberg 1988, Aaberge *et al.* 1992).

Materials and methods

Restrainment of the animal

The technique employs the lateral saphenous vein which runs dorsally and then laterally over the tarsal joint. A variety of restraint techniques may be used, depending on the species concerned. Animals that are not expected to bite, such as rats and mink, kits may be adequately immobilized simply by palming them in the operator's hand, or by wrapping them loosely in a laboratory coat or towel (Fig 1).

Sedation is not necessary for saphenous venepuncture, but it may be used on welfare grounds or in species such as the gerbil and hamster that are difficult to immobilize. Alternatively, the use of sedatives can be limited to the act of shaving the skin over the vein, which need only be repeated as the hair grows again. The vein is often so readily visible that it may not even be considered necessary to shave the leg, but it may be an advantage to wipe the area with 70% ethanol.

Sedatives containing peripheral vasodilators, such as Hypnorm® (0.315 mg/ml fentanyl and 10 mg/ml fluanisone; Janssen, Belgium) which is widely employed in European laboratory animal environments for rodents (Flecknell 1996), may be used, but doses should be low to avoid prolonged bleeding from the puncture site. Clear plastic restraining tubes of suitable diameter may also be used (Fig 2). Centrifuge tubes, with airholes drilled in the tip, are suitable for small mice. For larger animals, plastic tubes of varying diameters may be employed. These should be equipped with an endplate containing airholes, that can be inserted into one of a series of slots towards the end of the tube, so that animals of different lengths can be suitably restrained. Tubes are commercially available for animals of the size of an adult rat (B & K Universal, Hull, England). All forms of restraining equipment should be frequently washed to prevent cross-infection or pheromonally-induced stress.

The operator's other hand is used to extend the hindleg, which is immobilized in the extended position by applying gentle downward pressure immediately above the knee joint. This serves the additional function of stretching the skin over the ankle, making it easier to shave, and immobilizing the saphenous vein, which in many species is rather mobile. The preferred site for venepuncture may vary between operator and

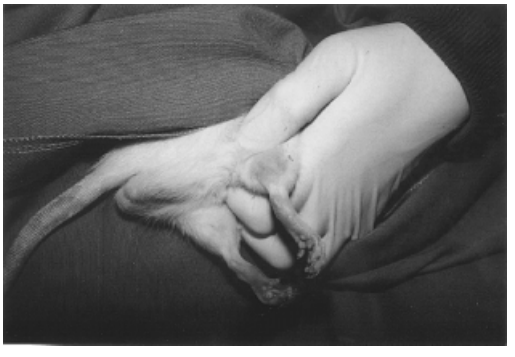

Fig 1 A method for physical restraint prior to saphenous vein puncture

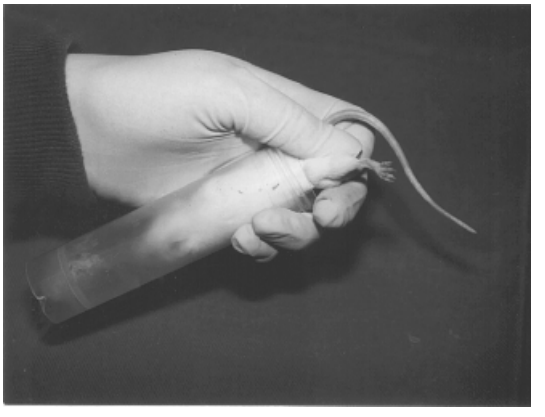

Fig 2 Use of a restraining tube to immobilize a mouse prior to saphenous vein puncture

species concerned, since the saphenous vein is usually visible both above and below the ankle joint.

Preparation of the sampling site

To shave the tarsal area, a small instrument is constructed by removing the wrapping paper from a number 11 scalpel blade (Swann-Morton Ltd, Sheffield, England) and folding it around the blade to form a short handle. The advantage of not using a standard scalpel handle is that the wrapping paper may then be curved slightly to fit the operator's grasp between the thumb and forefinger. This instrument is then used to shave the hair away from the lateral and dorsal area around the ankle joint, using a gentle stroking motion in the direction of the hairs. Care must be taken to hold the blade almost parallel to the skin, to avoid cutting it. The saphenous vein is readily visible, immediately under the skin. A small amount of silicon grease (Stopcock grease, Dow Corning, Midland, MI, USA) may be smeared over the site before puncture, to reduce the risk of clotting as the blood comes in contact with the skin. This is, however, usually unnecessary if the vein is punctured correctly and sufficient stasis is applied to maintain blood flow.

Saphenous venepuncture

A variety of methods may be used for venepuncture, depending on the size of the animal, number of blood samples to be drawn and type of blood sample required. The smallest possible gauge needle that enables sufficiently rapid blood withdrawal should be employed. In most cases this is adequately achieved using a sterile syringe needle. The diameter of the needle to be used should be assessed against the diameter of the vein of the animal in question. For mice an adequate puncture wound can often be achieved using a 25-gauge (0.6 mm) needle.

When performed correctly, a drop of blood forms immediately at the puncture site. A range of equipment may be used to collect the blood sample, depending on the amount to be removed and the specifications for the sample. As a general rule, a blood volume equivalent to about 0.5% of the animal's body weight may be safely drawn, as a single sample (Wolfensohn & Lloyd 1994), and this can usually be repeated at fortnightly intervals without disturbances to the animal's haematological status. Alternatively, daily samples corresponding to 0.05% bodyweight may be taken.

For small samples, standard micro-capillary tubes for haematocrit measurement, that hold 50–100 μl, may be used (Fig 3). These are filled by holding the tubes against the blood drop that forms on the skin. The end of the tube is then pushed into a plastic tray containing a clay sealant. For larger samples, Microvette® collection tubes (Sarstedt, D-51588 Numbrecht, Germany) may be used. These are sealed by a plastic cap and may then be centrifuged in an Eppendorf tube rotor head. Alternatively, several haematocrit tubes may be filled consecutively. All these tube types are available with or without anticoagulants.

Gentle pressure over the puncture site, or simply relaxation of the operator's grip on the hindleg, is usually more than adequate to stop further bleeding. Animal restraint time should be reduced to an absolute minimum, on welfare grounds. This will also reduce the risk of excessive bleeding caused by stress-induced increases in blood pressure. The scab that forms at the puncture site may be gently rubbed off at a later stage. Bleeding will then

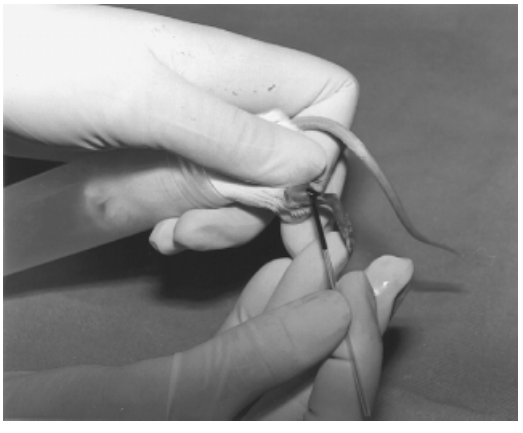

Fig 3 Blood collection from the saphenous vein using a haematocrit tube

resume, enabling serial sampling from the original puncture site. We have successfully used this method to withdraw more than 10 samples from one mouse during a 24-h period from one puncture site.

Discussion

Saphenous venepuncture is a fast, reliable method for blood collection in a range of species. The superficial position of the vein helps ensure that venepuncture is accurate, and allows easy observation of any post-collection haemorrhage. In our opinion the technique can be widely used as a replacement for techniques such as cardiac and retro-orbital puncture. It eliminates the need for anaesthesia and risk of reduced feed consumption associated with sublingual vein puncture. The most critical part of the technique is shaving the tarsal joint area prior to blood sampling, due to the risk of cutting the skin. However, this is soon mastered by experienced personnel and may be learned or carried out on sedated animals. Many alternative blood sampling techniques involve sedation or anaesthesia of the animal. If the use of a chemical restraint does not interfere with the experimental protocol, this should be used in conjunction with the present method to eliminate any risk of accidental injury, particularly before the staff are experienced with the technique. Small electrical clippers may also be used to shave the hair, if the noise of these machines is not expected to alarm the animal.

Retro-orbital blood sampling on mice and rats is widely reported in the literature. Dutch researchers concluded that retro-orbital puncture does not represent additional stress to mice that are anaesthetized for the procedure (van Herck *et al.* 1991a). However, the study employed ether anaesthesia, which in itself is a potent stressor (Gärtner 1980) and may have masked additional stress caused by retro-orbital puncture.

The authors observed a delay in the rate of regression of plasma noradrenaline levels, which they conclude may be due to tissue damage caused by the technique. They have subsequently reported histological changes in the orbital region in rats after retro-orbital puncture (van Herck *et al.* 1991b). These included haemorrhages and inflammatory reactions in the puncture track, retro-orbital periosteum, eye muscles and Harderian gland. Ether anaesthesia for laboratory rodents is virtually obsolete in Norway, having been replaced by injectables such as a fentanyl-fluanisone-midazolam mixture (Hypnorm® + Dormicum®) or volatile anaesthetics such as halothane and isoflurane (Flecknell 1996).

The size of the needle used to puncture the vein ought to be offered sufficient attention when introducing the technique to a laboratory. There is some evidence that larger bore needles may not result in more stress to mice and rats than small bore needles, based upon the use of a disturbance index (Barclay *et al.* 1988), but this may be related to the shorter handling time and faster blood removal associated with larger needles.

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