

FLSC Standard Operating Procedure for Femoral or Saphenous Vein Blood Sampling in the Rat

Purpose

The saphenous vein in the rat is a readily accessible venipuncture site as an alternative to retro-orbital bleeding. In addition, anesthesia is not required to obtain a sample. This method of blood collection provides a sample size from 75 microliters to 225 microliters.

Equipment

Rodent DecapiCone®	Electric clippers	Binder clip
Cotton tipped applicators	Cotton balls	22g x 1” needles
Triple antibiotic ointment	Scissors	Clay sealant
Eppendorf tubes	Hematocrit tubes (Plain or Heparinized)	

Procedure

1. Measure the DecapiCone® against the body of the rat and cut a hole at the level of the hind leg.
2. Restrain the rat in a plastic DecapiCone®.
 - a. The rat is placed into the DecapiCone® and the hind leg pulled through the hole.
 - b. The excess plastic is gathered around the tail to prevent the rat from backing out and secured with a binder clip.
3. Extend the back leg and shave the hair from the inner surface of the leg from the hock to the groin.
4. Apply a small amount of triple antibiotic ointment to the shaved area using the cotton tipped applicator.
5. Apply digital pressure at the groin to raise the femoral and saphenous veins. The saphenous vein crosses the leg above the ankle from the lateral to the medial surface and joins the femoral vessel above the knee joint.
6. Use a 22 g needle make a puncture by inserting the needle perpendicular to the vessel on the inner surface of the hind leg. The blood should immediately flow and form a drop on the skin.
7. Fill hematocrit tubes from the blood that pools on the surface of the skin. Up to 3 tubes can be collected.
8. Release the pressure at the groin and apply pressure to the puncture site to achieve hemostasis.
9. Return the animal to the cage after checking that bleeding has stopped.
10. DecapiCone® restrainers may be used for multiple rats and discarded when soiled or torn.

Precautions:

1. Although the procedure can be accomplished by a single technician it is recommended that two people perform the procedure, one restraining and one collecting the sample.
2. Large or fractious rats can be lightly anesthetized using inhalation anesthesia (Isoflurane) in a bell jar.
3. Support the back of the rat in the flexible restraint DecapiCone®. Use the free hand to hold off the blood vessel.
4. Only apply enough triple antibiotic ointment to form a thin layer as a barrier between the skin and the blood.
5. When sampling from the femoral vein you must ensure hemostasis to prevent subcutaneous bleeding that can form a hematoma large enough to impede ambulation.
6. Before returning the animals to their room, check again that hemostasis has been accomplished.
7. Blood samples can be spun in hematocrit tubes or transferred to an eppendorf tube to suit the needs of the protocol.