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DATE: 18.11.2010 Revised: 28.03.2013	**Standard Operating Procedure** Bleeding and Tail-Biopsies	Pages 1/3

Tail-Biopsies (only permitted as rare exceptions and if well justified)

1. Demobilize mouse by gripping neck-skin between thumb and index-finger
2. Use sterile razor blade or sharp scissors to cut no more than 5mm of tail-end
3. pad-dry tail end with sterile gaze

Tail-bleeding

Warm the mouse and place it in a restraining tube. (Do not attempt to increase blood flow by rubbing the tail from the base to the tip, as this will result in leukocytosis (increased white blood cell count)).

Using a scalpel, straight edge razor, or sharp scissors, quickly nick the tail vein. If indicated, cut no more than 5mm of tail-end instead (for example when repeated bleeding is necessary). Collect blood in a capillary tube as drops appear and collect up to 80 ul of blood into a microcentrifuge tube containing a drop of Heparin sulfate (10.000 USP). Apply pressure to stop the bleeding. (When several samples are needed within a short time period, the original wound can be reopened by removing the clot)

Retroorbital bleeding (only if tail bleeding is not possible)

1. Anesthesize mouse using isoflurane
2. Lay the anesthetized mouse on its side on a table or hold it in your hand with its head pointing down. With your first finger and thumb (finger above and thumb below the eye) pull the skin away from the eyeball, above and below the eye, so that the eyeball is protruding out of the socket as much as possible. Take care not to occlude the trachea with your thumb.
3. Insert the tip of a fine-walled Pasteur pipette (o.d. of 1 mm) or a microhematocrit blood tube into the corner of the eye socket underneath the eyeball, directing the tip at a 45-degree angle toward the middle of the eye socket. Rotate the pipette between your fingers during forward passage; do not move it from side to side or front to back. Apply gentle downward pressure and then release until the vein is broken and blood is visualized entering the pipette.
4. When a small amount of blood begins filling the pipette, withdraw slightly and allow the pipette to fill (maximal 60ul). Do not let the pipette come out of the eye socket. If the pipette is not withdrawn slightly, it may occlude the vein and blood will not flow freely. Cover the open end of the pipette with the tip of your finger before removing it from the orbital sinus to prevent blood from spilling out of the tube. It may be necessary to apply gentle pressure on the eyeball for a brief moment by closing the skin above and below the eye using your first finger and thumb. It is recommended that sample collection not be repeated on the same eye for at least two weeks.
5. Eject blood into an microcentrifuge tube containing a drop of Heparin sulfate (10.000 USP).

Sublingual bleeding (when larger volumes are needed, around 300 µl)

For blood collection, animals are anaesthetized with 3% isoflurane anaesthesia (Forene™, Abbott Laboratories SA, Switzerland, oxygen flow rate of 4 l/min). The mouse is picked up by a technician, and then, the neck skin is grasped in order to assure a partial congestion of the jugular and lingual veins. The animal is brought into a supine position. A second person extends the tongue by picking it up between the thumb and a cotton bud. The thick caudal part of the left *V. sublingualis* is punctured with a 24-gauge (24G x 1"; 0.55 x 0.25 mm) hypodermic needle. The animal is turned in a ventral position and held horizontally above the micro blood collection tube. A target volume of 300 µl from every mouse is set to be collected. In order to stop the blood flow, the animal's neck skin is released. The mouth is cleaned with a dry cotton bud to remove any remaining blood. The blood collection lasts only 20–30 s.

Blood collections from *Vena saphena*

The conscious mouse is restrained in a 50 ml Falcon tube with air holes drilled in its tip to ensure proper air accessibility and breathing. The hind leg is extended and fixed firmly by applying gentle pressure immediately above the knee joint with the same hand holding the restraining tube. The hair of puncture side is removed by electric shaving. Disinfect the puncture site with 70% ethanol and a piece of gauze. Make sure that the puncture side dried well afterwards, for example by wiping it with another piece of gauze. Blood collection can be improved by slightly warming the leg of the mouse 5 to 10 seconds under a heat lamp.
Puncture the *V. saphena* with a 18 to 26 gauge needle. Collect the blood drops into appropriate blood collection tubes. Stop the bleeding by gentle compression with a gauze compress.

- This method will be used for repetitive blood collections over at least 6 weeks, usually taking blood samples once a week (at least six times repetitive blood drawing).
- If this method is used repetitively, one should try to alternate between the left and right leg in between sequent blood samplings.
- The maximum of blood volume one is allowed to draw at once respectively within 14 days should not exceed 20% of the smaller estimated total blood volume (e.g. total blood volume of mice: 70-80ml per kg body weight ▸ results in 1.4-1.6ml total blood volume for a 20g mouse ▸ 20% of 1.4ml tot. blood vol. results in a maximum of 280ul of blood for sampling at once respectively within 14 days).

Blood collections from the tail vein by puncture or vein nicking

Gently warm the mouse under a heat lamp and place it in a restraining tube. Disinfect the puncture side (middle 1/3 of the tail) with 70% ethanol. Make sure that the puncture side dried well afterwards, for example by wiping it with another piece of gauze. Place a 22 to 23

gauge needle into the tail vein. Collect the blood drops into appropriate blood collection tubes. Blood can also be taken intravenously with U-100 0.5ml insulin syringes by aspirating very slow directly from the vein. For collecting blood by tail vein nicking, quickly nick the vein with a scalpel blade or razor blade and collect blood into microcentrifuge tubes. Apply slight pressure to stop the bleeding.

- These methods will be used for repetitive blood collections over at least 6 weeks, usually taking blood samples once a week (at least six times repetitive blood drawing).
- Alternate sides/veins of the tail should be used and successive needle punctures/nicks moved towards the tail base
- The maximum of blood volume one is allowed to draw at once respectively within 14 days should not exceed 20% of the smaller estimated total blood volume (e.g. total blood volume of mice: 70-80ml per kg body weight ▸ results in 1.4-1.6ml total blood volume for a 20g mouse ▸ 20% of 1.4ml tot. blood vol. results in a maximum of 280ul of blood for sampling at once respectively within 14 days).

References, literature and protocols:

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