

Tail-vein collection of blood: (mouse)

Mice will be either manually or mechanically restraint for blood collection. The tip of tail will be aseptically prepared or wiped with alcohol prior to the tail-snip or excision. The tail will be nicked with the sterile scalpel blade (< 0.5mm) and the blood will be collected in a sterile capillary tubes. Pressure will be applied to the tail to stop bleeding and the mouse will be returned to the cage. If bleeding persists, the nicked tail will be cauterized using a silver nitrate stick.

If the mouse is older than 21 days, the animals will be anesthetized (Isoflurane or Ketamine/Xylazine or Pentobarbital) prior to tail tip excision.

Retro-orbital bleeding: (mouse)

The mouse will be anesthetized using injectable anesthetics (Ketamine/Xylazine or pentobarbital), or a local ophthalmic anesthetic ointment will be applied for retro-orbital bleeding. Then, while properly holding/restraining the mouse (e.g., by the scruff of the neck) the blunt tip of sterile pasteur pipette will be inserted gently into the retro-orbital venous plexus to collect blood. If the blood collection is to be repeated, the alternate side/eye will be used.

Genotyping: GFP (mouse)

The investigator will wear specialized goggles to genotype live transgenic mice expressing GFP.

Genotyping: ear notches (mouse)

Genotyping will be done using an ear punch obtained from each mouse pup at the time of weaning (3 weeks of age). Stainless steel ear punches will be cleaned with clidox and wiped with alcohol between animals.

If biopsy of mice older than 3 weeks of age is required, 2 mm diameter ear notch samples will be collected, using a clean stainless steel ear punch. No anesthesia is required.

Genotyping: tail biopsy (mouse)

For genotyping, no more than 0.5 cm of tail will be cut with clean sharp scissors from young pups (3 weeks of age). This is a mild, rapid procedure, and animals experience little discomfort. Upon returning to their cages, pups immediately resume normal behavior, and show no signs of pain or discomfort. Post tail-clip bleeding is normally minimal; however, silver nitrated sticks will be used if necessary to stop any bleeding.

If a tail biopsy is required of adult mice, anesthesia will be used as per ACC policy.

Toe marking (for identification of mice)

Mice will be identified by marking their toes with non-toxic ink. The ink will be applied by piercing the toes with a 30 gauge needle. No anesthesia is required.

Gavage: (mouse)

Mice will be restrained by hand without anesthesia, then a feeding needle (attached to a syringe) will be placed next to the mouse so that the end of the needle is adjacent to the last rib. The position on the needle that is adjacent to the tip of the nose will be marked to indicate the distance that the needle should be advanced into the oral cavity to deliver the solution into the stomach. The feeding needle/syringe containing vehicle of interest will then be introduced near the rear of the mouth/pharynx to induce swallowing, such that the needle enters the esophagus. The needle will be advanced to the stomach, and then the vehicle will be injected into the stomach. When the feeding needle is appropriately placed, there is no discomfort to the animal.

If signs of distress occur (e.g., labored breathing or swelling in an axilla), the mouse will be immediately euthanized.

(The volumes and names of the solutions used for gavage should be included in the description.)

Imaging (Faxitron and MicroCT): (mouse or rat)

Animals will be subjected to in vivo imaging using digital Faxitron and MicroCT instruments. Animals will be anesthetized during the imaging procedures.

(Anesthetics to be used during the imaging procedures should be indicated in the description.)

Superovulation Procedure: (mouse)

Super-ovulation is administered by an initial IP injection of 5 IU of pregnant mares serum (PMS) in 0.1ml sterile saline using a 27 or 30G syringe, followed by a second IP injection of 5 IU of human chorionic gonadotropin (hCG) in 0.1 ml sterile saline 46~48 hours later.