

## Mouse Procedures: ARC Approved Standard Techniques

### Mouse Handling and Restraint

#### **General Considerations:**

Disinfect gloves, forceps and any other item used to pick up a rodent prior to use and between animals. Mice may bite so use caution when handling. Restraining awake mice is stressful so when performing procedures on awake animals work quickly and release animals as soon as possible. Mice should not be suspended by the base of the tail for more than a few seconds (grabbing at the end of the tail will cause a degloving injury) Always handle rodents gently. If unable to capture or restrain animal after a few attempts, stop and allow animal to calm down before you try again.

#### A. Tunnel or Cusp method of handling mice for acclimation and stress reduction:

Picking up mice by the tail has been shown to induce aversion and create high anxiety levels, as assessed by a range of measures, which can be minimized by using a tunnel or a cupped hand method. The tunnel method involves bringing a clear acrylic tunnel towards the mouse. The mouse will voluntarily walk into the tunnel, allowing it to be lifted without direct contact.

With the cupped hand method, the handler can cup the mouse in his or her hands and allow it to walk freely over the open gloved hands. As unfamiliar mice tend to jump immediately away, closing the hands loosely around the mouse on the first time until it becomes accustomed to the experience will extinguish the behavior. Do not perform this method with mice that bite.

#### B. Decapicone method of restraint:

The DecapiCone™ is a triangular shaped bag made of heavy plastic, and at the tip there is a small opening so the animal can breathe. The mouse's head is directed into the large opening of the DecapiCone™, and the animal is advanced to the narrow tip, orienting its nose in the small opening. The loose material is then closed at the animal's hindquarters. A twist-tie may be placed around the bag, at the base of the mouse's tail. This would secure the DecapiCone™ around the animal for such procedures as a tail vein injection. The restraint will be for short periods (several minutes at most), and only long enough to accomplish the dosing/sample collection technique.

### C. Acrylic tube restrainer with access ports:

An appropriately sized acrylic tube restrainer with adjustable head plate and endplates allows the animal to walk into the restrainer and for the handler to then adjust the length based on the animal's size. Alternatively, one can purchase a tube with only an adjustable head plate, although it is recommended that both ends be adjustable. If the restrainer is too big, it will allow the mouse to turn around. The mouse should have just enough space to breathe comfortably without being able to move around. Varying access ports will allow for injections and blood sampling.

## **Mouse Injection Techniques**

### **General Considerations:**

The injection of substances directly into the body requires asepsis to avoid complications. Injected substances and the needles/syringes used to inject substances should be sterile. Potential complications for all routes of injection include infection, local irritation, pain, and damage to surrounding tissue. Video demonstrations of various injection routes are available on the [Procedures With Care](#) website

Factors to be considered when selecting an injection route include:

- Pharmacology of the substance administered
- Final effect desired (e.g., local or systemic)
- Minimization of stress and discomfort to the animal

### A. Lateral Tail Vein Injection

The mouse is placed in a mechanical tube or decapicone restrainer. To facilitate dilation of the tail blood vessels, heat in the form of warm water in a beaker or similar vessel or a warm fluids bag may be applied to the tail for up to several minutes. The area over the tail vein is cleansed using a moistened gauze sponge or similar material. A 28 gauge or smaller needle is attached to a syringe and is inserted into the vein. No more than 0.2 ml is injected. Following withdrawal of the needle, direct pressure is applied to the puncture site until bleeding has stopped.

### B. Intramuscular Injection

The animal is restrained manually. A 27 gauge or smaller needle attached to a syringe is inserted into the heavy musculature of the upper thigh. Before injecting, the plunger of the syringe is withdrawn to verify that the needle is not in a blood vessel. No more than 0.05 ml is injected.

### C. Subcutaneous Injection

While manually restrained, the skin over the back is lifted to form a tent. A 25 gauge or smaller needle attached to a syringe is inserted at the tent base. The needle/syringe is held parallel to the animal's body to also avoid puncturing underlying structures. Before injecting, the syringe plunger is withdrawn to ensure that the needle has not entered a blood vessel. No more than 1 ml is injected per site.

### D. Intraperitoneal Injection

The mouse is restrained manually. The head should be tilted down at a 45 degree angle such that abdominal organs move cranially. A 25 gauge or smaller needle attached to a syringe is inserted into the lower right quadrant of the abdomen. Before injecting, the syringe plunger is withdrawn to ensure that the needle has not entered a blood vessel or possibly the bowel. No more than 1 ml is injected. IF multiple IP injections are given, the lab will alternate sides being careful not to puncture organs and watching for signs of infection or soft tissue damage.

### E. Retro-Orbital Injection

The mouse is first anesthetized with isoflurane. Once anesthetized, it will be placed on its side on a clean towel or absorbent pad. We will either immediately proceed with the injection or place a small face mask over the mouse's muzzle to deliver isoflurane (if maintenance is needed during the injection). Tetracaine may be used for pain control. Gentocin ophthalmic drops may be used to prevent infection. Using the index finger and thumb, the skin next to and below the eye will be gently pulled to protrude the globe from the socket. A 1 cc syringe with a 27 gauge or an insulin syringe with the needle attached (bevel facing outward) will be inserted at a 30-45 degree angle from the medial canthus towards the center of the area behind the eye. The needle will be inserted gently into the retro-orbital sinus. No more than 200  $\mu$ l will be injected slowly. The needle will be removed keeping the bevel facing outward to prevent scratching the eye. Gentle digital pressure will be placed over the eye to promote hemostasis. The isoflurane will be removed and the mouse will be continuously monitored until fully awake.

## **Mouse Blood Collection Techniques**

### **General Considerations:**

In general, removal of <10% of TBV not more than every 2-3 weeks will avoid adverse effects and allow adequate physiologic recovery time. However, TBV can be significantly lower than mean estimates in animals that are older, obese, or otherwise physiologically abnormal. The NC3R's site provides a table showing [practical blood sample volumes](#) for laboratory animals as well as a [Decision Tree](#) to assist in choosing the most appropriate technique.

#### A. Blood Collection from the Tail Vein

The mouse is placed in a mechanical restrainer. To facilitate dilation of the tail blood vessels, heat in the form of warm water in a beaker (or similar vessel) or a warm fluids bag may be applied to the tail for up to several minutes. The area over the venipuncture site is cleansed using a moistened gauze sponge or similar material. A 26 gauge or smaller needle attached to a syringe is inserted into the vein. No more than \_\_\_ ml blood is withdrawn into the syringe. Following withdrawal of the needle, direct pressure is applied to the puncture site until bleeding has stopped.

#### A. Blood Collection from the Saphenous Vein

The mouse is restrained in a mechanical restrainer with the rear limbs protruding. The restrainer and the mouse are held such that the rear limb is immobilized. The hair covering the lateral saphenous vein is removed with a scalpel blade. The shaved area is cleansed with a gauze pad or similar material wetted with isopropyl alcohol or other appropriate disinfectant. A lubricant may be applied so that the blood forms a droplet. Gentle pressure is applied around the leg above the knee to help improve venous filling. The saphenous vein is punctured with a 23 gauge or smaller needle or similarly sized lancet. The resulting blood flow is collected using a hematocrit tube or other suitable collecting device. Gentle pressure is applied to stop the bleeding. Two attempts per side per session will be permitted.

#### B. Blood Collection from the Submandibular Vein

The mouse is scruffed and held upright to provide a good view of the mandibular area. The fur of the area may be wetted or shaved with a scalpel blade to provide a better view of the vein. A 22 gauge or smaller needle or similarly sized lancet is used to puncture the vein. The resulting blood flow is collected using a hematocrit tube or other suitable collecting device. No more than \_\_\_ ml blood is collected. Direct pressure is applied to the puncture site until bleeding has stopped. Two attempts per side per session will be permitted.

### C. Blood Collection by Submental Vein

The mouse is scruffed so that all skin around the ventral area of the neck is taut. The vascular target is 1-2 mm off the midline of the mouse chin. An appropriate sized lancet is used to puncture the vein (5mm - 3mm) at a angle of 10-15 degrees. The resulting blood flow is collected using a hematocrit tube or other suitable collecting device. Two attempts per side per session will be permitted.

### **Oral Gavage**

The animal is restrained manually. The length of a gavage needle (sized 22 gauge or smaller) is measured against the outside of the animal's body ensuring that the tip of the needle reaches to the last rib. The gavage needle attached to a syringe is lubricated and inserted in the mouth along the back/dorsal aspect of the throat, beginning at approximately 45° angle and then gradually becoming vertical. The syringe plunger is then depressed. No more than 10 ml/kg is administered.

### **Tail Tip Excision for PCR Analysis**

The animal is restrained manually. Using a sterile scalpel blade, up to 0.5 cm of distal tail is quickly excised. A styptic powder, e.g., Kwik Stop<sup>®</sup>, is then applied to the tail tip to ensure hemostasis. Upon return to its cage the animal is observed to ensure that bleeding has ceased. If the procedure will be performed at >21 days of age, the animal will be anesthetized.

### **Ear Tag Placement**

The animal is restrained manually. An ear tag is placed in a commercial ear tag applicator. The tag is applied by crimping on the ear behind the ring of cartilage. One tag may be applied once to each ear.

### **Ear Notching/Punching**

The animal is restrained manually. A commercial ear punch tool is positioned at the appropriate portion of the ear at the cutting end of the tool. The tool is quickly depressed, cutting a hole or notch in the ear.