

## **Rat Procedures: ARC Approved Standard Techniques**

### **Rat Handling and Restraint**

#### **General Considerations:**

Disinfect gloves, forceps and any other item used to pick up a rodent prior to use and between animals. Rats may bite so use caution when handling. Restraining awake rats is stressful so when performing procedures on awake animals work quickly and release animals as soon as possible. Rats 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. Mechanical Restraint:**

The animal will be grasped gently and lifted from the cage and then placed in one of several mechanical restraint devices which are appropriately sized such as a Broome restrainer or similar. Mechanical restrainers will be cleaned to avoid pheromone induced stress or cross-infection between animals. Alternatively, a DecapiCone™ may be used. The DecapiCone™ is a triangular shaped bag made of heavy plastic with a small hole at the tip so the animal can breathe. The rat'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 rat's tail to secure the DecapiCone™ around the animal for the procedure. The restraint will be for short periods (several minutes at most), and only long enough to demonstrate the use of the device and/or to accomplish an accompanying dosing or sample collection technique.

#### **B. Manual Restraint:**

The handler's dominant hand will form a V shape with the index and middle fingers apart. The rat will be grabbed with the head between those two fingers and the rest of the hand wrapped around the body of the rat. The handlers thumb and ring finger will be used to move the arms away from the body. The non-dominant hand is then brought underneath the rat for more support. Alternatively, the index finger and thumb can be placed behind the arms, pushing the arms forward into a crossed position which prevents the rat from moving it's head to bite.

## Rat 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 rat is placed in an appropriately sized mechanical restrainer as described in ARC veterinary standards. To facilitate dilation of the tail blood vessels, heat in the form of warm water in a beaker, warm fluid bag, or similar vessel will be applied to the tail for a few minutes. The area over the tail vein approximately 3 cm from the base of the tail is cleansed using a moistened gauze sponge or similar material. A 23 gauge or smaller needle attached to a syringe is inserted into the vein. No more than 5 ml/kg is injected. Following withdrawal of the needle, direct pressure is applied to the puncture site until bleeding has stopped. The vein will be flushed with normal saline if irritation is expected.

#### B. Intramuscular Injection

The animal is restrained manually. A 25 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 0.2 ml/kg is injected.

#### C. Subcutaneous Injection

While manually restrained, the skin is lifted to form a tent. A 21 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 25 ml/kg is injected total with not more than 5 mls per site.

#### D. Intraperitoneal Injection

The rat is restrained manually and tilted with head down at a 45-degree angle. A 23 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 25 ml/kg is injected.

## **Rat 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 rat is placed in a mechanical restrainer as described in ARC standards. To facilitate dilation of the tail blood vessels, heat in the form of warm water in a beaker (or similar vessel) or a warm fluid bag is 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 23 gauge or smaller needle attached to a syringe is inserted into the vein and the blood is withdrawn. Following withdrawal of the needle, direct pressure is applied to the puncture site until bleeding has stopped. No more than 4 attempts per session.

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

The animal is restrained manually as described in ARC standards. The hair covering the lateral saphenous vein is shaved with a scalpel blade. The shaved area is cleansed with isopropyl alcohol or other appropriate disinfectant. 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. The resulting blood flow is collected using a hematocrit tube or other suitable collecting device. Direct pressure is applied to the site until the bleeding has stopped. No more than two attempts per side will be permitted.

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

The rat is anesthetized. The facial vein is located along the jaw line, directly below the lateral canthus of the eye. The site may be shaved to permit better visualization. A lancet size 5.5-8.0 mm (depending on the size of the rat) is inserted at a 30-degree angle and gently pressed to obtain a blood sample. Once the sample is collected, pressure is applied to the area until the bleeding has stopped. The animal is observed until fully awake.

### C. Blood Collection from the Jugular Vein

This procedure is performed with 2 people: one for restraint and the other for blood collection. The rat's forepaws are held on sloped restraint board and the rat held in dorsal recumbency with one person holding the abdomen and legs. The person taking the blood sample controls the head using a head cap/collar fashioned from a plastic cup or similar container. The head is tilted at an angle in the head cap, to make the site of sampling prominent. A 23 gauge or smaller needle attached to a syringe is inserted into the vein and the blood is collected followed by direct pressure until the bleeding has stopped.

An alternative method may also be done by one person on either anesthetized or conscious animals. The operator restrains the animal with one hand, typically positioning the forelimbs in the caudodorsal direction using the thumb and middle finger and securing the head with the index finger. Using the other hand, the operator inserts a 23 gauge or smaller needle into the center of the jugular fossa to puncture the jugular vein.

Not more than two attempts per side per session are permitted with either method.

### **Oral Gavage**

The animal is restrained manually with index and middle fingers, or thumb and index fingers braced along the sides of the jaw. The length of a gavage needle (sized 15-18 gauge) 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 1 % of the animal's body weight will be administered. The animal will be monitored for any signs of complications.

### **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. Note: ear punches can may at times be used for PCR analysis and are preferred to tail snips.