1.0 OBJECTIVE

1.1 The objective of this procedure is to describe the procedures for the collection of blood, urine and feces in mice and rats.

2.0 SCOPE

2.1 This procedure applies to rats and mice in the MMRI, Inc. animal facility.

3.0 POLICY

3.1 It is the policy of MMRI that this procedure be followed to ensure blood, urine and fecal samples are collected in an approved manner.

4.0 RESPONSIBILITIES

4.1 It is the responsibility of the Animal Facility Manager or designated alternate to implement this procedure and revise it when necessary.

5.0 REFERENCES

5.1 SOP # ANP007, Euthanasia of Rats and Mice

5.2 SOP # ANP016, General Animal Anesthesia

5.3 Oral Chromium Picolinate Improves Carbohydrate and Lipid Metabolism and Enhances Skeletal Muscle Glut-4 Translocation in Obese, Hyperinsulinemic (JCR-LA Corpulent) Rats, William T. Cefalu1 et al. The American Society for Nutritional Sciences J. Nutr. 2002, 132:1107-1114

6.0 PROCEDURE

6.1 General Considerations

6.1.1 The technician responsible for the sample collection during a particular study will prepare, when possible, all necessary equipment and utensils prior to the day of biological sample collection. This preparation includes removal of disposable needles and syringes from their paper wrappings and putting the needles on the syringes, with the sterile hubs left on the needles. Further preparations include identification of test tubes, urine collectors, or other receptacles. Identification labels should include study number, animal number, and collection date (type of sample if applicable).

6.1.2 Rapid or forceful withdrawal of blood from the animal may hemolyze the sample and/or the vessel from which it is collected may collapse. Furthermore, rapid or forceful ejection of a blood sample from the syringe into the test tube may similarly break platelets and red corpuscles. Both techniques should be avoided since hemolysis may interfere with laboratory tests.

6.1.3 Blood can be collected with a needle and syringe or with a needle and Pediatric sized (2.5-3.0 ml) Vacutainer^{R}. Introduce a needle into the heart or vessel, withdraw the blood sample slowly. The vacuum action of the tube, in Vacutainers^{R} will cause it to fill to the proper level. To reduce hemolysis, angle the needle so that the blood flows down the inside wall of the tube. Remove the needle.

6.1.3.1 For whole blood, or plasma, collect the blood in a tube containing anticoagulant. (Heparin, EDTA, or Citrate) When collection is complete, gently invert the tube two or three times. Place the sample on a blood rocker or mix the sample by gentle inversion of the tube at least ten times. Do not shake.

6.1.3.2 To collect blood for serum; fill the appropriate test tube or serum separator tube with blood and place it into the tube rack. Allow the sample to stand undisturbed for 20 minutes at room temperature and form a clot. Centrifuge at appropriate speed to separate cells from serum.

6.2 Terminal Blood Collection - Rodents

6.2.1 Dorsal Aorta

6.2.1.1 Anesthetize animal per SOP # ANP016.

6.2.1.2 When the animal is properly anesthetized, turn the animal on its back. Raising the loose skin on the lower abdomen, make a three to four inch incision through the skin and the abdominal wall.

6.2.1.3 Expose the dorsal aorta and, with a pair of hemostats, clamp off the lower portion of the exposed artery. Cranial to the hemostats, introduce a slightly bent needle attached to syringe or a butterfly catheter, bevel down, into the artery. Withdraw the required volume.

6.2.1.4 Euthanize the animal by an approved method per SOP # ANP005.

6.2.2 Cardiac Puncture

6.2.2.1 Anesthetize animal SOP # ANP016.

6.2.2.2 Pick the animal up by the nape of the neck and hold the animal vertically in one hand or place on a solid surface in dorsal recumbency. Palpate the apex heart beat and the xiphoid area to feel and visualize the insertion point.

6.2.2.3 Take a previously prepared syringe with the appropriate size needle (usually 22 gauge) in the other hand. Introduce the needle at a 30 to 45 degree angle to the left of the xiphoid process into the thorax and the heart. The site of the puncture should be deep in the xiphoid pit and as close to the last costal rib as possible to prevent puncture of the diaphragm.

6.2.2.4 Aspirate slowly and gently. After collection of the required volume of blood, withdraw the needle.

6.2.2.5 This is a terminal bleeding procedure. Subsequent euthanasia per SOP # ANP005 is required.

6.3 Survival Blood Collection - Rodents

6.3.1 Retro-orbital sinus bleeding

6.3.1.1 Lightly anesthetize animal per SOP # ANP016.

6.3.1.2 Position the animal on a table top so that it is laying on its side exposing the eye to be used. Use the index finger to make the eye protrude. Gently push in the heparin zed capillary tube alongside the orbit past the ventrolateral surface of the eye into the retro orbital plexus. Twist the tube gently to rupture the blood vessels. Maintain tube in place, turn animal over on its back, place the distal end of the tube into a prelabelled blood tube and let blood flow freely into the tube. When sufficient sample has been collected, remove the tube from the animal's eye. Gently wipe the residual blood from around the eye and return animal to cage.

6.3.1.3 Volumes up to 1 mL from rats and 200 ul from mice may be collected.

6.3.2 Saphenous vein bleeding procedure

6.3.2.1 Place animal in Broome restrainer, head first

6.3.2.2 Extend either hindleg and fix by holding the fold of skin in front of the thigh

6.3.2.3 Shave the hair over the vein using a clipper or scalpel blade.

6.3.2.4 Apply Vaseline or any ointment on the shaved area

6.3.2.5 Puncture the vein using a gauge 21 or 23 needle (for mice) or gauge 18 or 20 (for rats)

6.3.2.6 Collect blood by letting it drop into a blood tube or a capillary tube.

6.3.2.7 Using a piece of gauze, apply pressure to the punctured vein to stop the bleeding.

6.3.3 Tail Caudal Artery or Vein

6.3.3.1 Note: this procedure can be performed with anesthesia OR by warming the animal with an external heat source.

6.3.3.2 Warm the animal under a heat lamp for a few minutes. Watch the animal constantly (especially the mouse) for signs of overheating, or skin burn.

6.3.3.3 Remove the animal from heat source and place in restraining cylinder.
Put a warm (not hot) isothermal pad on top of the cylinder.

6.3.3.4 Place the animal/cylinder/pad on work surface. Swab the tail lightly with alcohol swab. Insert needle into the caudal artery, or vein and collect blood into prelabelled tubes.

6.3.3.5 When sufficient sample has been collected, remove the needle and gently apply pressure on the site to stop bleeding. Return animal to cage.

6.3.3.6 Volumes up to 25% of the total blood volume of a survival animal may be collected. Total blood volume is approximately 6% of the total body weight.

6.4 Urine

6.4.1 Urine samples are generally collected from mice and rats using a metabolic cage.

6.4.2 A metabolic cage is an enclosure onto which animals are placed for the collection of body metabolism samples such as urine and feces. These cages generally have a wire mesh or ribbed floor through which urine and feces can freely pass. A collection system separates the urine and feces and diverts them to different containers.

6.4.3 Place animal in metabolic cage and observe periodically or as specified by protocol. Collect urine from the urine container and place into appropriate container for analysis.

6.4.4 Replace the urine sample container with a fresh one daily.

6.5 Feces

6.5.1 Feces samples are also collected from metabolic cages.

6.5.2 Place animal in metabolic cage and observe periodically or as specified by protocol. Collect feces from the feces container and place into appropriate container for analysis.

6.5.3 Replace the feces sample container with a fresh one daily.