Shock Center Protocol

Protocol: Blood Collection RO

Date: 2/22/17

Originator: J Neal

Note:

Following is the SOP for RO Blood Collection.

1. Procedure Description

Include exact details of any/all chemical, biological, radiation, or physical agents as well as route(s)/ dose(s)/ volume(s)/ frequency and duration

This procedure involves collecting blood from behind the eye (globe) of the mouse where the venous plexus is located. The lateral (caudal) or medial canthus (rostral) may be used to access the venous plexus.

This procedure may be performed with local anesthesia in an awake mouse or with isoflurane as a general anesthetic.

NOTE: Retro-orbital bleeding of mice less than 16 days of age is not a routine procedure and requires special review and approval by the ACUC.

Procedure:

A. Lateral canthus (technician dominant side):

1. Restrain the mouse manually.

   Restrain the animal by grasping the base of the tail with dominant hand. With non-dominant hand, place the thumb and index finger, or second knuckle, on either side of the neck and draw up the loose skin, trapping the fold gently between the finger and thumb to ensure that the weight of the animal is supported. The tail may then be released or restrained.

Alternative method of restraint (one-handed method):

Place the tail of the animal between two fingers of the non-dominant hand or secure tail with pinkie finger. Place the same hand over the back of the animal and proceed to "scruff" the mouse as described above.

2. Place 1 drop of topical anesthetic onto the eye to be bled and allow to set for 15 seconds or more. Wick away excess anesthetic with a tissue or paper towel.

   NOTE: Avoid touching the surface of the eye with any material, e.g. tip of dispenser bottle, blood collection tube.

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3. Use ring finger of non-dominant hand to pull back the eyelid to cause eye to proptose and allow access to the membrane.

4. Position the blood collection tube as shown in Figure A paying particular attention to the orientation and angle of the tube relative to the eye of the animal.

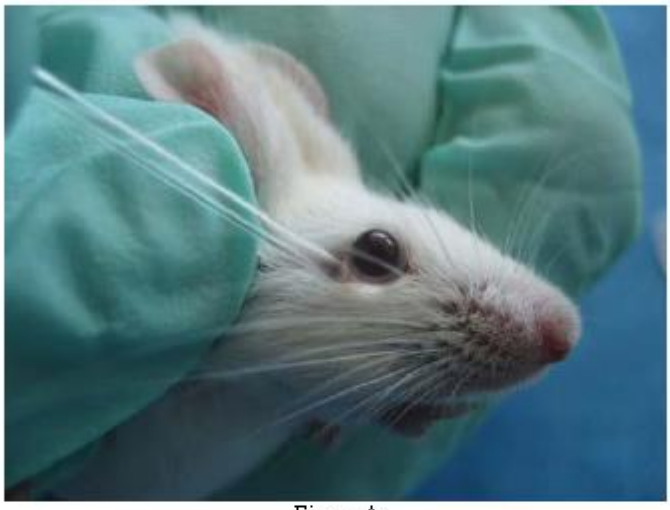

Figure A

5. Apply gentle inward pressure and rotate the hematocrit tube between your fingers until the tube punctures through the sclera and blood enters the tube.

6. Release the forward pressure, withdraw the tube slightly and allow the tube to fill. Modify blood flow as needed by changing the angle of the tube.

   NOTE: Refer to *Blood Collection - Volumes* for acceptable collection amounts (http://myjax.jax.org/acuc/procedures/index.html).

7. After the required amount of blood is obtained, withdraw the tube from the canthus. Bleeding usually ceases immediately. If necessary, control bleeding by applying direct pressure with a sterile gauze pad over the eyelids.

8. Return the mouse to its cage.

9. Repeat steps 1-8 for each additional animal.

B. <u>Lateral canthus (technician non-dominant side)</u>:

1. Restrain the mouse manually.

   Restrain the animal by grasping the base of the tail with dominant hand. With non-dominant hand, place thumb and index finger, or second knuckle, on either side of the neck and draw up the loose skin over the head toward the thumb. Thumb will brace the cheek of the animal. The tail may then be released or restrained.

Alternative method of restraint (one-handed method):

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Restrain the animal by grasping the base of the tail with non-dominant hand. With dominant hand, place thumb and index finger, or second knuckle, on either side of the neck and draw up the loose skin, trapping the fold gently between the finger and thumb to ensure that the weight of the animal is supported. The tail may then be released or restrained.

2. Repeat steps 2-9 in section A.

C. Medial canthus:

1. Place the mouse on a table or cage lid in lateral recumbency. Restrain the body of the mouse against the table using the palm of the hand.

2. Use ring finger of non-dominant hand to pull back the eyelid to cause eye to proptose and allow access to the membrane.

3. Place 1 drop of topical anesthetic onto the eye to be bled and allow to set for 15 seconds or more. Wick away excess anesthetic with a tissue or paper towel.

   NOTE: Avoid touching the surface of the eye with any material, e.g. tip of dispenser bottle, blood collection tube.

4. Position the blood collection tube as shown in Figure B paying particular attention to the orientation and angle of the tube relative to the eye of the animal.

Figure B

5. Quickly rotate the tube between your fingers. Apply gentle forward pressure until the blood vessels are broken and blood enters the tube.

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- **Potential complications:** corneal ulcerations, infection, puncture of the globe, blindness, or adverse reaction to anesthetic

- **Tetracaine has been shown to produce seizure activity in some strains of mice.** When seizures are observed: stop the procedure and allow the animal to recover for 24 hours before attempting again. Use an alternative topical anesthetic for the second attempt. If sampling must occur that day, allow the animal to recover for a minimum of 1 hour and sample from the other canthus.

4. References if applicable: