Johns Hopkins University
Animal Care and Use Committee

Retro-orbital Blood Collection from Mice and Rats

PURPOSE:

To collect blood from the retro-orbital sinus (mouse) or plexus (rat).

INTRODUCTION:

The retro-orbital (RO) sinus or plexus are good routes to collect medium to large volumes of blood. This technique is suitable for collecting up to the maximum allowable volume for the given animal weight. Additionally, blood can be collected in a brief period making it a good route for exsanguination.

Factors to consider when selecting the appropriate blood collection route:

- The quantity of blood required, including extraneous blood loss due to a selected route.
- The health status of the animal's health (e.g., hydration status, condition of the route).
- The frequency of sampling, i.e., sampling interval. See the ACUC *Multiple Blood Draws* Guideline Animal Care and Use Committee.
- The size and type of capillary tube, lancet, or needle.
- The quality of the sample required (e.g., sterility, hemolysis, tissue fluid contamination).
- The training and experience of the phlebotomist.

PROCEDURE:

A. Retro-orbital Blood Collection Parameters

1) As a survival procedure, the maximum amount of blood that may be drawn from this route is 1%.
2) Based on the amount, blood can only be collected once per week from one eye. Subsequent bleeds should use alternate eyes resulting in a 2-week healing time for each eye.
3) The maximum number of survival bleeds for each animal is two bleeds per eye, with a third if terminal, for a total of five.
4) To prevent corneal damage due to drying, ophthalmic ointment is applied to the eye that is not to be bled.

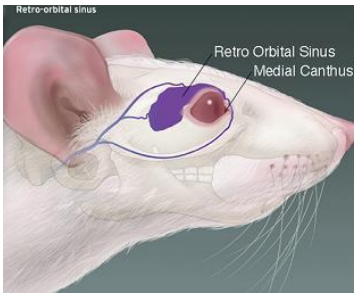
B. Anesthesia – This is a category D procedure; if adding by amendment, mark the *Modify Pain Category* option.

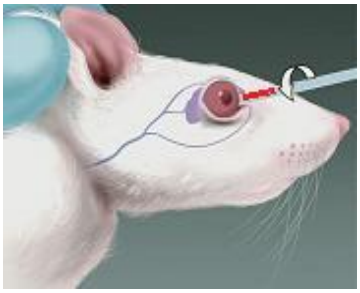
General anesthesia is required. When selecting the anesthetic drug keep in mind the procedure does not take long to perform, and therefore a relatively short acting drug is recommended.
Consider the anesthetic options below.

1) Injectable anesthetic drugs
   a) For Drugs and doses see the *RAR formulary*: Research Animal Resources
2) Gas anesthetic drugs
   a) The "Drop Method" see the ACUC guidelines, *Anesthesia-Gas: Drop Method:* Animal Care and Use Committee
   b) For a system that uses anesthesia machines and vaporizers
      i) The recommended doses are: 3-5% for induction and 1-3% for maintenance
      ii) See the ACUC guidelines for *Anesthesia-Gas: Use and Vaporizer Calibration:* Animal Care and Use Committee

C. Blood Collection

1) This is done using aseptic technique.
2) The animal is held by the scruff of the neck, and the skin of the head is tightened with the thumb and middle finger.
3) The tip of the capillary tube is placed at the medial canthus of the eye under the nictitating membrane or third eyelid.
4) Either rotate the tube or provide a gentle thrust as you press the tube past the eyeball to enter the slightly resistant membrane of the sinus/plexus. The eyeball itself remains uninjured.
5) As soon as the sinus/plexus is punctured, blood enters the collection tube by capillary action. It may be helpful to retract the tube a bit to facilitate blood flow.
6) For exsanguination, angling the tube downward can help in increasing the blood flow.
7) When, based on the number of bleeds, the allowable amount of blood is collected, the tube is withdrawn and slight pressure with sterile gauze/cotton tipped applicator is used to ensure hemostasis.
8) Care is taken not to scratch the cornea.





Images source: www.theodora.com/rodent_laboratory/blood_collection.html

D. Analgesia

Pre-emptive analgesia is preferred for this procedure. Use either a local anesthetic such as Proparacaine (1 drop per eye, applied approximately 1 minute prior to the procedure, with analgesia lasting ~15 minutes after application) or a systemic analgesia such as buprenorphine, see the *RAR formulary*: Research Animal Resources.

E. Anesthesia Recovery Monitoring

1) During recovery from anesthesia, the following clinical parameters must be monitored and documented at 10-to-15-minute intervals until the animal is fully recovered. Ensure the following:
    a) Hemostasis is maintained
    b) Adiquate respiratory, not too shallow, or too rapid
    c) Ability to maintain sternal recumbency
2) To protect the animal from hypothermia place them on a water recirculating heating pad or provided with an overhead heat lamp. The animal should never be returned to the housing facility until ambulatory.

F. Post-procedure care and monitoring for adverse effects: Consult a veterinarian

1) Monitor 2-3 times in the week following each blood collection
2) Squinting of the eye
3) Signs of infection such as discharge or redness
4) Peri-orbital swelling
5) Shrunken appearing eye
6) Evidence of self-trauma
7) Corneal signs such as ulceration or opaqueness

G. Humane Endpoints

Animals should be euthanized if: the eyeball is acutely damaged, treatment of an injured/infected eye is unsuccessful, and/or bilateral blindness occurs.