

SOP No: AHT 30(b)

SUBJECT: Retro-Orbital Plexus Bleed in the Rat

POLICY: Rats must be bled under anaesthetic
Operator must be experienced and competent in this technique.
Bleeding may only be performed once unless otherwise approved by the AEC.

PRECAUTIONS: Gloves, eye protection, long sleeve gown, closed in shoes.

EQUIPMENT: anaesthetic
Capillary tubes
Eppendorf tubes

PROCEDURE:

- 1. Anaesthetise rat with anaesthetic of choice:**
Injectable - specify dose rate, route of injection and volume.
Gaseous - Isoflurane 3% in oxygen adequate for rapid induction and rapid recovery
- 2. Restrain the animal and placing thumb just below the eye, pull lower eyelid downward**
- 3. Using a 90° twist gently push the fire polished (red marking) exposed end of the capillary tube behind the eyeball into the eye socket at the outer edge of the eye. The tube should be held at 90° to the plane of the side of the head and if the eye-socket is considered a clock-face, the tube should be pressed gently to perforate the tissues at approximately the 8 o'clock position.**
- 4. Hold the rat horizontally but rotate the rat in the axial plane by about 45° so that the capillary tube still at 90° to the side of the head is inclined downward at 45°.**
Without moving the capillary tube release the inward pressure and allow blood to flow down the tube and drop into an Eppendorf tube. Each drop will be approximately 50µl.
- 5. Expect to collect no more than 200µl/bleed from a 200g rat. All bleeds must represent less than 5% of calculated total blood volume to avoid hemorrhagic shock (resulting if 10% or more of TBV is collected). If multiple bleeds have been approved, do not bleed again for at least 14 days**
- 6. Expect to collect no more than 200µl/bleed. If multiple bleeds have been approved, do not bleed again for at least 14 days.**

- 7. Withdraw capillary tube from plexus and immediately close the eye and apply gentle pressure using sterile gauze pad. Maintain this light pressure for one minute and check that blood is not leaking from the sinus. If it is, repeat the application of pressure until bleeding has stopped. Unchecked bleeding behind the eye and subsequent clot formation can result in blindness.**
- 8. Remove excess blood from around the eye.**

RECOMMENDATIONS:

DATE ISSUED:

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REFERENCES

- 1. Perspectives on Animal Use Biological Effects of Blood Loss: Implications for Sampling Volumes and Techniques. In: ILAR News (1989), 31(4).**
- 2. Removal of blood from laboratory mammals and birds: First report of the BVA/FRAME/RSPCA/UFAW Joint working group on refinement. Lab Anim (1993) 27, 1-22.**
- 3. J. Donovan and P. Brown. Blood Collection. In: Current Protocols in Immunology, (eds: A. Kruisbeek et al), John Wiley & Sons, New York, NY, USA, Unit 1.7.**
- 4. Guidelines for Retro-orbital Bleeding in Laboratory Rats and Mice <http://iacuc.yale.edu/policies/retro-orbital.html>**