1. PURPOSE

1.1. The purpose of this Animal Care and Use Procedure (ACUP) is to describe the method for obtaining blood from the retro-orbital sinus / plexus of mice and rats. This ACUP is approved by the Cornell Institutional Care and Use Committee (IACUC). Any deviation must be approved by the IACUC prior to its implementation.

2. SCOPE

2.1. This ACUP is intended for use by all personnel that plan to use this technique for blood sampling in mice and rats.

3. INTRODUCTION

3.1. Retro-orbital blood collection in rodents can provide moderate to large amounts of blood when performed by trained personnel; however, severe injuries may occur to the animal if this procedure is not done properly. Satisfactory alternatives to retro-orbital bleeding are available and should be considered. Since rats have a retro-orbital venous plexus rather than a venous sinus (mouse), retro-orbital bleeding may result in greater tissue damage than in the mouse; therefore, alternative blood collection sites are strongly recommended for the rat.

3.2. Center for Animal Resources and Education (CARE) at Cornell University staff must observe and approve the technique of personnel that will use this procedure. Contact CARE training at (607) 253-3831, dem23@cornell.edu or cmp15@cornell.edu for more information.

3.3. Contact CARE at care@cornell.edu for more information or for other assistance.

4. MATERIALS AND EQUIPMENT

4.1. Blood collection tubes (e.g., capillary tubes or Pasteur pipettes).
4.2. Blood collection containers (e.g., microfuge tubes, cryo-vials).
4.3. Gauze sponges.
4.4. General anesthetic agents and equipment (see ACUP 101 Rodent Anesthesia).
5. **PROCEDURE**

5.1. **General Considerations and Precautions**

5.1.1. Retro-orbital blood collection should be done under general anesthesia unless the operator is highly experienced and proficient in the procedure.

5.1.2. Never attempt to collect from both eyes at the same session unless it is a terminal collection.

5.1.3. Check the animal once a day for at least 3 days for complications as a result of this procedure. Record these checks in the animal’s record or on the cage card.

5.1.4. Call CARE veterinary staff if ocular damage is apparent.

5.1.4.1. Evidence of ocular damage include: squinting, bulging, swelling, opaque appearance of the eye, and orbital discharge following the procedure.

5.1.5. If an eye is damaged from a previous procedure that eye may not be used again for blood collection.

5.1.6. Perform no more than one retro-orbital bleed per animal per week.

5.1.7. Alternate right and left eyes so that each eye is used no more than twice a month.

5.1.8. No more than 3 procedures may be performed per eye.

5.1.9. Observe guidelines for blood collection volume (see ACUP 403 Recommended Blood Collection Volume and Frequency).

5.1.10. Clearly document all procedures (including blood collection and post collection health checks) with specific procedure cards or detailed notes on cage cards, see ACUP 542 Maintaining Health and Procedure Records for Research and Teaching Animals.

5.2. **Collection Procedure**

5.2.1. Follow ACUP 101 Rodent Anesthesia if applicable. Establish that the animal is adequately anesthetized before commencing blood collection procedure.

5.2.2. Place the animal on the table in lateral recumbency. Scruff the animal with the thumb and forefinger of the non-dominant hand, firmly enough to pull the skin taut around the eye.

5.2.3. Insert a collection tube into the medial canthus of the eye under the nictitating membrane at approximately a 30 degree angle lateral to the nose.

5.2.4. Direct the collection tube gently in a ventrolateral direction while rotating the tube (rolling it between the thumb and forefinger of the dominant hand).

5.2.5. Position the animal in such a way that the end of the collection tube, in the medial canthus, is higher than the end which will drip into the blood collection container.

5.2.6. A slight thrust may be needed to puncture the tissue and enter the plexus or sinus. Do not push too hard – it is possible to push through the skull if using too much force.

5.2.7. Blood will begin to flow into the capillary tube. If you need to obtain more blood than the volume of the capillary tube, the blood may be allowed to run through the tube and drip into the collection container.
5.2.8. Bleeding usually ceases upon withdrawal of the collection tube. If bleeding continues, apply gentle pressure over eyelids with gauze sponge until bleeding ceases.

6. PERSONNEL SAFETY

6.1. Medical Emergencies: CALL 911.
6.2. When working with animals wear appropriate PPE, observe proper hygiene, and be aware of allergy, zoonosis, and injury risks. Refer to the CARE Occupational Health and Safety webpage for more information.

7. ANIMAL RELATED CONTINGENCIES

7.1. Post contact information for emergency assistance in a conspicuous location within the animal facility.
7.2. Non-emergency veterinary questions and requests for care, email CARE veterinary staff at care@cornell.edu.
7.3. Emergency veterinary care is available at all times including after working hours and on weekends and holidays by calling the CARE pager (1-800-329-2456).

8. REFERENCES

8.1. ACUP 101 Rodent Anesthesia:
http://ras.research.cornell.edu/care/documents_k/ACUPs/ACUP101.pdf
8.2. ACUP 403 Recommended Blood Collection Volume and Frequency:
http://ras.research.cornell.edu/care/documents_k/ACUPs/ACUP403.pdf
8.3. ACUP 542 Maintaining Health and Procedure Records for Research and Teaching Animals:
http://ras.research.cornell.edu/care/documents_k/ACUPs/ACUP542.pdf
8.4. CARE Occupational Health and Safety webpage:
http://ras.research.cornell.edu/care/OHS.html

9. APPENDIX

9.1. None

10. HISTORY

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