

Aim: Common laboratory techniques. Blood withdrawal, serum and plasma separation, anesthetics and euthanasia used for animal studies.

References:

- CPCSEA Guidelines for Laboratory Animal Facility, Ministry of Environment, Forest and Climate Change, Government of India.
- Institutional Animal Ethics Committee (IAEC) Standard Operating Procedures.
- AVMA Guidelines for the Euthanasia of Animals.

Objective:

To outline the detailed procedures for blood withdrawal, serum and plasma separation, use of anesthetics, and euthanasia in laboratory animals, ensuring compliance with ethical standards and best practices.

Materials and Equipment:

- Sterile syringes and needles
- Capillary tubes
- Blood collection tubes (with and without anticoagulants)
- Centrifuge
- Anesthetics (e.g., isoflurane, ketamine, xylazine)
- Euthanasia agents (e.g., sodium pentobarbital, CO₂)
- PPE (gloves, masks, lab coats)
- Animal restraining devices
- Disinfectants
- Record-keeping materials

Procedure:

1. Blood Withdrawal:

1. Preparation

- Ensure all equipment is sterile and ready for use.
- Wear appropriate PPE.
- Calm the animal to minimize stress.

2. Site Selection and Techniques

- **Rodents:** Common sites include the tail vein, retro-orbital sinus, and cardiac puncture.
 - **Tail Vein:** Warm the tail to dilate veins. Clean the site with an alcohol swab. Insert a 25-27 gauge needle into the vein and gently withdraw blood.
 - **Retro-Orbital Sinus:** Anesthetize the animal. Use a capillary tube to puncture the sinus medially, ensuring proper positioning to avoid injury.
 - **Cardiac Puncture:** Performed under deep anesthesia. Insert the needle between the ribs into the heart, ensuring minimal trauma.
- **Rabbits:** Common sites include the marginal ear vein and the central auricular artery.
 - **Marginal Ear Vein:** Clean the ear with an alcohol swab. Insert a 21-23 gauge needle and withdraw blood gently.
 - **Central Auricular Artery:** Requires anesthesia. Insert the needle carefully to avoid excessive bleeding.

3. Post-Procedure Care

- Apply gentle pressure to the puncture site to prevent hematoma.
- Monitor the animal for any signs of distress or complications.
- Record the procedure details and the amount of blood collected.

2. Serum and Plasma Separation:

1. Collection and Handling

- **Collect blood into appropriate tubes:**
 - **Serum:** Use clot activator tubes (red-top).
 - **Plasma:** Use anticoagulant tubes (e.g., EDTA, heparin; purple or green-top).
- Allow blood in serum tubes to clot at room temperature for 30 minutes.

2. Centrifugation

- Place the tubes in a balanced centrifuge.
- Centrifuge at 1500-2000 x g for 10-15 minutes.
- **For serum:** Carefully pipette the clear supernatant (serum) into a clean tube.
- **For plasma:** Ensure complete separation of plasma from the cells, then pipette the plasma into a clean tube.

3. Storage

- Store serum and plasma at -20°C or -80°C for long-term preservation.
- Label tubes with relevant details (animal ID, date, type of sample).

3. Anesthetics

1. Preparation

- Choose the appropriate anesthetic based on the species and procedure.
- Calculate the correct dosage based on the animal's weight.
- Ensure all anesthetic equipment (e.g., vaporizers, injectors) is functional and clean.

2. Common Anesthetics

- **Isoflurane:** Inhalant anesthetic.
 - Place the animal in an induction chamber.
 - Administer 2-5% isoflurane in oxygen for induction.
 - Maintain anesthesia with 1-3% isoflurane.
- **Ketamine/Xylazine:** Injectable anesthetics.
 - Administer ketamine (80-100 mg/kg) and xylazine (5-10 mg/kg) intraperitoneally or intramuscularly.
 - Monitor the depth of anesthesia by checking reflexes (e.g., toe pinch).

3. Monitoring

- Continuously monitor vital signs (respiratory rate, heart rate, temperature).

- Adjust anesthetic levels as necessary to maintain appropriate depth.

4. Recovery

- Place the animal in a warm, quiet area for recovery.
- Monitor until the animal regains consciousness and can maintain sternal recumbency.

4. Euthanasia:

1. Preparation

- Select the appropriate euthanasia method based on the species and ethical guidelines.
- Prepare all necessary equipment and agents.

2. Common Euthanasia Methods

- **Sodium Pentobarbital:** Injectable euthanasia solution.
 - Administer intravenously (50-100 mg/kg).
 - Ensure deep anesthesia followed by cessation of vital signs.
- **Carbon Dioxide (CO₂) Inhalation:**
 - Place the animal in a chamber.
 - Gradually fill the chamber with CO₂ (20-30% per minute).
 - Continue until the animal shows no signs of respiration for at least 2 minutes.
- **Cervical Dislocation (small rodents):**
 - Ensure the animal is deeply anesthetized.
 - Dislocate the cervical vertebrae quickly and humanely.

3. Confirmation of Death

- Confirm death by checking for the absence of vital signs (heartbeat, respiration).
- Perform secondary methods if necessary (e.g., exsanguination).

4. Disposal

- Dispose of carcasses according to institutional and regulatory guidelines.

- Record the euthanasia details in the animal's health log.

Safety Precautions

- Always wear appropriate PPE.
- Handle all animals gently and with respect.
- Ensure all procedures are performed by trained personnel.
- Adhere to institutional and regulatory guidelines for animal care and use.

Ethical Considerations

- Obtain necessary approvals from the Institutional Animal Ethics Committee (IAEC).
- Adhere to the principles of the 3Rs (Replacement, Reduction, Refinement).
- Ensure all procedures are justified and minimize animal suffering.

Conclusion

This manual provides detailed protocols for blood withdrawal, serum and plasma separation, use of anesthetics, and euthanasia in laboratory animals, ensuring adherence to ethical standards and best practices for animal welfare.

Notes

- Regular training and competency assessments should be conducted for all personnel involved in animal handling and procedures.
- Any deviations from standard procedures should be documented and justified, with measures taken to minimize any negative impact on animal welfare.