Research and Innovation Division

Research Animal Standard Operating Procedure

SOP#43

Research Animal Standard Operating Procedures (SOP) must meet the following criteria:

1. Describe procedures or activities involving research animal(s) common to more than one research project.

2. Support the handling and or performance or undertaking of a procedure(s), involving an animal, in the same way on each occasion it is performed.

3. Describe a procedure or activity involving a research animal(s) undertaken by more than one person; and

4. Describe a procedure or activity involving a research animal(s) that will be undertaken in more than one location.

Name of Procedure	Intraperitoneal Anaesthesia: Ketamine + xylazine or Ketamine + medetomidine (non-recovery)	
Species	Mouse	
ACEC	**Reference**	SOP#43 – Jun 23 - IP Anaesthesia: Ketamine /xylazine or medetomidine-Non-Recovery - mouse
	Author	Dirk Van Helden
	Version	1.3
	Date approved	23 June 2023
	Date for review	23 June 2026
	Procedure classification 1. Observation involving minor interference 2. Animal unconscious without recovery 3. Minor conscious intervention 4. Minor surgery with recovery 5. Major surgery with recovery 6. Minor physiological challenge 7. Major physiological challenge	3
Ethical considerations	1. Respect for animals must underpin all decisions and actions involving the care and use of animals for scientific purposes. 2. The procedure must be performed according to current best practice to support the wellbeing of the animal. 3. Persons performing this procedure must be competent in the procedure or be under the direct supervision of someone who is competent.	

1. Purpose

This Standard Operating Procedure describes the method for induction and maintenance of anaesthesia in the mouse for a **non-recovery** procedure using the anaesthetic regime, ketamine + xylazine or ketamine + medetomidine.

2. Introduction

2.1. Mice are not routinely fasted prior to anaesthesia due to their inability to vomit.

2.2. Heat loss is rapid in anesthetised rodents. Keep animals warm from the time of initiation of anaesthesia, and for the duration of anaesthesia until the animal is euthanased.

2.2.1. Methods include:

(i) General: Warming of the room environment - maintain at a high ambient temperature of 25-30^{0}C for adult animals, 35-37^{0}C for neonates.

(ii) During procedure: Use of heating pad, warm packs, insulation material (towels, bubble wrap)

2.2.2. Monitor the animal's body temperature for the effectiveness of the method used, and to ensure that overheating does not occur.

2.3. Never leave an anesthetised animal unattended.

3. Essential knowledge and skills

3.1. Ability to restrain a mouse properly, with minimal stress to the animal.

3.2. Knowledge of needle safety.

3.3. Knowledge of how to draw up correct volumes of injectable solution.

3.4. Ability to correctly administer substances via the intraperitoneal route.

3.5. Knowledge of the use of a gaseous anaesthetic machine for delivery of oxygen.

3.6. Knowledge of stages of anaesthesia, assessment of depth of anaesthesia, methods for monitoring anaesthesia, methods for ensuring the well-being of the animal during anaesthesia.

3.7. Knowledge of correct procedures in the event of anaesthetic overdose.

4. Materials and equipment

4.1. Weighing machine

4.2. Drugs – ketamine, xylazine or medetomidine

4.3. Sterile "Water for Injection" for dilution of ketamine, xylazine or medetomidine needle (25-27 gauge, 13-25 mm) and syringe

4.4. Sharps Container

4.5. 0.5% chlorhexidine in 70% alcohol solution for cleaning and disinfection of skin

4.6. Method to restrain the mouse (e.g. towel, mouse bag, restraining tube)

4.7. Equipment to provide or conserve body heat (e.g. heating pad, heat lamp)

4.8. Rectal thermometer

4.9. Ophthalmic ointment

4.10. Equipment for delivery of gaseous oxygen. Note: Oxygen is normally delivered via use of

a gaseous anaesthetic machine. When delivering oxygen alone, the anaesthetic chamber and scavenging system is not used. Relevant part of the anaesthetic machine for delivery of oxygen are:

4.10.1. Anaesthetic machine (calibrated within the last 12 months).

4.10.2. Anaesthetic breathing circuit such as paediatric Bain coaxial circuit or Ayres T-piece and rebreathing bag

4.10.3. Mask

4.10.4. Medical grade oxygen cylinder, regulator and oxygen tubing.

5. Specific safety notes

5.1. Needle safety

5.1.1. Gloves must be worn.

5.1.2. Do not uncap the needle until ready.

5.1.3. Once a needle is uncapped it is never to be recapped.

5.1.4. Disposal of needles must be in a sharps container.

5.1.5. Do not leave uncapped needles lying on the bench after they have been used, place them straight in the sharps container.

6. Description of procedure.

6.1. Preparation:

6.1.1. Prepare area where anaesthetised animal will be placed:

(i) Prepare equipment for keeping animal warm. If using a heating pad, turn it on.

(ii) Prepare equipment for delivery of oxygen.

6.1.2. Weigh animal and return to its cage.

6.1.3. Prepare drug solution(s) in syringe, ready to administer to the animal.

(i) Calculate dose rate for the anaesthetic drug based upon the weight of the animal. Drugs and dose rates – see Section 8.

(ii) Prepare drugs, ensuring that sterility of the solution is maintained by use of sterile needles and syringes, disinfection of the top of the bottle prior to penetration with a needle; use of new needles between bottle of diluent and drug, use of new needles and syringes for each animal.

(iii) Ensure there are no air bubbles present in the syringe. Air bubbles can be removed by flicking the syringe a few times with your finger with the needle pointing upwards until all of the bubbles have reached the top. Then you draw the solution back and then forward slowly till the solution fills the top of the needle. This may need to be done a few times to remove all the bubbles.

(iv) Label syringe as necessary (drug name, concentration).

(v) Lay syringe down, ensuring that the needle does not touch an unsterile surface (e.g. rest syringe on the needle cap so that needle does not touch any surface, or rest needle on sterile swab).

6.2. Induction:

6.2.1. To restrain the mouse for intraperitoneal injection:

(i) Place the wire of the cage on top of the cage base or the cage lid.

(ii) Pick up a mouse close to the base of the tail and hold the mouse in place with one hand (right handed people restrain with left hand, left handed restrain with right hand).

(iii) Make a V shape with your bent index finger and your thumb. Use this hand and gently slide it up the back of the mouse towards the head.

(iv) Do not close your grip until you reach the lower base of the skull.

(v) Draw your grip together until the skin is pulled firm across the mouse's stomach and head.

(vi) Wrap the tail around your hand and secure it with your little finger.

(vii) The mouse should now be in a secure restraint, and be unable to move its head or legs but able to breathe freely. Be sure to hold enough skin so that it cannot bite you or kick.

6.2.2. Locate the midline (this is a line of fur that runs down the centre of the mouse).

6.2.3. Prepare to inject on the right side of the mouse, half way in between the midline and the top of the hind leg. The right side is used to avoid the bladder, spleen and stomach, which are situated on the left side of the mouse.

6.2.4. Hold the mouse at a slightly downward angle (to allow the organs to drop towards the upper body).

6.2.5. Wipe skin against the lay of the hair with a swab wetted with Alcoholic chlorhexidine, ensuring that the antiseptic contacts the skin.

6.2.6. Insert the needle, bevel tip up, into the mouse about 0.5cm deep. Once needle inserted, ensure a steady and still hold to avoid laceration of abdominal organs or blood vessels.

6.2.7. Pull back slightly on needle plunger to check that the needle is not in an organ or bladder. If the needle is correctly positioned in the peritoneal cavity a vacuum should be formed when an attempt to draw back the plunger is made.

6.2.8. Inject the solution slowly and at a constant rate.

6.2.9. Pause for a couple seconds so that the liquid does not seep out.

6.2.10. Remove the needle and place the mouse down gently back into its cage.

6.2.11. Dispose of the needle in the sharps bin.

6.2.12. Monitor the mouse until it loses it righting reflex (this should occur within 5 minutes). Remove the mouse from its cage and position it in the area required for the procedure.

6.2.13. Attach oxygen mask. Turn the oxygen flow rate to 1 litre per minute.

6.2.14. Ensure equipment for maintaining body temperature is in place and switched on.

6.2.15. Insert the rectal thermometer and note the temperature of the mouse at anaesthetic induction.

6.2.16. Apply ophthalmic ointment to both eyes.

6.3. Maintenance and monitoring:

6.3.1. Monitor respiratory frequency to ensure slow constant breathing.

6.3.2. Check adequacy of the depth of anaesthesia using lack or reflexes such as the withdrawal reflex (flexion of the leg following a firm pinch of the paw or interdigital skin) or the palpebral reflex (in response to stroking the eyelids). These reflexes should be absent if the mouse is adequately anaesthetised.

6.3.3. Monitor body temperature every 20 minutes using the rectal thermometer.

6.3.4. Administer 0.9 % normal saline via IP route (or IV route – see separate procedure). As a general guide, total fluid infusion of up to 10% of circulating blood volume per hour (7 ml/kg/hr) are well tolerated by most animals.

6.3.5. Animal's posture is to be adjusted every hour (e.g. roll over to change sides).

6.4. Conclusion of the procedure:

6.4.1. At the conclusion of the procedure, the mouse must be euthanased before awaking using a suitable method (separate Standard Operating Procedure).

7. Drug details

Drug name (**generic** name, not trade name)	Dose rate (**mg/kg body weight**)	Route	Timing of administration, and frequency *(e.g. 30 minutes pre-operative, to induce anaesthesia, during procedure, at specific intervals during the procedure)*
Anaesthetic			
Ketamine + medetomidine	75 mg/kg ketamine + 1.0 mg/kg medetomidine	IP	Once. Produces surgical anaesthesia for 20-30 minutes with a total sleep time of 60-120 minutes. Top-up dose: One-third of initial dose.
Ketamine + xylazine	80 – 100 mg/kg ketamine + 10 mg/kg xylazine	IP	Once. Produces surgical anaesthesia for 20-30 minutes with a total sleep time of 60-120 minutes. Top-up dose: One-third of initial dose.

8. Refinement

Potential adverse effects	Frequency of adverse effects	Methods to minimise adverse effects
Stress from inappropriate handling and restraint	Infrequent if performed by skilled personnel	1. Personnel trained to perform handling and restraint or under the direct supervision of a skilled person. 2. Acclimatisation of the animals to handling and the presence of the operator. 3. Use of clean mouse bag or towel, or cleaning of restraint tube to minimise pheromonally-induced stress.
Pain and distress because of incorrect administration of pre-medication via subcutaneous route.	Infrequent if performed by skilled personnel	1. Personnel trained to perform subcutaneous injection or under the direct supervision of a skilled person.

Inadvertent puncture of major blood vessel, gut loop, bladder during intraperitoneal injection procedure.	Infrequent if performed by skilled personnel	1. Personnel trained to perform intraperitoneal injection or under the direct supervision of a skilled person. 2. Drawing back with the syringe prior to injection to ensure correct placement.
Pain and distress from procedure if anaesthetic depth is inadequate	Infrequent if performed by skilled personnel	1. Personnel trained to perform anaesthesia or under the direct supervision of a skilled person. 2. Depth of anaesthesia is monitored frequently.
Anaesthetic overdose and death	Infrequent if performed by skilled personnel	1. Personnel trained to perform anaesthesia or under the direct supervision of a skilled person. 2. Animal is weighed and dose rates calculated accurately.
Drying of cornea	Infrequent	Use of ophthalmic ointment.
Hypothermia	Frequent	1. Provide external source of heat. 2. Monitor body temperature. If body temperature falls below 35^0C, provide supplemental heat.
Respiratory difficulties because of build-up of salivary and bronchial secretions.	Infrequent	1. Personnel trained to perform anaesthesia or under the direct supervision of a skilled person. 2. Administration of atropine prior to anaesthesia. 3. Monitoring of animal, and action taken if respiratory compromise is noted (eg. suction).
Individual variation in the animal's response to the drug with potential for pain and distress from inadequate anaesthesia, or death from anaesthetic overdose.	Frequent	The use of gaseous anaesthetic will be considered as an option to intraperitoneal anaesthesia so that the anaesthetic depth can be more accurately controlled.

9. Monitoring

9.1. Monitoring during maintenance of anaesthesia is outlined in the procedure above.

9.2. Monitoring checklist is attached.

10. Potential effect on research results

10.1. Ketamine has been reported to be associated with the following effects which may influence research results:

10.1.1. Mechanism of action is controversial. No effect on GABA although it does block

ion channels. Inhibits excitatory polysynaptic pathways mediated by acetylcholine and L-glutamate in the spinal cord and N-methylaspartate (NMA) in the brain.

10.1.2. Sympathomimetic actions.

10.1.3. Metabolised by the liver.

10.1.4. Effects in rodents are variable and high dose rates are required for surgical anaesthesia. Most useful in combination with other drugs.

10.1.5. Respiratory effects are relatively minor. However, high dose rates required for surgical anaesthesia result in severe respiratory depression.

10.1.6.

10.2. Alpha-2 adrenergic agonists (xylazine and medetomidine) have been reported to be associated with the following effects which may influence research results:

10.2.1. Sedatives not anaesthetics; markedly potentiate the action of anaesthetic drugs.

10.2.2. Cardiovascular and respiratory depression.

10.2.3. In CNS, inhibit presynaptic calcium influx and neurotransmitter release.

10.2.4. Alpha-2 agonism in periphery causes vasoconstriction, decreased insulin release, diuresis, and decreased gastrointestinal motility.

10.2.5. Metabolised by the liver.

11. For additional information and references

11.1. NHMRC Guidelines to promote the wellbeing of animals used for scientific purposes. http://www.nhmrc.gov.au/health_ethics/animal/issues.htm#b

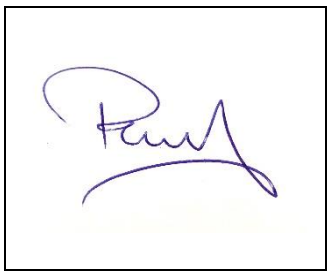
11.2. Flecknell P. 2015. Laboratory animal anaesthesia. 4th edition. Academic Press, London.

11.3. Kohn DF, Wixson SK, White WJ, Benson GJ (Eds). 1997. Anaesthesia and analgesia in laboratory animals. American College of Laboratory Animal Medicine Series. Academic Press, San Diego.

12. Abbreviations

IP intraperitoneal
IV intravenous
SC subcutaneous

ACEC Chair



ANAESTHETIC RECORD – (Intraperitoneal)

Premedication	Induction	Maintenance
Time:	Time:	Time:
Drug:	Drug:	Drug:
Dose:	Dose:	Dose:

Withdrawal reflex (Y/N)																					
Palpebral reflex (Y/N)																					
Mucous membrane colour **P=Pink. B=Blue**																					
Administration of fluids *(tick when done)*																					
Change animal's posture *(tick when done)*																					
Chart physiological measures *(to insert ranges)*																					
x---x---x---x Heart rate																					
●---●---● Breathing Frequency																					
▲---▲---▲ Arterial Blood Pressure																					
Time Intervals(15 minutes)																					