Research and Innovation Division

Research Animal Standard Operating Procedure

SOP# 207

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	Anaesthesia- Ketamine / medetomidine	
Species	Mouse	
ACEC	**Reference**	SOP#207 – Feb 21- Anaesthesia- Ketamine, medetomidine - mouse
	Author	Jenny Smart
	Version	1.1
	Date approved	26 February 2021
	Date for review	25 February 2024
	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.	

Details

Description of procedure

EQUIPMENT

1. 0.3ml or 1 ml syringe
2. 29-30G needle
3. Ketamine 100mg/ml*
4. Medetomidine 1 mg/ml
5. Atipamezole 5mg/ml
6. Antiseptic (eg chlorhexidine in 70% ethanol in water)
7. Cotton gauze swabs

*Ketamine is a S8 drug and a drug authority from the Department of Health is required for its use.

PROCEDURE

Ketamine/ medetomidine may be administered as a two step procedure (preferred) or made up as a fresh stock solution daily. This anaesthetic combination is administered by intraperitoneal injection, and as a general guide will provide 20-30 minutes duration of anaesthesia and a sleep time (loss of righting reflex) of 60-120 minutes.

Two step Administration

1. Using a cotton gauze swab, wipe the rubber diaphragm of the mutidose medetomidine and ketamine bottles with antiseptic.
2. Using aseptic technique draw up 1mg/kg of medetomidine into a syringe (for a 20g mouse this is 0.02mls).
3. Restrain the mouse, wipe the skin of the abdomen with antiseptic and inject the medetomidine intraperitoneally (follow SOP#78- Injection, intraperitoneal- rodents- Aug 11) Return mouse to holding cage for 5-10 minutes.
4. For female mice: using aseptic technique draw up 75 mg/kg of ketamine into a syringe (for a 20g female mouse this is 0.015mls).
5. For male mice: using aseptic technique draw up 50mg/kg of ketamine into a syringe (for a 20g male mouse this is 0.01mls).
6. Restrain the mouse, wipe the skin of the abdomen with antiseptic and inject the ketamine intraperitoneally. Return mouse to holding cage and observe until the animal loses its righting reflex.
7. Carry out procedure when testing of reflexes reveals that the mouse is adequately anaesthetised for the required procedure.
8. Reverse the medetomidine with atipamezole when recovery from anaesthesia is required. Using aseptic technique draw up 1ml/kg of atipamezole into a syringe (for a 20g mouse this is 0.004mls).
9. Wipe the skin of the mouse with antiseptic and inject the atipamezole subcutaneously (follow SOP#80-Injection, subcutaneous- rodents and rabbits- Aug 11)
10. Monitor the mouse until it is moving around normally.

NOTE: as the volumes above are very small, diluting the drugs may assist administration as follows:

Drug	Drug Volume	Sterile water for injection Volume	Final concentration	Dose for 20 g mouse
Medetomidine 1 mg/ml	0.1 ml	0.9ml	0.1mg/ml	0.2 ml
Ketamine 100 mg/ml	0.1 ml	0.9 ml	10mg/ml	0.15ml **Female** mouse 0.10ml **Male** mouse
Atipamezole 5 mg/l	0.02ml	0.98ml	0.1 mg/ml	0.2 ml

Stock Solution

Make up the stock solution **aseptically** just prior to injecting mice.

1. Ensure that you are using the correct drug concentrations (see table above and in section 2.)
2. For **female** mice: Mix 1 ml of medetomidine and 0.75 ml ketamine in 8.25 ml sterile water for injection. This provides a stock solution of 7.5 mg/ml of ketamine and 0.1 mg/ml of medetomidine.
3. For **male** mice: Mix 1ml of medetomidine and 0.50ml of ketamine in 8.5ml sterile water for injection. This provides a stock solution of 5.0 mg/ml of ketamine and 0.1mg/ml of medetomidine.
4. Dilute atipamezole by mixing 0.2 ml of atipamezole in 9.8 mls of sterile water for injection. This provides a stock solution with a concentration of 0.1 mg/ml.
5. Aseptically draw up 0.2 ml /20 g mouse of the ketamine/ medetomidine solution.
6. Restrain the mouse, wipe the skin of the abdomen with antiseptic and inject the stock solution intraperitoneally. Return mouse to holding cage and observe until the animal loses its righting reflex.
7. Carry out procedure when testing of reflexes reveals that the mouse is adequately anaesthetised for the required procedure.
8. If the initial injection is wearing off, and a top-up is needed, redose using one third (1/3) of the ketamine dose alone, as the duration of action of medetomidine is much longer than the duration of effect of ketamine.
9. Reverse the medetomidine with atipamezole when recovery from anaesthesia is required. Using aseptic technique draw up 0.2 mls for a 20g mouse of the diluted atipamezole solution.
10. Wipe the skin of the mouse with antiseptic and inject the atipamezole subcutaneously (follow SOP#80-Injection, subcutaneous- rodents and rabbits- Aug 11)
11. Monitor the mouse until it is moving around normally.

When diluting drugs or making up stock solutions MIX well prior to drawing up doses for injection.

Drug details. Give details of the anaesthetic agent(s) and technique to be used. Include details of sedatives or tranquilisers.

Drug name (**generic** name, not trade name)	Dose rate (**mg/kg body weight**)	**Route**	Timing of administration, and frequency *(eg. 30 minutes pre-operative, to induce anaesthesia, during procedure, at specific intervals during the procedure)*
Medetomidine 1 mg/ml	1 mg/kg	IP	5 mins pre operative
Ketamine 100 mg/ml	75 mg/kg (female mice) 50mg/kg (male mice)	IP	To induce anaesthesia
Atipamezole 5 mg/ml	1 mg/kg	SC	To reverse medetomidine at end of procedure

Monitoring

During anaesthesia:

- Respiratory frequency will be monitored to ensure slow constant breathing
- The adequacy of depth of anaesthesia will be checked intermittently using lack of 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)
- Animals will be kept warm using a warming pad

Recovery period:

Mice will be kept warm during recovery either by using warming pads, heaters or by placing into a warm room. They will observed continuously until they can move around the cage normally.

References

Flecknell, P. 2016. Laboratory Animal Anaesthesia. 4th ed. Oxford Associated Press

ACEC Chair