Research and Innovation Division

Research Animal Standard Operating Procedure

SOP# 114

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	Intratracheal inoculation	
Species	Mouse	
ACEC	**Reference**	SOP#114 - Feb 21 - Intratracheal inoculation-mouse
	Author	Ming Yang
	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	6
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

Purpose

Challenge of mice with varying inoculants via the lungs to monitor response

Description of procedure

1. Equipment:

1.1 Pipettes and associated tips
1.2 Alfaxalone: 10mg/ml stock diluted 1:4 in sterile PBS
1.3 Inoculant

2. Method:

2.1 Prepare inoculant material so that the concentration required is in a volume of up to 50µl.
2.2 Lightly anaesthetise each mouse following SOP# 8 Intravenous anaesthesia using Alfaxan - Mouse.
2.3 Pick the mouse up by grasping the skin on the back of the neck firmly with your thumb and pointer finger. Gently turn the animal over so it faces the operator in vertical position.
2.4 Open the animal's mouth gently using tweezers while viewing under a microscope.
2.5 Insert a polyethylene cannula in very carefully and gently into the trachea of mouse. Using either a P20 or P200 pipette and an associated tip, collect volume of the inoculant material to be administered.
2.6 Place the pipette tip near the cannula and gently expel the contents of the pipette.
2.7 Once the mouse has breathed in all of the inoculum place it on its back in the bottom of its cage and watch that its breathing is normal and it rights itself (this should occur in the next 5-10 seconds).
2.8 During operation, anaesthesia is maintained to ensure lack of pain perception and non-responsiveness. The adequacy of the depth of anaesthesia is checked intermittently using the withdrawal reflex (flexion of the leg following a firm pinch of the paw or interdigital skin) and palpebral reflex (gentle touch of the eyelid with a finger). Mice are continuously observed by the researcher during and after operation.

Substances administered

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)
Alfaxalone	10 mg/kg (100ul of 10mg/ml stock diluted 1:4 in sterile PBS)	IV	Mice injected immediately prior to procedure

ACEC Chair