Research and Innovation Division

Research Animal Standard Operating Procedure

SOP# 78

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 injection in small rodents	
Species	Rat, mouse, guinea pig	
ACEC	**Reference**	SOP#78- Jan 22- Injection, intraperitoneal-rodents
	Author	Jenny Smart
	Version	1.1
	Date approved	28 January 2022
	Date for review	27 January 2025
	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

1. Purpose

To describe the procedure for safely performing an intraperitoneal injection in rats, mice and guinea pigs with a minimum of stress to the injected animal.

2. Description of procedure

EQUIPMENT

1. Sterile syringe of sufficient size to contain the volume of injectate
2. Sterile hypodermic needle. The needle should be of the smallest gauge possible through which the injectate can pass. Viscous solutions will require a larger gauge needle. In general use a 23-26G needle for rats and guinea pigs and a 25-27G needle for mice.
3. Antiseptic such as chlorhexidine in 70% ethanol in water
4. Cotton gauze swabs
5. Sharps container
6. Injection solution

NOTE:

1. Injections should be carried out in a quiet area away from other animals.
2. Animals should be acclimatised to handling before attempting injections. Use the acclimatisation period between delivery of the animal to the research holding facility and the first injection to condition the animal to the catching and restraint techniques used for the injection.
3. The maximum volume of injection via the intraperitoneal route should be no more than:

Species	Mouse	Rat	Guinea Pig
Maximum Volume for adult animal	2 ml	5ml	10ml

PROCEDURE

1. Attach the needle to the syringe, apply antiseptic to the rubber diaphragm of the bottle of injectate, carefully uncap the needle and insert it through the rubber diaphragm.
2. Invert the bottle of injectate and draw up the calculated volume of injectate into the syringe.
3. Put the needle and syringe to one side. DO NOT recap needle (to avoid needle stick injuries). Avoid contaminating the sterile needle by resting the needle hub or top of syringe against the needle cap so that the needle is elevated.
4. Catch and restrain animal to be injected as follows:
    a. **Mice**- grasp the base of the tail and restrain by “scruffing” (holding the skin over the shoulders between the thumb and forefinger)
    b. **Rats**- grasp the rat gently around the upper body. If a single operator is performing the injection, place the rat into a ‘Rat Bag’ for restraint. If using an assistant, the assistant restrains the rat by grasping the upper body in one hand and holding the hindlegs in the other

c. **Guinea Pigs**- grasp the guinea pig gently around the upper body.

5. Tip the animal onto its back and identify the xiphisternum and pubis. The midline of the animal's abdomen runs between these two points. Imagine a line bisecting this midline and dividing the abdomen into quadrants. To avoid injecting into an abdominal organ, the needle should be inserted into the lower left or lower right quadrant of the abdomen, just below the bisecting line.
6. Tilt the animal's head slightly down to move the small intestine away from the lower abdominal quadrants.
7. Using a cotton gauze swab soaked with antiseptic, apply the antiseptic to the injection site by wiping against the lay of the hair so that the antiseptic reaches the skin.
8. Insert the needle through the skin and muscle, into the peritoneal cavity at a 45 degree angle.
9. Draw back on the hub of the needle to ensure that the needle has not penetrated abdominal organs or blood vessels. If blood, urine or intestinal content are seen in the syringe, withdraw the needle, prepare a new injection and start again.
10. If the needle is in the peritoneal cavity, inject and remove the needle quickly and smoothly.
11. Place needle and syringe into sharps container.
12. Release the animal back into its cage and observe for any signs of abnormal behaviour.

3. Images of Intraperitoneal Injections

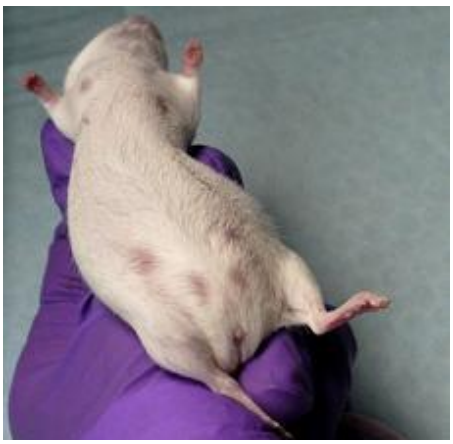
RAT

Catching

"Rat Bags"

Rat restrained in 'rat bag'

MOUSE



Mouse scruffed and restrained on back

Injection into lower left quadrant- abdomen

GUINEA PIG

Injection into lower left quadrant of abdomen

References

1. Basic Biomethodology for laboratory mice

   http://www.theodora.com/rodent_laboratory/injections.html

2. NC3Rs - Procedures with care
   https://researchanimaltraining.com/articles/intraperitoneal-injection-in-the-mouse/
   https://researchanimaltraining.com/articles/intraperitoneal-injection-in-the-rat/

   Note; Photos also courtesy of these sites

ACEC Chair