



# Curtin University Standard Operating Procedure

## INTERNAL NECROPSY PROTOCOL FOR RODENTS

Number: TEC 19

Version: 1.2

Date: 30/10/2014

**Aim/Purpose:** To provide guidelines on the correct procedure to carry out a thorough necropsy on any rodents which have become ill or have died unexpectedly during research projects at Curtin University which are to be carried out by staff and researchers of the university. Necropsy must be carried out in such a way as to satisfy the requirements of the Code (sections 2.1.5, 2.5.17, 3.1.25)

### Definitions

**The Code:** The Australian Code for the care and use of animals for scientific purposes 8th Edition, 2013

**Necropsy (viewing the dead):** refers to the examination of a dead animal and their organs and tissues in order to establish the cause and circumstances of death.

**Formalin:** a solution of formaldehyde in water (10%) which is used for the preservation of organic specimens. It can be purchased in the appropriate concentration.

### Kit Contents

- 1) Buffered formalin 10%
- 2) Plastic leak proof containers for collecting samples- ensure all correctly labelled
- 3) Air flow cabinet
- 4) 10ml syringe for faecal washing
- 5) 1ml syringes for blood and nasal tracheal wash
- 6) 1ml peptone water for nasal tracheal wash
- 7) Needles for infusion eg 20-22G needles
- 8) Cellophane tape for tape test
- 9) Diluted iodine (betadine) for duodenal and caecal wet preparations
- 10) Tooth picks for taking faecal samples.
- 11) Glass slides and cover slips
- 12) Tray to carry mouse carcass
- 13) Cutting board
- 14) Small metric ruler



- 15) Scalpel blades
- 16) Pair of forceps
- 17) Scissors
- 18) Gloves and personal protective equipment, such as eye protection, face protection, lab coats, and an appropriate work station.
- 19) Any appropriate blood tubes if collecting blood samples.
- 20) Weighing machine

## Procedure:

**Ideally the animal should be evaluated immediately after death. If this is not possible, refrigeration (not freezing) can slow any tissue decomposition.**

- 1) Record all findings. Ensure the animal is correctly identified, and that all the information and observations noted during the necropsy must be written down. Look for any abnormalities and changes to the normal anatomy of the mouse such as changes to the size and shape of organs, absence of organs, ulcers in the skin, discharges, and presence of fluids in the thorax and abdomen.
- 2) Ensure all specimens are correctly and clearly labelled. The best way to store small flaccid organs is to place them on a small square of paper and put into the formalin jars.
- 3) If the animal has just been euthanased, collect a sample of blood using the cardiac puncture technique. Collect the blood into the blood labelled red tube. Leave a sample of whole blood for 30 minutes, then place the tube in a centrifuge and spin for 5 mins at approximately 1300RPM. Carefully remove the top layer of the blood with a pipette and store in the Serum labelled red top tube.
- 4) External Examination
  - a. Note the condition of the animal i.e. thin, obese, normal
  - b. Note the condition of the skin egg scaly, normal, hair loss
  - c. Examine the external openings for any signs of blood or discharge i.e. nose, eyes, ears, anus, and vagina. Is there any evidence of diarrhoea or haemorrhage?
  - d. Check for any lumps or external lesions or wounds
- 5) Use sticky tape to place on perianal area under tail, and place this sticky tape onto a glass slide and label it.
- 6) Use a piece of sticky tape and run it over the back of the animal and then along the abdomen. Place this tape onto another labelled slide.

- 7) Position the mouse correctly for the necropsy procedure which is with the mouse flat on its back with its paws pinned to the underlying foam.
- 8) Remove the skin- a small cut is made at the level of the pubis, and then a longitudinal cut is made along the central midline, through to the chin. The skin can then be dissected off the body. You can then see the sub cutis, the superficial lymph nodes, the mammary glands and the skeletal muscles.
- 9) Expose the salivary glands in the neck and dissect out. These should be split into 2, one for PCR testing (saline) and one for histology (formalin). See Figure 2

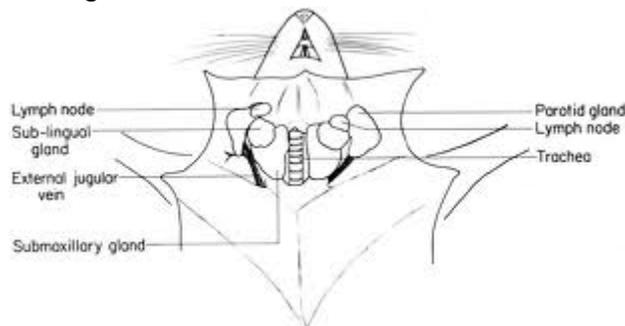


Figure 2- the anatomy around the neck

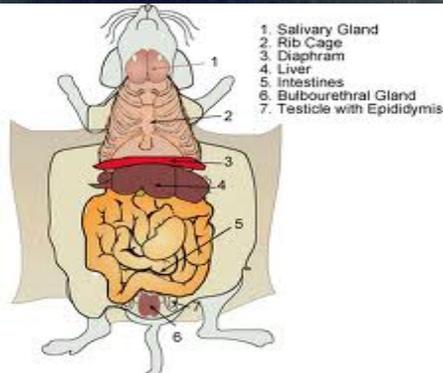
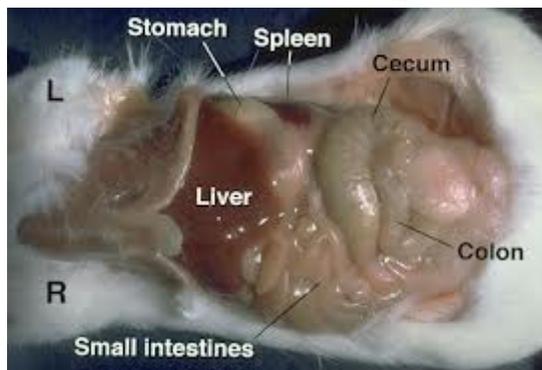


Figure 3 – the abdominal cavity

- 10) Collect the triangle of skin and lymph nodes on the right side of the abdomen joining down to the groin area.
- 11) Open the abdominal cavity and expose the organs. Note the stomach contents (is it full or empty) and the bladder content (full or empty)



- 12) Lay out the intestinal tract.
- 13) Use a toothpick to take a sample of the caecal contents from the caecum if you suspect a gastrointestinal problem. (send for PCR)
- 14) If you can identify the ileocaecal lymph nodes near the junction of the ileum and the caecum, collect these as they are good to check for parvo viruses
- 15) Fill the intestines with 10ml of formalin- inject at multiple places along the length of the intestinal track. Place formalin and intestinal contents into plastic container. This will be used for the faecal flotation
- 16) Collect the kidneys – if possible try and remove them with the adrenal glands intact. Keep one of the kidneys for PCR testing, and the other for histopathology.
- 17) Collect the other organs for histopathology. Weigh and record weights for the liver and spleen.
  - a. Cut out 1cm pieces of liver and collect in an empty pot, and place the remaining liver tissue in the formalin pot.
  - b. Cut out the spleen, weigh it, then cut it in half and place ½ in a labelled container and the other 1/2 in the formalin pot
- 18) Remove the bladder and uterus (if female) and down to the vagina. Place onto paper and into formalin
- 19) Remove the ribcage to expose the lungs and heart
- 20) Use scissors or forceps to raise the trachea for the nasotracheal wash. Express 0.1ml of peptone water into the trachea using a needle, and pull back on the plunger to aspirate the nasotracheal wash back into the syringe.
- 21) Place the nasotracheal wash in a sterile screw topped container and label it.
- 22) Using sterile scissors cut the trachea out (approximately 1cm) and place in the peptone water sterile container with the nasotracheal wash.
- 23) Remove the heart and lungs by cutting the oesophagus and aorta at the diaphragm
- 24) Weigh the heart, then store it in formalin.
- 25) Keep one half of the lungs in a plain tube
- 26) Now perfuse the other half of the lungs with formalin
  - a. Insert the needle attached to a filled 20ml syringe into the main bronchi
  - b. Grasp the bronchi around the inserted needle with forceps to prevent backflow of formalin.
  - c. Push the syringe plunger to inflate lungs with formalin. Lungs are adequately inflated when the formalin reaches the margins of all lung (Should be able to see the lungs increase in size)
- 27) If you see an abscess, take a swab for microbiology.



28) Removal of the brain:

- a. The skin must be cut with a median longitudinal cur from the nape of the neck to the nose.
- b. Remove eyes. Should see Hadrian glands, which lie in the orbital cavity behind the eye ball.
- c. Insert the scissors into the opening at the back of the brain and make two incisions around the edge of the brain
- d. Remove cranium
- e. This is very important if showing any signs of immuno-compromise or displaying neurological clinical signs
- f. With forceps or fingers, very carefully pull the brain out of the skull and place in a formalin pot.

29) Collecting and fixing tissue samples

- a. Fix tissues as soon as possible
- b. The fixative is generally a 10% buffered formalin solution
- c. It is not advisable to fix large entire organs- take only some sections, preferably those showing the lesion.

30) If you cannot see any obvious abnormalities....

- a. Collect small and large intestine, stomach, salivary glands, kidney, heart, brain and liver into 10% buffered formalin.

31) Submit the specimens to the appropriate people for analysis.

32) If there is an obvious lesion and a sample is to be collected, ensure a sample from the edge of the lesion bordering normal tissue is collected.

A Necropsy Examination Guide form can be used to record your findings. This can be obtained through the Animal Facility.

If histopathology is required, samples should be sent to the appropriate person or agency for this to be carried out, with an appropriate history.

References:

John Hopkins Medicine: Laboratory Manual Practical Mouse Evaluation and Pathology  
[www.hopkinsmedicine.org](http://www.hopkinsmedicine.org)

Animal Health Laboratories, Agriculture WA. Pathology Laboratory Procedure. Issue Number 3.

Proceedings from the 2013 ANZLAA Conference, UWA.

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