Mouse Necropsy Protocol

1. **Purpose**
The purpose of this Standard Operating Procedure (SOP) is to describe the process of performing a complete necropsy on a mouse.

2. **Scope/Responsibilities**
Animal Health Staff, Veterinary Staff.

3. **Prerequisites**

4. **Procedure**

**Day 1 - Preparation**

a) Record information from cage card of animal in the Necropsy Log Book and assign each case a new number.

b) Euthanize mouse using approved method (CCAC Guidelines on the Euthanasia of Animals Used in Science, CCAC Training Module on Euthanasia of Experimental Animals 1,2)

c) Collect blood from mouse if required for serology (speak with Veterinarian).

d) Place mouse in dorsal recumbency.

e) Make a linear incision through the skin and subcutaneous tissues starting from the urogenital area and extending upwards to the chin.

f) Make a 'T-shaped' incision through the peritoneum from the pelvis to the sternum and extend across the bottom ribs on either side to expose the abdominal organs.

g) Make a linear incision from right to left through the diaphragm.

h) Use scissors to cut the lateral aspects of the rib cage, up to the thoracic inlet. Remove the sternum and rib cage and place in formalin jar.

i) Place the rest of the mouse in the formalin jar ensure the formalin has access to all organs.

**Day 2 - Necropsy**

a) Record identification number from the Necropsy Log Book on all cassettes that are being used.

b) **Part A - The Sternum**
   - Using a scalpel blade, cut a square shaped piece of tissue of the sternum.
   - Make sure to cut right on the outside of the vessels (looks like railroad tracks)
   - Place tissue in cassette, railroad tracks down.

c) **Part B - Lymph Nodes**
- Note: lymph nodes are difficult to identify. If you can find a lymph node, there is a good chance it is enlarged. If it is large enough to section, section it and place it cut side down in the cassette. If it is not large enough to section, place it directly in the cassette.

d) Part C - The Pluck
- Lift the lungs away from the body and cut any connecting tissue.
- Lay your scissors on the trachea and cut and overlying soft tissues. Place the point of the scissors within the mandibular symphysis and cut. Separate the mandibular symphysis manually.
- Grasp the tongue with your forceps and gently pull caudally (downwards) and away. Cut and tissues underneath the trachea and esophagus that are connecting them to the deeper tissues. You may need to insert your scissors on either side of the trachea at the level of the thoracic inlet to cut away the last rib.
- Using scalpel blade, make two parallel cuts right below the esophagus/tracheal opening. Place tissue in cassette, cut side down. This section contains the trachea, esophagus, thyroid and parathyroid.
- Using a scalpel blade, cut through the largest lung lobe on the left. Place tissue in cassette, cut side down.
- Using scissors, cut through the heart, great vessels and thymus in sagittal section. Place tissue in cassette, cut side down.

e) Part D - The Abdominal Organs
- Using a scalpel blade, cut a section of the largest liver lobe on the left side (it should be crescent-shaped). Place tissue in cassette, cut side down.
- Using scalpel blade or scissors, cut a cross-sectional portion of the spleen (it should appear triangle shaped). Place tissue in cassette, cut side down.
- Using scissors, cut a small section of the duodenum, jejunum, ileum and colon.
- The pancreas is a tan-coloured soft tissue surrounding the duodenum. When cutting a section of the duodenum, try to include this tissue as well. Ensure that the colon has feces in it. Place in cassette longitudinally.
- Using scissors, cut the top off the cecum. Try to obtain a section of cecum that has material in it. Place tissue in cassette, cut side down.
- Using scalpel blade, cut the ‘cap’ off the stomach. Make sure that your section includes both the glandular and non-glandular portion of the stomach and keep stomach contents within the tissue. Place tissues in the cassette, cut side down.
- Using scalpel blade, cut a sagittal section of the left kidney and left adrenal gland. The adrenal gland is very small (1mm in diameter, tan-coloured nodule) and is embedded within the fat above the kidney. Place tissue in cassette, cut side down.

f) Part E - Brain
- Note: only take the brain if the animal is demonstrating neurological clinical signs (ex. seizure, ataxia, drooling)
- Using your hands, bend the head of the mouse backwards to expose the base of the skull and the atlanto-occipital joint. Using scissors, cut through the skin of the neck and the atlanto-occipital joint.
- Peel back the skin over the skill.
- Place the skull in fast decal for 30 minutes or in slow decal overnight.
- Using a scalpel blade, make 4 sagittal cuts. The first cut is in front of the eyes. The second is right behind the eyes. The third is through the ear canal and the last cut is through the cerebellum close to the base of the skull.

g) In addition: always section tissues that are abnormal (ex. tumour, enlarged lymph nodes etc) and place cut section down in cassette.

5. References

Mouse Lymph Nodes adapted from Dunn 1954
(+Van den Braeck, et al. 2006)

1. Superficial cervical (or mandibular) Nodes
2. Deep cervical node
3. Mediastinal Nodes
4. Axillary Nodes
5. Branchial Nodes
   A. Thymus
   B. Spleen
6. Pancreatic (or pancreaticoduodenal) Node
7. Renal Nodes
8. Mesenteric (or jejunal) Nodes
9. Inguinal (or iliac) Node
10. Lumbar Nodes
11. Sacral Nodes
12. Sciatic Nodes
   NS  Popliteal Node - Behind Knee

Lymph Nodes
Brain

Figure 1: Mouse head, anatomic landmarks, and sectioning decalcified specimens

<table>
<thead>
<tr>
<th>Eye</th>
<th>Ear</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Adapted from Paxinos & Franklin 2001

Adapted from Popesko et al. 1992. Vol 2
Order of Tissue Collection
1. Sternum
2. Lymph Node(s)
3. Trachea, Esophagus, Thyroid, Parathyroid
4. Lung
5. Heart
6. Liver
7. Spleen
8. Duodenum, Pancreas
9. Jejunum
10. Ileum
11. Descending colon
12. Cecum
13. Stomach
14. Left Kidney, Adrenal Gland
15. Brain (optional)

http://www.ccac.ca/en_/education/niaut/vivaria/euthanasia
http://www.mc.vanderbilt.edu/diabetes/msshortcourse/presentations/MMPC_boyd.pdf