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Rodent Necropsy and Tissue Processing Guidelines

Anatomic Pathology
In contrast to clinical pathology, which generally refers to the evaluation of the fluids or excreta from an animal, anatomic pathology refers to the morphologic features of various anatomical structures, organs, and tissues.

Necropsy
Necropsy, which literally means viewing or examining the dead, is an extremely important technique that can provide very valuable diagnostic and/or experimental information. The necropsy technique is best performed in a consistent and efficient manner which ensures the systematic evaluation of all organ systems, collection of appropriate tissues.

General Tissue Handling/Fixation/Submission Guidelines
- Tissues decompose or autolyze rapidly following death. Autolyzed tissues tend to defy microscopic diagnosis, and are of little diagnostic or experimental value. Postmortem autolysis can be minimized with prompt (<5 minutes) immersion of the tissues in the adequate amounts of an appropriate fixative at room temperature. Typically the recommended ratio of specimen to fixative is a minimum of 1:10 (v:v). If the formalin is dark brown after 24 hours the formalin should be poured off and replaced fresh formalin. For routine histologic analysis, room temperature 10% neutral buffered formalin is the recommended fixative. Following fixation, bones can be decalcified and by using a formic acid solution like Shandon TBD2.
- Attaching freshly harvested small or flexible flimsy tissues to a small piece of a dry index card for 30seconds prior to placing them in the fixative will help with maintaining tissue identity and orientation. A lead pencil can be used to write notes on the index card.
- Handle unfixed tissues gently with forceps and avoid grasping tissues directly with forceps. Use forceps to grasp the adventitial tissues surrounding the organs to avoid crush artifacts in the tissues.

Necropsy / Tissue Collection Procedure
- **External Examination** -- The animal should be weighed external features including assessment of the body condition and identifying marks (e.g. toe snips, ear punches) should be recorded.
- **Dissection/Tissue Collection**:
  1. It is helpful to always **orient animals in the same direction**.
  2. Make a midline incision from the mandibular symphysis to the pubis and reflect the skin laterally.
  3. Attaching the animals in dorsal recumbency by pinning the legs to a layer of paraffin or a cork board is helpful.
  4. Reflect the cut skin edges laterally with blunt dissection.
  5. Remove the parotid and submandibular salivary glands intact. Place the salivary gland pluck on a piece of a index card before placing in fixative
  6. Open the abdomen, xiphoid to pubis, cut the diaphragm from the ribs and cut the rib cage through the middle of the ribs on both sides of the thorax. Place the sternum in the decalcifying solution.
  7. Examine contents of the abdominal and thoracic cavities in situ (in the body).
  8. Remove the spleen.
  9. Grasp the most digital portion of the rectum with forceps and cut with scissors. Gently lift the rectum while stripping the colon, cecum and small intestine from the mesentery until reaching the stomach. Cut the esophagus at the diaphragm and place the gastrointestinal tract with the attached pancreas in the fixative.

10. Split the pubic symphysis with scissors and remove the urogenital tract intact. Place urogenital tract (dorsal side down) on index card before placing in fixative.
11. Remove the right and left kidneys with the adrenal glands attached.
12. Gently lift the cervical trachea. Cut the esophagus at the diaphragm to remove the larynx, trachea, esophagus and lung (pluck) intact. Use a 3ml syringe/21g needle placed in the lumen of the trachea to infuse and fully expand the lung with fixative.
13. Remove attached fat, mesentery and pancreas.
14. Remove the liver and place in formalin.
15. Disarticulate both femurs at the coxofemoral joint and place rear legs in fixative.
16. Using scissors cut the cervical spinal cord immediately caudal to the head and dissect the spinal column and pelvis from the skin. With scissors trim ribs adjacent to the vertebrae and place the spinal column and pelvis in decalcifying solution.
17. Dissect the head from the skin and place in decalcifying solution.
18. Collect a representative 1cm square piece of the pelt (include mammary glands tissue from female rodents). Place subcutaneous tissue side down on index card to keep flat.

Fixed Specimen Preparation/Submission (Trim-in) Guidelines

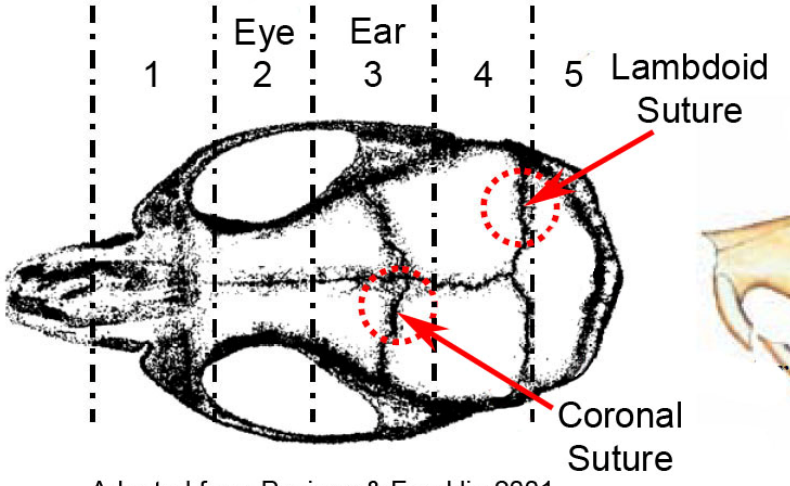
AFTER tissues have been in room temperature 10% buffered formalin for a minimum of 24 hours

- **General**
  1. Pre-label cassettes with AP# and sequential cassette numbers (i.e. 69245 1, 69245 2, etc.)
  2. Place maximum of 5 tissue samples per cassette.
  3. Careful selection and sampling of tissue will result in increase diagnostic quality of the sections. Placing a large amount of tissue (i.e. entire small and large intestine) in a cassette typically results in reduced quality of the sections.
  4. Group tissues of similar size and tissue density in cassettes.
     a. Samples of the liver, spleen, and kidney can be placed in the same cassettes
     b. Samples of the gastrointestinal tract can be grouped in cassettes.
  5. Small tissues like the adrenal glands and ovaries can be grouped in cassettes.
  6. Tissues are placed in cassettes so that the side to be cut is down.

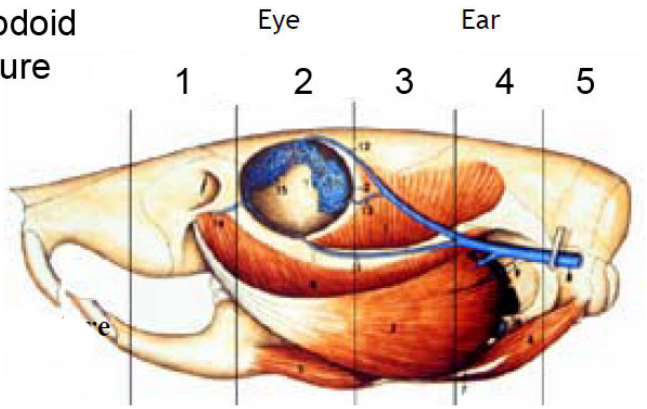
7. **Specific Tissue Handling Suggestions**
   1. Salivary gland pluck can be removed from the index card and the flat side placed down in the cassette.
   2. The fixed heart can be hemisected (cut in half, longitudinally) to expose all chambers and valves.
   3. Both kidneys can be hemisected to form cross sections through the hilus. The left kidney can be cut longitudinally only if important to distinguish the sections from each kidney.

   4. Cut 2-3 mm wide slices of a single liver lobe. Include the gall bladder in one of the slices if possible.
   5. Cut stomach in half longitudinally along greater curvature and place in cassette.
   6. Small intestine
      - Cut 3mm long segments of duodenum, jejunum, and pylorus so that the segments can be embedded on end for **O**-like cross sections.
      - Alternatively cut 1cm long segments of the duodenum, jejunum, and pylorus so the segments can be embedded on their sides for longitudinal sections.
   7. Cut cecum in half and place cut side down in cassette.
   8. Cut multiple 3mm cross sections of colon for **O**-like cross sections
   9. The female urogenital tract can be removed from the index card and placed flat slide down in a cassette for longitudinal sections of the uterus, cervix, and vagina and sections of the ovaries. Remove urinary bladder by cutting it at the neck of the bladder and place in it separately in a cassette.

10. The male urogenital tract can be removed from the index card and placed flat slide down in a cassette for longitudinal sections of the seminal vesicles, coagulating glands, prostate and testicles. Remove urinary bladder by cutting it at the neck of the bladder and place in it separately in a cassette.
11. The decalcified hind limb should be disarticulated at the knee and placed in the cassette with the medial (inner thigh down) so that when sectioned the entire length of the femur and associated bone marrow and skeletal muscle can be examined.
12. Decalcified head
    a. Using a single edged razor blade slice the decalcified head using smooth long slicing single cutting strokes into 5 slices using the eye, ear, and the lambdoid and coronal anatomic land marks illustrated in the figure below.

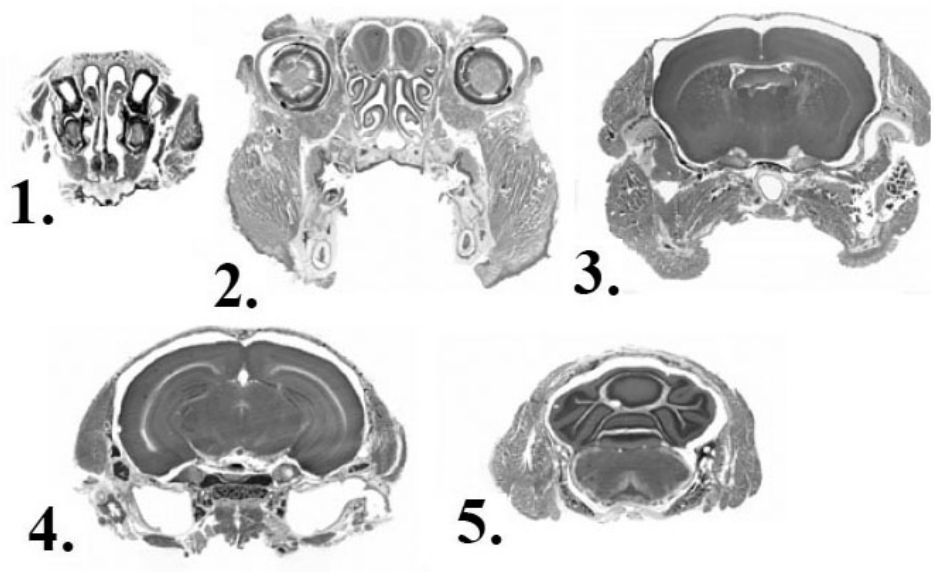


Adapted from Paxinos & Franklin 2001



Adapted from Popesko et al. 1992 Vol 2

    b. Place caudal surfaces of slices 1, 2, and 3 down in one cassette and the rostral surfaces of slices 4, and 5 down in a second cassette which result in sections similar to those in the figure below.



13. Segments of the decalcified spinal column and/or sternum can be cut longitudinally. If detailed examination of the spinal cord is appropriate then selected 4mm cross sections of the cervical, thoracic and lumbar spinal column should be cut.