



MMPC Mouse

Gross Necropsy with Histology

Version: 1

Edited by: Stephen M. Griffey, University of California, Davis.

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Summary:

Gross necropsy with documentation of changes present. Collection and histologic processing (paraffin blocks/H&E slides) and interpretation of kidneys, liver, spleen, pancreas, heart, lungs, esophagus, trachea, thymus, mesenteric lymph nodes, GI Tract, cerebrum, cerebellum, urinary bladder, and reproductive tract and additional tissues with gross changes. This includes photodocumentation of significant findings. This assay can be customized to include other target organs based on findings from other MMPC assays.

This procedure is to be followed by trained Comparative Pathology Laboratory (CPL) staff responsible for performing necropsy diagnostics procedures at CPL.

Reagents and Materials:

Submitted animals; adults, weanlings, or neonates.

Necropsy Profile Form, Histopathology Request Form.

Sample containers: tissue cassettes, weigh boats, formalin, microcentrifuge or click seal containers.

Equipment/Instruments: Carbon Dioxide (CO₂) gas chamber, Ohaus scale, razor blades, scalpel, iris and metzenbaum scissors, forceps, pencil, sharpie pen, 1-3 CC syringe with blunt aluminum hub needle, microscope.

Chemicals: 95% ethanol, saline, 10% phosphate buffered formalin, TBD Decalcifier solution, 1:10 Bleach and Lysol Solutions.

Gowning: Wear gloves, eye protection, and standard lab gowning (lab coat or scrubs) while performing described procedures.

Protocol:

1. Procedure: **Gross necropsy with tissue collection**

- 1.1 All animals submitted to CPL for necropsy, are assigned a CPL accession number. **Note:** Make sure to match the animal's ID number and the CPL accession number. Some animal ID may be in the form of ear notch or toe snip.
- 1.2 Prior to starting, record the CPL accession number onto each sample container using a permanent pen, (Sharpie).
- 1.3 Document "additional" diagnostic requests (other than standard necropsy as described in this procedure).
- 1.4 Animal Inspection: Observe animals in cage for visible abnormalities, unusual body movements, tremors, head tilt, generalized muscular weakness, tumors, wounds, alopecia, dermatitis, eye lesions, abscesses, etc. External orifices are examined for evidence of diarrhea, excessive salivation (indicating overheating or suffocation) and other evidence of altered health. Record observations on the profile form.
- 1.5 Euthanasia: **Note:** Clean the CO2 chamber prior to use with use a 1:10 bleach solution spray on all areas within the chamber and lid, saturate well, then follow by wiping with a dry paper towel.
Euthanize each animal following the Rodent Euthanasia procedure.
- 1.6 Animal Preparation: Weigh the whole animal on a tared scale. Record each animal weight on the profile form. Clean the scale with a paper towel and 1:10 Lysol solution after weighing all animals.
 - 1.6.1 Place a euthanized animal in dorsal recumbancy position on the necropsy table in the small lab, and perform a thorough physical examination. Record observations on the profile form.
 - 1.6.2 Wet the ventrum thoroughly using a squeeze bottle with 95% ethanol.
 - 1.6.3 Using forceps and or scissors, incise the skin along ventral midline on both sides to reflect the skin/mammary glands back from the abdomen and thorax area to mandible cranially. Put the skin/mammary glands into a 10% formalin container.
 - 1.6.4 Organ Inspection: Inspect all organs for lesions or gross abnormalities, (take digital pictures of any noted lesions). Record all observations on the profile form.
- 1.7 Sample Collection: Using forceps and scissors collect the following tissues and place in container containing 10:1 ratio of formalin to tissue.
 - 1.7.1 Gently remove all abdominal tissues including liver, kidneys (notch right kidney), spleen, gastrointestinal tract, pancreas, urinary bladder, and reproductive tract. Place in 10% Formalin.
 - 1.7.2 With Iris scissors, carefully notch the trachea. Insert a blunt aluminum hub needle attached to a syringe containing 10% formalin, and gently perfuse both right and left lung with formalin to approximately inspiratory volume. Gently remove the pluck containing lungs, heart, and thymus. Place in formalin.
 - 1.7.3 Using Metzenbaum scissors, carefully decapitate animal. Switching back to Iris scissors, carefully cut open the skull cap, exposing the brain but not removing it from the skull. Place the head in formalin.
 - 1.7.4 Place the remaining carcass into formalin.

2. Procedure: **Tissue trimming for histologic processing**

- 2.1 Prepared tissues or organs are fixed into labeled formalin containers for a minimum of 24 hours before the samples can be trimmed. Samples are placed on the Tissue Trimming lab shelf or into a labeled container, to await trimming.
- 2.2 Preparation: Prepare the following materials at the trimming location, before beginning: 1 –container of formalin soaked sponges, clean plastic cutting board, 1 pair of forceps, new one sided razor blade, small scissors, a 1000 µl beaker with a strainer, stock of plastic sample cassettes and a permanent black marking pen.
- 2.3 Labeling: Use a number 2-3 hardness pencil to label each cassette with the year and corresponding CPL accession number from the sample container. Example: 09-1365. If

- the trimmed samples require four cassettes label as (example): 09-1365-A, 09-1365-B, 09-1365-C, 09-1365-D etc. If submitted samples require sub-accessions, label these cassettes as (example): 09-1365-A1, 09-1365-A2, 09-1365-A3... For B sub-accession label as: 09-1365-B1, 09-1365-B2, 09-1365-B3, 09-1365-B4.
- 2.4 In general, submissions require at least five cassettes (where applicable more cassettes may be required for other lesioned organs or special requests).
 - 2.5 Obtain samples from the shelf or labeled container, and set at the trimming location.
 - 2.6 Uncap one sample container and pour out the formalin into a beaker with a strainer set on top.
 - 2.7 Remove sample with forceps and place on the cutting board.
 - 2.8 Proceed to dissect the tissue or organ sample as specified in the applicable sections of this procedure.
 - 2.9 Tissues are trimmed to a thickness of 2-3 mm using a razor blade or scalpel. Place trimmed tissue into a labeled cassette as follows:
 - 2.9.1 The first cassette will contain: liver, spleen, pancreas and kidney tissue sections. Livers are trimmed to evaluate the two lobes adjacent to the gall bladder. Kidneys are trimmed in cross section at the hilus (right kidney) and longitudinally in half (left kidney). The spleen is trimmed in cross section.
 - 2.9.2 The second cassette will contain: hearts and lung and mediastinal tissue (esophagus, trachea, and thymus). Hearts are trimmed below the auricles and the lungs are trimmed in cross section at the level of the bronchus.
 - 2.9.3 The third cassette will contain: sections of the mesenteric lymph node, stomach, duodenum, jejunum, and colon. The stomach and duodenum are trimmed together and placed in the cassette. This cassette also contains: section of the ileocecal colic junction to contain cecum.
 - 2.9.4 The fourth cassette contains 5 cross sections of brain including brain stem, cerebellum, and cerebrum.
 - 2.9.5 The fifth cassette contains the entire reproductive tract and urinary bladder, cross sectioned.
 - 2.9.6 Place cassette(s) into a container filled with 10% buffered formalin. Record tissue collection information on Necropsy Worksheet.
 - 2.9.7 At end of trimming procedures, dispose of used formalin in the beaker into the labeled formalin waste container – wear eye protection while performing this step.
 - 2.9.8 Upon completion of procedures, clean lab and instruments following PAT-08 Cleaning the Small Animal Necropsy Lab and or PAT-09 Cleaning the Necropsy Tissue Trimming Workstation procedure(s).
 - 2.8 Cassettes are submitted to histology laboratory for routine processing to paraffin blocks. 5 μ m thick sections are cut and stained with Hematoxylin and Eosin for histologic examination. (Additional sections with special stains may be processed based on study findings).

3. Procedure: **Histologic analysis**

- 3.1 Each H&E stained slide will be examined microscopically for pathologic changes or changes related to a respective study.
- 3.2 Results with summary will be supplied in an excel workbook if multiple animals are examined from a respective study.
- 3.3 Results will be discussed with submitting PI and histologic photos documenting representative changes will be made if warranted.
- 3.4 Based on findings, further recommendations to PI for pathologic analysis may be made.

Reagent Preparation: *Not applicable*

