

JHUSOM Phenotyping Core Mouse Necropsy

Anatomic Pathology

In contrast to clinical pathology, which generally refers to evaluation of fluids or excreta from an animal, Anatomic Pathology refers to the pathology of the anatomical structures, organs and tissues. At least in larger animals, clinical pathology specimens often can be obtained in a clinical setting from live animals, and contribute diagnostic information to influence therapeutic interventions. Anatomic Pathology typically involves specimens obtained by biopsy or at necropsy.

a. Biopsy

While it is not common practice to submit only a piece of tissue from a mouse, in chronic or long term studies of valuable mice, such practices may become more common.

i. Specimen Preparation/Submission

Biopsy specimens, i.e. tissues from live animals, should be fixed immediately, and adequate volumes of fixative should be used. A specimen to fixative ratio of 1:10 (v:v) is recommended. Unfixed animal tissues may decompose or autolyze rapidly depending on tissue, temperature, microbial content and other factors. Autolyzed tissues tend to defy microscopic diagnosis, and are likely to have limited diagnostic or experimental value. Fixation in 10% neutral buffered formalin can provide useful histology after only hours of fixation, or even after years of preservation. Other fixatives or snap freezing may be required for immunohistochemistry or other techniques.

b. Necropsy

The necropsy (viewing or examining the dead) can provide quantitative as well as qualitative diagnostic or experimental data. A useful necropsy technique can be performed consistently and efficiently, ensure systematic evaluation of all organ systems and lesions, ensure collection of all tissues, and be taught reasonably easily to others. While procedures such as microbiological specimen collection, photographs, radiographs or other procedures may be added to suit specific diagnostic, health surveillance or experimental needs, the development of a **specific and systematic method of dissection or prosection** will facilitate and expedite the necropsy and will improve the information derived from it. Pathologists, investigators and experienced technicians should develop techniques that specifically suit their situation, resources, protocols or purposes of investigation. This document intends only to provide a basic outline.

Whole animal perfusion provides optimal fixation of all tissues and permits dissection and tissue collection at the prosector's convenience. However perfused tissues cannot be cultured, some lesions may be difficult to identify, due to absence of blood, and similarity in color and consistency. Also fresh and perfused tissue weights may differ.

i. General Procedure

PLAN

Before euthanasia and necropsy, develop a PLAN that outlines all procedures to be performed (e.g. photography, radiography), all specimens to be collected (e.g. blood, urine, swabs, feces, tissues), all tissues or organs to be weighed, and in what order they will be done. A **check list** may facilitate the procedure.

MATERIALS

1. **Prosector** -- person who performs the necropsy. With multiple animals to examine, division of labor can facilitate and expedite the process: e.g. one person (prosector) to examine and dissect, one person (recorder) to record, label, weigh, collect specimens for microbiology or parasitology.
2. **Ventilated work station** or other means to protect the prosector from formalin fumes should be used.
3. **Glasses or goggles** -- while infectious or zoonotic disease should not be a concern with mice from clean sources, glasses or goggles protect eyes from formalin or other fixatives. Magnifying reading glasses also facilitate dissection and examination of small animals or tissues.
4. **Gloves** -- Hand lotion and double gloving can be especially useful when there are many animals to examine. Replace the outer gloves when damaged or soiled, so there is less exposure of hands to drying or contaminating materials. Vinyl gloves may be less allergenic than latex.

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5. **Lab coat or other protective uniform** should be used to protect skin and clothes from contaminating material or fixatives.
6. **Cutting board** - Inexpensive, plastic cutting boards are adequate for most purposes. They should be easy to clean and reuse, and not ruin blades too quickly. Wax and cork cannot be sanitized.
7. **Paper towels** - some tissues (e.g. skin and reproductive tract) can be 'laid out' on a dry paper towel and will adhere to it to facilitate examination and ensure uniform fixation.
8. **Small Metric ruler** - should usually be included in photographs. When a lesion or organ is described as 'large' or 'small' it should be measured, and if possible weighed. Mass lesions and organs have 3 dimensions and their measurements should be recorded in 3 dimensions. E.g. a 'spot' may be 2x2mm, but a 'mass' is 2x2x2mm.
9. **Forceps** - blunt ended, serrated or toothed forceps may cause less damage. Fine pointed forceps can puncture and tear. Smooth forceps require considerable pressure to 'grip' a slippery tissue.
10. **Scissors** - fine, blunt ended scissors may cause less damage. Sharp tipped corneal scissors are used commonly but are likely to tear or puncture tissues.
11. **Blades** - not so useful in mouse necropsy dissections. But sharp fresh blades are critical to trimming tissues for histology. Single edge blades in hundred packs are adequate and inexpensive, but some prosectors may prefer scalpels with handles or other special blades.
12. **Syringe and needle** - 3ml syringe with a 21 gauge needle works well for infusing lung and gastrointestinal tract with fixative.
13. **Fixatives** - 10% neutral buffered formalin (10%NBF) is suitable for soft tissues for many situations. Acid alcohol fixatives and other options are being used more frequently. The primary goal (histology, immunohistochemistry, in situ hybridization) should influence the choice of fixative.
14. **Decalcifying solutions** - Head and other bones must be decalcified for paraffin processing for histology. Several formic acid solutions (TBD2, Formical etc) simultaneously fix and decalcify in < 24hr. Bouin's solution also fixes and decalcifies and maybe especially useful for mice < 2w old.
15. **Specimen containers, cassettes, labels, pencils, markers** - Label containers, cassettes, etc before starting. Pencils work best for many things. Even 'permanent' markers disappear with alcohols, fixatives.

PROCEDURE

- A External examination** - (see necropsy form below) Weigh the intact animal. Note colors of coat/skin and eyes. Note identifying marks (e.g. toe snips, ear punches), presence/absence of whiskers. External lesions (e.g. domed head, microphthalmia, skin lesions) should be recorded, and measured whenever possible (e.g. left flank, subcutaneous mass 4x4x5mm). Body condition should be assessed (e.g. thin, adequate or good body condition, obese), or scored, e.g. per Ullman & Fultz 1999.
- B Palpation** -- Gentle palpation may reveal pups or abdominal masses, or suggest the presence of abdominal fluid. When the abdomen is distended by fluid (e.g. ascites), a sterile sample can be obtained with a needle and syringe for cytologic, chemical or microbiologic evaluation. Mass lesion should be measured, weighed when feasible. Their consistency can be described as soft, fluctuant, firm or hard. 'Hard' should be reserved for boney or mineralized masses.

C Dissection:

1. It is helpful to always **orient animals in the same direction**, e.g. head up or head right, so that the side of the lesion can be recalled. If the animal always is examined 'right-side-up' (i.e. left lateral recumbency), and the prosector remembers that the 'top' kidney was cystic, the prosector will be certain that the **right** kidney was cystic.
2. **Pelt removal** facilitates assessment of subcutaneous fat (minimal, adequate, abundant), and reveals subcutaneous lesions and abdominal organs in situ. Cervical and submandibular lymphadenomegaly (enlarged lymph nodes) is evident, as is splenomegaly, or cystic kidneys, pregnancy or other lesions. The pelt can be removed easily by pinching or incising ventral abdominal skin and exerting gentle pressure

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cranially and caudally until the animal is 'degloved'. Cut the ear canals, around the eyes, muzzle and extremities to complete pelt removal. Eyes can be left in situ and decalcified with the head.

➤ For certain experimental evaluations, specific dissections with minimal skin trauma may be required.

3. After examining the animal with the pelt removed, remove the 'chain' of salivary glands (parotid, sublingual, submandibular) and lymph nodes, that extend from ear to ear under the chin. Place the whole chain in a cassette (#4). Further trim-in is not required unless for tumors or other lesions.
4. Open the abdomen, xiphoid to pubis, and examine contents in situ (in the body).
5. Lift the sternum by the xiphoid process and cut on both sides of it to remove it intact and expose the thoracic cavity. Place sternum flat on paper, or directly in cassette (#1), inside (marrow side) down, to fix. Examine thoracic contents, note fluid or masses, absence or enlargement of the thymus over the heart, megaesophagus.
6. Expose the cervical trachea by blunt dissection and use the 3ml syringe/21g needle to infuse the lung with fixative. The lungs should expand fully and excess fixative will reflux up the trachea. It is not necessary to clamp or tie the trachea. Generally this technique will not overfill the lungs because there is ample space around the needle for fixative to reflux. Take care after infusion to not compress the lungs during subsequent dissection.
7. Split the mandibular symphysis with scissors. Grasp the tongue with forceps and retract gently to remove tongue, larynx, trachea and esophagus from the head and neck. Continue retracting to remove heart, thymus and lungs from the thorax. Use blunt dissection (scissors) to free these tissues. Examine thoracic contents ex situ (out of the body). Note megaesophagus, aortic or other lesions. Separate heart, thymus, lungs. Weigh heart, thymus. Place thymus in a cassette (#1), so it is not lost. If you can not find the thymus, note/record its absence. Heart should fix intact. Place lungs (dorsum down), plus larynx/trachea with thyroid glands in a cassette (#2).
8. Thyroid glands are immediately caudal to the larynx on both sides of the trachea. They may be difficult to see without magnification, so include 2-3mm of trachea caudal to the larynx. The histology technician should understand if a cross section of trachea is desired ⇒ 'O', or a longitudinal section. ⇒ ==
9. Split the pelvis at the pubic symphysis to facilitate removal of ALL abdominal contents, including the urogenital tract.
10. Grasp the diaphragm with forceps, cut at its deepest extent and retract gently, lifting and dissecting free abdominal contents to remove them in toto from the abdominal cavity. Very little cutting should be necessary. Adrenal glands and kidneys tend to stay deep in the retroperitoneal space. They should be identified early, and blunt dissection may be necessary to remove them.
 - Nick the right kidney with a scissors or blade to facilitate subsequent identification. If you forget this, note that the right kidney normally is 'higher' (more cranial) than the left.
 - Splitting the pelvis facilitates removal of urogenital tract and rectum intact.
11. Examine abdominal and pelvic contents ex situ. Measure, weigh, record any abnormalities.
12. Isolate the urogenital tract, with rectum attached, from other viscera. Lay it flat on a dry piece of paper. Consistently orient the rectum (dorsal aspect) 'down' on the paper, and urinary bladder (ventral aspect) 'up' facing you, with left and right sides identified easily. Dissect out testes, or ovaries and uterus if these are to be weighed. These tissues also can be placed directly in fixative on the paper towel. Small ovaries or testes can be placed in a cassette (#8) at the time of dissection so that they are not lost. Testes should not be cut until they are fixed.
13. Dissect kidneys (with adrenal glands attached) from the other abdominal contents. Even *ex situ* the right kidney should be higher (more cranial, closer to liver) than the left. Remove adrenals and other attached tissue (fat, lymph nodes) from the kidneys and place them in a cassette (#3). Weigh the kidneys. Female mice usually have larger adrenals than male mice.
14. Dissect the spleen from other abdominal contents. Remove attached fat, mesentery and pancreas before weighing it.
15. Dissect the liver from the GI tract. A small lobe usually is folded into the lesser curvature of the stomach. Take care to remove it so that the entire liver is examined and weighed. Also remove remnants of diaphragm, fat and other tissue to obtain an accurate weight. When manipulating the liver,

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lift it gently, or grasp parts that will not be submitted for histology, e.g. diaphragm, smaller lobes, or fibrous attachments. Unless there are lesions in other lobes or protocol requires examination of other lobes, the **median and left lateral lobes are the biggest** and yield comparable sections for histology.

- Median lobe is closest to the diaphragm and looks like 2 lobes with the gall bladder in middle.
- The left lateral lobe is the large lobe immediately beneath the median lobe. Separate these lobes from other lobes to ensure good immersion fixation.

16. **The gastrointestinal (GI) tract** should be all that remains of the abdominal contents at this point. Different methods to dissect, trim-in and section GI tract may be required for specific purposes. For a reproducible method to evaluate representative sections from different segments: Hold stomach in one hand and rectum (fecal balls) in other and stretch gently. The GI tract should stretch out but remain intact. Bluntly dissect off fatty tissues and pancreas including mesentery and lymph nodes. Place these *in toto* in a cassette (#5). Further trim-in usually is not required unless there are tumors or enlarged lymph nodes. If necessary tissues can be identified by spreading them out on dry paper.
17. With a 3 ml syringe/21g needle, infuse approximately ½-1 ml fixative gently into stomach, duodenum, ileum, cecum, colon. Unless analysis of contents of specific segments is required, tying off of these segments should not be necessary. The entire GI tract may be placed intact into fixative to facilitate identification of segments at trim-in.

D. Decalcification:

1. Simultaneous immersion fixation plus decalcification of mouse heads and bones can be accomplished with formic acid solutions such as TBD2 (Thermo Shandon), or Formical, Immunocal (Decal). Mouse tissues usually are sufficiently decalcified and fixed for trim-in by 24 hours, so that they can be trimmed with other fixed tissues. Advantages of this method are that it involves only a single solution; soft tissues and bones can be trimmed at the same time (sometimes <12 hours after dissection of small mice); and some immunohistochemical techniques may work. Other decalcification protocols are more complex (e.g. perfusion and/or prolonged immersion in Bouin's solution; fixation followed by EDTA or nitric acid, but may be required for specific purposes). 50ml conical centrifuge tubes are a useful size for decalcifying a typical mouse head, limb and spine. The ratio of tissue to solution should be at least 1:10 (as with other fixatives) and the tissue should be covered completely by the solution. Gentle agitation (e.g. rocker) may improve exposure to solution. Although these formic acid solutions are relatively gentle, mouse tissues may become over-decalcified within 48-72hrs. Over decalcified tissues are mushy and histology is uniformly 'pink', without cellular detail.
2. **Head** with skin removed can be placed directly into the decalcification (decal) solution. The tongue should have been removed during necropsy dissection (to facilitate removal of the pluck, and to examine the oral cavity and teeth. Skin and tongue will interfere with fixation and decalcification.
3. **Hind limb** with skin removed can be placed directly into the decalcification (decal) solution. Limbs can be saved in formalin for subsequent decalcification.
4. **Spine / vertebrae** (backbone) should be trimmed of extraneous tissue (e.g. ribs and body wall muscle) and placed intact into the decalcification (decal) solution. It is not necessary to remove paravertebral muscles as with larger animals.
5. **Sternum** usually does not require decalcification, and can be placed (marrow side down) in a cassette (#1). Cell detail of bone marrow is better when not decalcified. Most of its firm tissue is cartilage. But old, large or osteopetrotic mice may have boney sterna.

E. Specimen Preparation/submission - Trimming tissues into cassettes for paraffin processing)

During dissection some tissues are placed in cassettes and may be submitted for histologic processing 'as is':

Cassette 1: Thymus, sternum - *Add heart after trimming - see below.*

Cassette 2: Lung, thyroid/larynx/trachea.

Cassette 4: Salivary glands and associated lymph nodes.

Cassette 5: Pancreas, mesentery, lymph nodes

(Cassette 8: *entire reproductive tract when it is small & fits nicely*)

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Other tissues must be 'trimmed' to fit into cassettes for histologic processing. Trimming of tissue should be performed in a well ventilated area or hood.

- Used fixative should be discarded according to institutional guidelines for hazardous materials.
- After trimming, labeled cassettes should be submitted in clean formalin or alcohol depending on the processor schedule. Check with the histology lab and technician.
- Tissues to be saved should be kept in clean formalin.
- During trimming, tissues should be cut with a single clean swipe, not squished or sawed. Inexpensive single edge blades are suitable for trimming most tissues, and should be replaced as soon as they become dull so that tissue is not squished. Longer Weck-type blades facilitate trimming decalcified heads. These are sharper and have a longer cutting edge, to achieve a single clean cut.
- Decalcified tissues should cut easily (quietly). Crunchy tissues require additional decalcification. Mushy tissues are over-decalcified and the decal period should be reduced for subsequent similar specimens. Decalcified specimens should be rinsed in water (preferably distilled, deionized), and cassettes should be kept in water until histology processing within 24hrs. Any remaining, rinsed, decalcified tissue can be saved with formalin fixed tissues.
- Specimens should be not more than 3mm deep to fit into the cassette without generating grid marks and 'squish artifact'.
- Systematic numbering of cassettes facilitates retrieval of archived material. The numbering system in Table 1 is an example. Lesions in an organ that require additional cassettes can be labeled with the same number followed by a, b, c, d, e.g. Liver tumors can be labeled 7a, 7b, 7c.

Table 1: Example of tissue cassette and slide numbering system for mouse histopathology

Cassette #	Tissues
1	♥Heart (+ Thymus) (+ muscle e.g. tongue, diaphragm, soleus) (+ nondecalcified sternum if it fits)
2	Lung -- Entire, formalin-infused + Thyroid/trachea
3	Kidneys - right/cross-section, left /longitudinal section + Adrenals
4	Salivary glands with lymph nodes (ear to ear)
5	Pancreas + mesentery, lymph nodes (any enlarged nodes → 5a,b,c etc)
6	GI (Stom, Duod, Ileum, Cecum, Prox colon, Rectum)
7	Liver (Left Lateral + Median Lobe/G bladder sections) + Spleen (1 or 2 hemisections)
8	Female (Uterus + Ovaries, Vagina/bladder/rectum) Intact tract if small enough Male (Testes/Epi, Sem ves, Bladder/rectum/prostate)
9	Skin - dorsal neck + inguinal (mammary + clitoral/preputial gl)
10	Decal Head ears (pituitary, thalamus, hippocampus, cortex), eyes (olfactory lobes, molars), nose (olfactory + respiratory, incisors), cerebellum, medulla
11	Decal hind limb (knee + tarsus)
12a,b,c...	Decal Spine (cervical, thoracic, lumbar-sacral)
13	LESIONS - e.g. tumors, abscesses

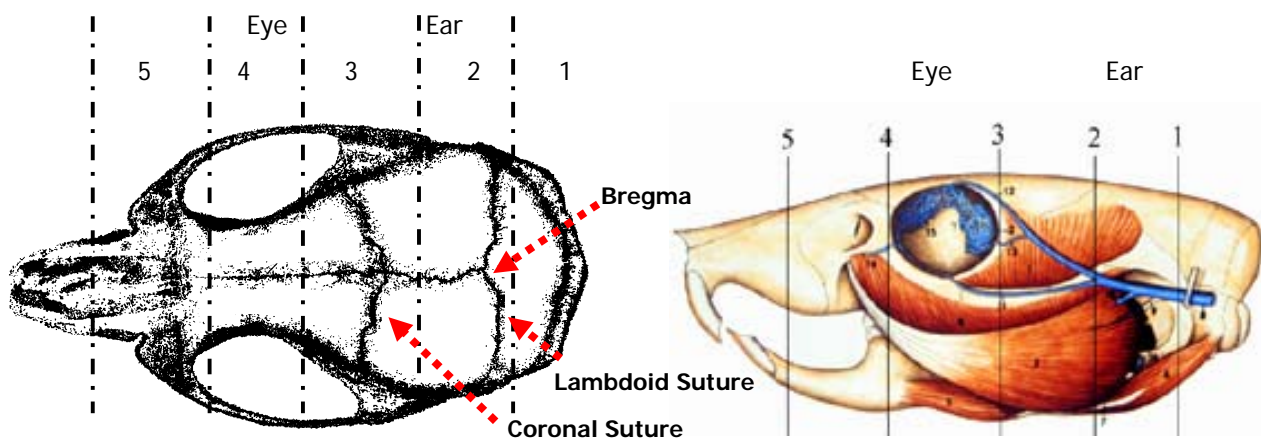
TRIMMING SUGGESTIONS (trimming tissues into cassettes for paraffin processing)

1. **Cassette/slide #1:** The fixed heart can be hemisected (cut in half, longitudinally) to expose all chambers and valves, and both halves submitted. The right ventricle has a thinner wall than the left, and may 'wrinkle' slightly. With a little practice, the correct orientation will be identified easily. For some purposes multiple cross-sections of heart are preferred. Additional tissues in cassette #1 may include thymus (inserted during necropsy dissection), sternum (non decalcified, marrow side down), representative muscle such as tongue (cross section of its thickest part), diaphragm.
2. **Cassette/slide #3:** The right kidney is transected (cross section), and the left kidney is cut longitudinally (longitudinal section) and placed in cassette (#3) with the adrenal glands. Kidney sections should include cortex and medulla. Submit 2-4 pieces (halves) of kidney if they fit easily

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3. **Cassette/slide #6:** The following GI tract segments can be included in a single cassette (#6): Any lesions can be placed in separate cassettes 6a,b, c etc.
 - a. Stomach - squamous (white) + glandular (pink) portions
 - b. Duodenum with pancreas ~ 1 cm from pylorus, \hat{O} -like cross section
 - c. Mid-jejunum ~ several cm from pylorus, O -like cross section
 - d. Ileum - from last 2cm before cecum, O -like cross section, include lymphoid nodules (Peyer's patch) if you identify them grossly
 - e. Cecum - from the tip -- V - or U -like section, include pale lymphoid nodule (Peyer's patch) if you identify it grossly
 - f. Proximal colon - from 2cm after cecum, 2 OO -like cross sections are nice to examine for protozoa and pinworms. Diagonal stripes discerned though the serosa are due to deep mucosal folds
 - g. Distal colon/rectum - an o -like cross section from between fecal balls. Feces contain indigestible material that damages microtome knives, and are not appreciated by histology technicians
4. **Cassette/slide #7:** Cut the liver's median lobe is to include the gall bladder between the left and right parts. Cut a section of the left lateral lobe from hilus to edge. Hemisect spleen longitudinally/sagittally. The 2 liver sections and both spleen sections normally fit easily in 1 cassette.
5. **Cassette/slide #8: female reproductive tract** - when small may fit *in toto* in the cassette. Or trim to provide representative sections of ovaries and uterus, and 1 or 2 cross sections through rectum, vagina and urinary bladder demonstrate their anatomical relationships nicely.
6. **Cassette/slide #8: male reproductive tract** - when small may fit in toto in the cassette. Or trim to provide representative sections of testes, epididymis, seminal vesicle and coagulating gland, and a cross section through rectum and the neck of the urinary bladder to include prostate gland.
7. **Cassette/slide #10: Decalcified head**, External ear canal openings and eyes are primary land marks. Lambdoid and coronal sutures also are useful landmarks. A right-handed prosector usually holds the nose in the left hand and cuts/sections with the right hand, starting with the most posterior/caudal section (1), and progressing anteriorly/rostrally. Use a single (1) clean stroke for each section.
 - a. Cut on caudal then rostral side of ear canals for section 1 (cerebellum), and section 2 (middle and/or internal ear, pituitary, hippocampus).
 - b. Cut on caudal then rostral side of eyes for section 3 (cerebrum), section 4 (eyes, Harderian glands, olfactory lobes, molar teeth), section 5 (nose, incisor roots).
 - c. Long nosed (dolicocephalic) mice may require a 5th cut.
 - d. Place sections 1 and 2 front/rostral down in the cassette.
 - e. Place sections 3,4,5 back/caudal down in the cassette.

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

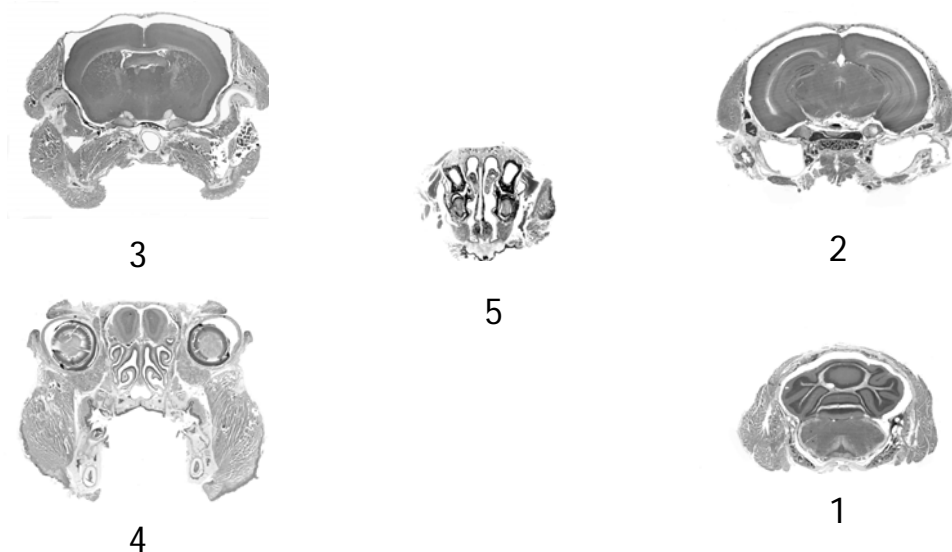


Adapted from Paxinos & Franklin 2001

Adapted from Popesko et al. 1992. Vol 2

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Figure 2. Decalcified Mouse head - 5 transverse histology sections. Numbered in order of cut, caudal to rostral: 1. Cerebellum; 2. Ears/hippocampus/Pituitary; 3. Cerebrum; 4. Eyes, oral cavity; 5. Nose.



8. **Cassette/slide #11: Decalcified hind limb** - remove lateral muscles by a clean cut so that the knee and long bones can be seen on the flat (cut) surface, and minimal sectioning by the microtome will enter the bone to result in a useful histologic section. One specimen can include femur and knee joint. The other specimen can include tibia and tarsus (heel joint). Toes with claws and fur interfere with decalcification, damage microtome knives, and tend not to be appreciated by histology technicians.
9. **Cassette/slide #12:** Pathologists or investigators may have very specific methods for evaluation of spinal cord and vertebrae. For simple survey screening of bone, musculature and spinal cord, cervicothoracic and lumbosacral specimens can be accommodated in 2 cassettes. Cut transverse sections at each end of the cervicothoracic and lumbosacral segments, then hemisect each segment (split it longitudinally) and put the 'best' half in the cassette, along with the cross sections from the same segments. Biopsy ink or a distinctive nick in the section can be used to identify left or right sides, if desired.

Suggested References

- The Virtual Mouse Necropsy -- <http://www.niaid.nih.gov/dir/services/animalcare/MouseNecropsy/Necropsy.html>
 EULEP Mouse Necropsy http://www.eulep.org/Necropsy_of_the_Mouse/index.php
 RENI Tissue trimming guide <http://reni.item.fraunhofer.de/reni/trimming/index.php>
 Columbia U mouse pathology <http://icg.cpmc.columbia.edu/cattoretto/Protocol/mousepathology/mouseTissue.html>
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 Ward JM, J Mahler, RR Maronpot, JP Sundberg and R Frederickson. 2000. *Pathology of Genetically Engineered Mice*. Iowa State University Press - out of print

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Figure 3. Mouse Lymph Nodes (adapted from Van den Broeck, et al. (2006). Anatomy and nomenclature of murine lymph nodes *J. Immunol Meth* 312(1-2): 12-19.)

#	English name	Official name
1	Mandibular lymph node	Ln. mandibularis
2	Accessory mandibular ln.	Ln. mandibularis accessorius
3	Superficial parotid ln.	Ln. parotideus superficialis
4	Cranial deep cervical ln.	Ln. cervicalis profundus cranialis
5	Proper axillary ln.	Ln. axillaris proprius
6	Accessory axillary ln.	Ln. axillaris accessorius
7	Subiliac ln.	Ln. subiliacus
8	Sciatic ln.	Ln. ischiadicus
9	Popliteal ln.	Ln. popliteus
10	Cranial mediastinal lnn.	Lnn. mediastinales craniales
11	Tracheobronchial ln.	Ln. tracheobronchalis
12	Caudal mediastinal ln.	Ln. mediastinalis caudalis
13	Gastric ln.	Ln. gastricus
14	Pancreaticoduodenal ln.	Ln. pancreaticoduodenalis
15	Jejunal lnn.	Lnn. jejunales
16	Colic ln.	Ln. colicus
17	Caudal mesenteric ln.	Ln. mesentericus caudalis
18	Renal ln.	Ln. renalis
19	Lumbar aortic ln.	Ln. lumbalis aorticus
20	Lateral iliac ln.	Ln. iliacus lateralis
21	Medial iliac ln.	Ln. iliacus medialis
22	External iliac ln.	Ln. iliacus externus

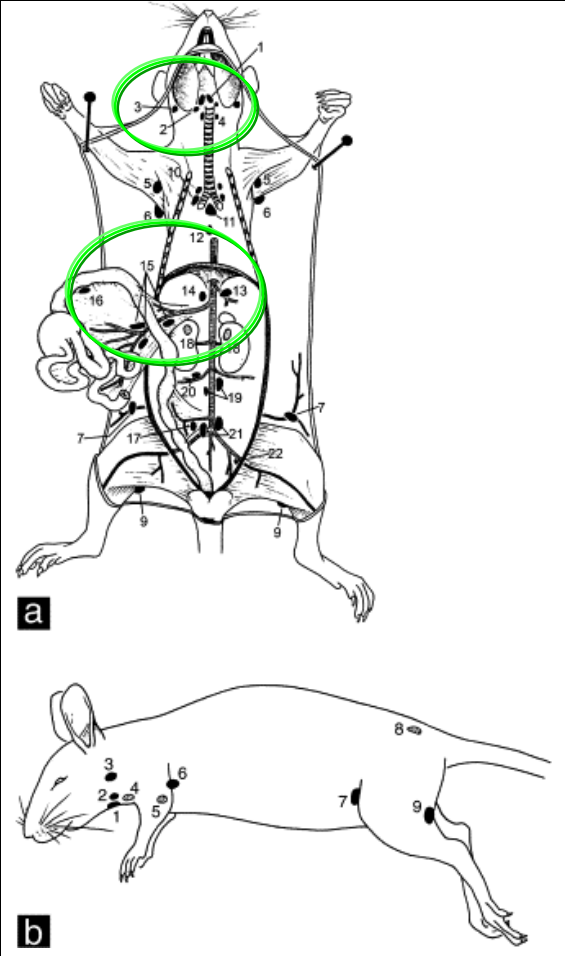
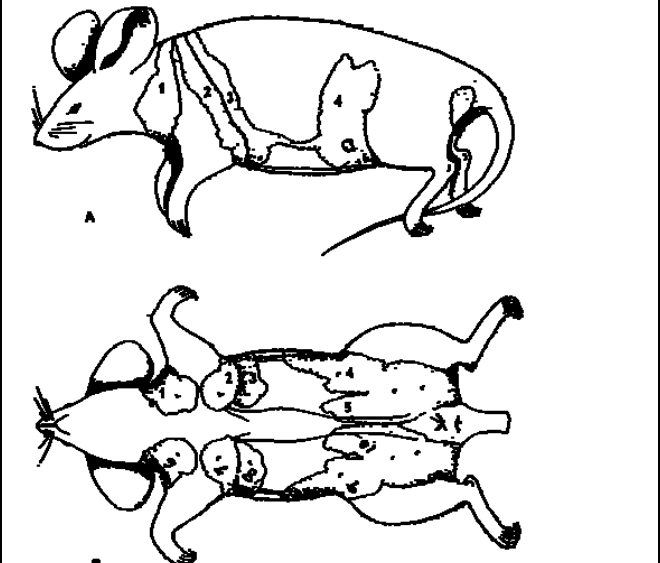
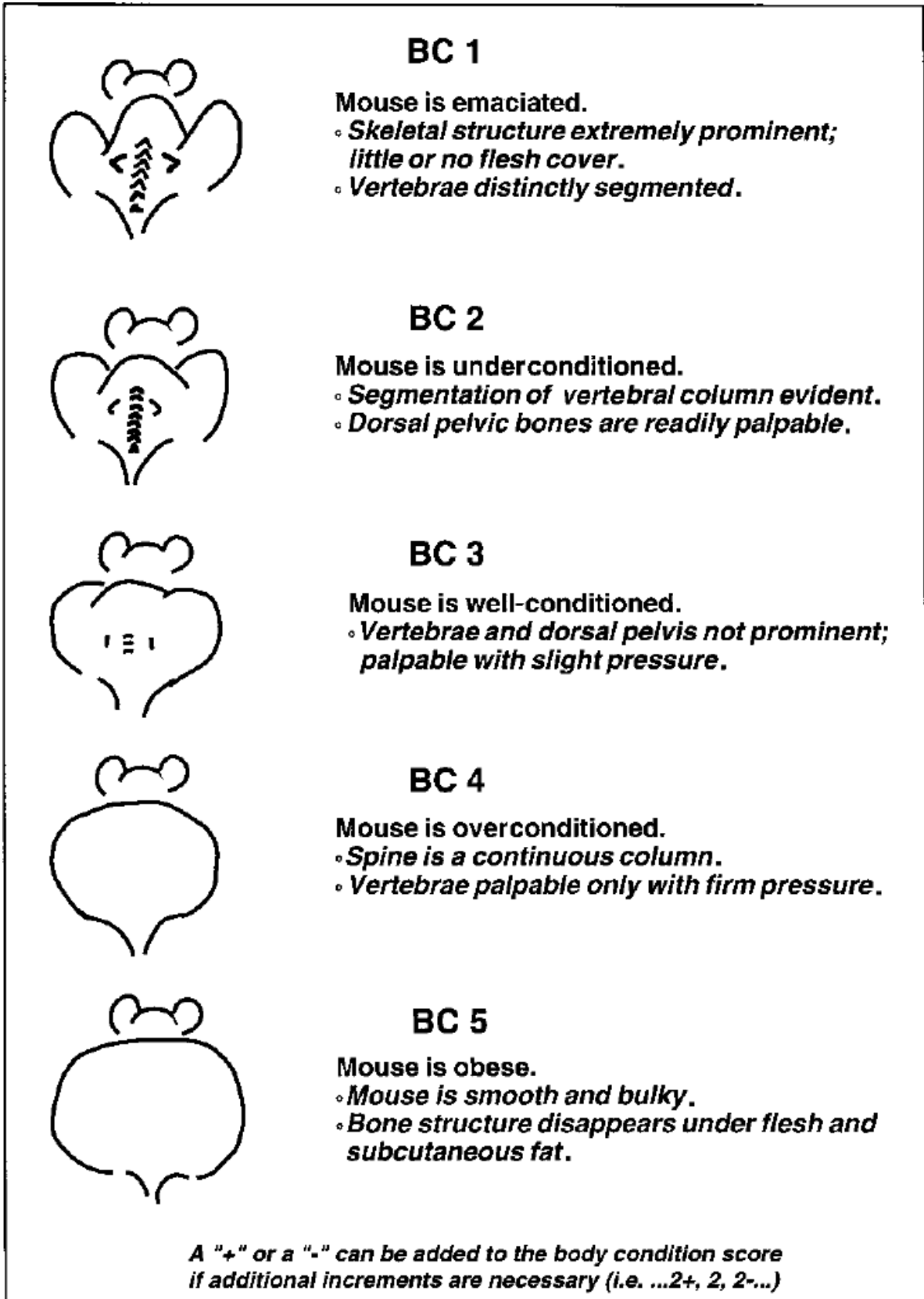


Figure 4. Mouse Mammary glands (adapted from Dunn 1951 &/or Cloudman 1936, 1941)

<p><i>A (lateral view)</i></p> <ol style="list-style-type: none"> 1. Mammary Gland-Left Cervical 2. Mammary Gland-Left Thoracic 3. Mammary Gland-Left Thoracic 4. Mammary Gland-Left Abdominal <p><i>B (ventral view)</i></p> <ol style="list-style-type: none"> 1. Mammary Gland-Left Cervical 2. Mammary Gland-Left Thoracic 3. Mammary Gland-Left Thoracic 4. Mammary Gland-Left Abdominal 5. Mammary Gland-Left Inguinal 6. Mammary Gland-Right Cervical 7. Mammary Gland-Right Thoracic 8. Mammary Gland-Right Thoracic 9. Mammary Gland-right Abdominal 10. Mammary Gland-Right Inguinal 	
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Figure 5. Mouse Body Condition scoring (from Ullman & Foltz. 1999)



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 Department of Molecular and Comparative Pathobiology
 ANATOMIC PATHOLOGY SUBMISSION FORM

Necropsy / Biopsy Date _____ Prosector _____ DCM # _____
 Investigator/Dept _____ Contact _____ Contact # _____
 Research Diag Teaching Charge #: _____ Room # _____

Species Mouse Strain/Geno _____ Mutant Y / N _____ Animal ID# _____
 Source _____ Sex F / M Color BL / Ag / AL: _____ Age / DoB _____
 Found Dead Y / N Euth CO2 / CD / Other: _____

Ancillary Tests (check '✓' applicable boxes) Other: _____

CBC / Diff	Clin Chem	UA	Serology	Micro	Fur	PWP/OP	Urinalysis	Behav JW	Imaging

Site Retroorbital / Facial / Saphenous / Tail / Cardiac / Other: _____ Volume: ~ _____ ml.

History and Clinical Signs (Include correct nomenclature, indicate background strains, genetic manipulations/ mutation(s), generations of backcrossing, experimental manipulations including bleeding, special diets, drug treatments e.g. Baytril, Ivermectin, Fenbendazole; reason(s) for submission; clinical signs):

Gross Findings (Include weights/measurements for lesions, diagrams to indicate external lesions whenever possible.):

Body Condition: 1 (emaciated) 2 3 (normal) 4 5 (hugely obese > 45g)

Body Wt (g)	Liver (g)	Spleen(g)	♥(g)			

ID Tags/#'s (save with tissues if possible): _____

Ear Punch: R  L

