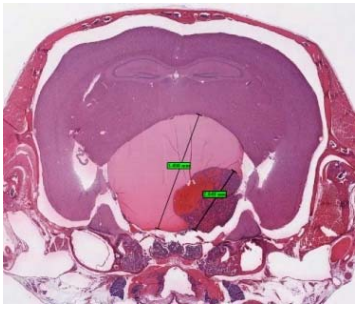
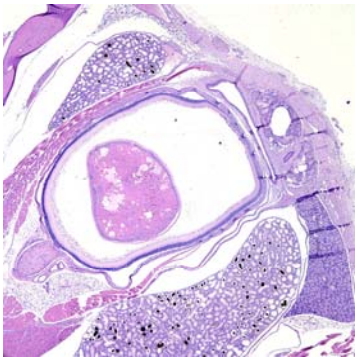
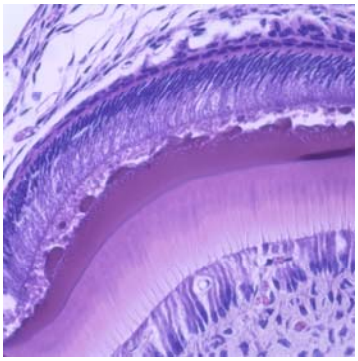
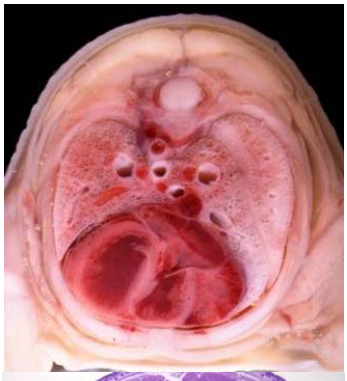
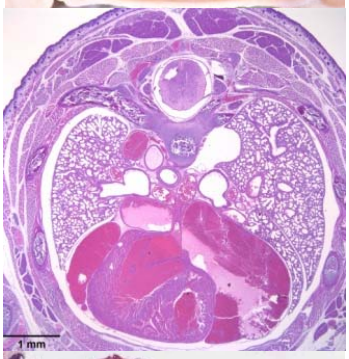
Mouse Pathobiology & Phenotyping

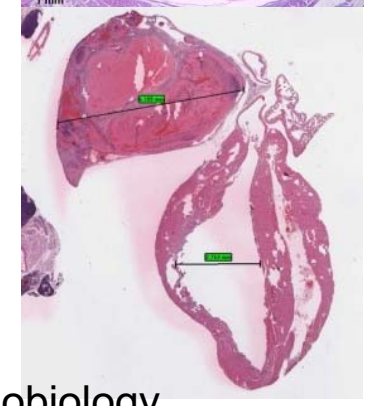












Short Course

2025

Lab Manual

6th ed Rev 2025

Department of Molecular & Comparative Pathobiology
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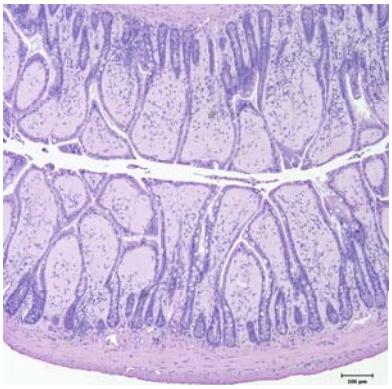
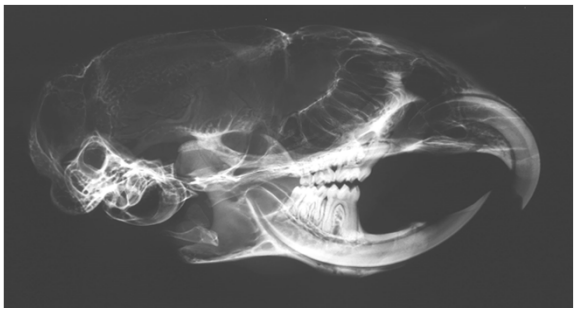
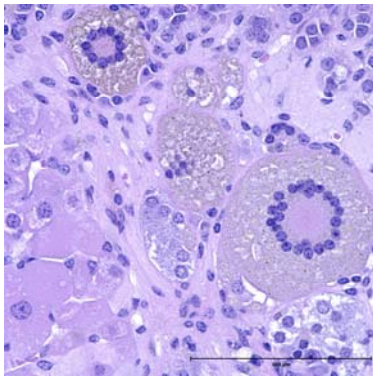






Lab Manual Mouse Pathobiology and Phenotyping

Contents

** **ALL LAB participants** should arrive promptly or early.*

** **ALL LAB participants** are expected to clean up after themselves, AND to assist in clean-up of their work area.*
***raduate students** taking this course for credit MUST complete 4 worksheets & hand them in for laboratory credit.*

Lab 1 2

LAB 1 Modified SHIRPA Clinical Examination

Modified from Julie Watson 2019 (CR Moats 2024)

This screen should take about 10 minutes per mouse and provides a basic evaluation for physical abnormalities (dysmorphologies), clinical abnormalities and abnormal behaviors, including absent or reduced normal behaviors or reflex responses. The assessment can be useful for clinical diagnostic and experimental (phenotyping) purposes.

Materials/Supplies:

1. Mice (sufficient n of experimental animals and concurrent relevant controls)
2. Quiet area,
3. Clean cage(s)
4. Wire bar cage lid
5. Cotton tip applicator (long wood disposable preferred)
6. Clicker (or other standardized noise/ tone)
7. Timer (phone)

Methods/Procedures: ~'Normal'/ 'expected' results in bold.

1. **Condition Score:** (1-5) (per Ullman & Foltz 1999.
   1 = Emaciated = vertebrae palpable as distinctly segmented. Little or no flesh covering.
   2 = Thin = segmentation of vertebrae evident, and dorsal pelvic bones are palpable.
   3 = 'Normal' = well conditioned = Vertebrae and dorsal pelvis not prominent, but palpable with slight pressure
   4 = Over conditioned = Vertebrae palpable only with firm pressure
   5 = Obese. Bone structure disappears under flesh and subcutaneous fat

2. **Gait abnormal** (Y or N) Abnormal: Hopping rather than running, exaggerated limb movements, limbs kicking out or dragging, lack of bilaterally symmetrical movement, uneven cadence, unable to move in a straight line, falling.

3. **Posture abnormal** (Y or N) Abnormal: rounded / hunched, Head tilt, or other head or body asymmetry, tail dragging or held rigid. Image shows hunched (abnormal) position.

4. **Aggression:** (Y or N) A wooden stick is placed in front of the mouse's mouth. The most common reaction is to ignore or turn away from the stick. **N = no biting,** or Y = biting.

5. **Body Tone:** (0-3) Hold mouse by tail base on a hard surface. With 2 fingers gently press down over the mid dorsum. '**Normal' tone: resists depression somewhat** – not allowing depression to the floor. 0 = flaccid; 1 = allows depression to floor; **2 = allows some flattening;** 3 = hunches back to completely resist compression

6. **Petting Escape:** (0-3) Hold mouse by tail base on a hard surface. With finger and thumb stroke down the flanks (sides) from front to back. 0 = no reaction; 1 = difficult to elicit escape response; **2 = easy to elicit escape response;** 3 = Difficult to test because of spontaneous escape attempts

7. **Passivity:** (0-3) Hold mouse by the tail and place front paws on the edge of the cage lid. **Normal response is climb promptly up to the top of the lid.** Falling or hanging without climbing is abnormal. This test is often used to evaluate drugs for sedative effects.
   0 = falls; 1 = delayed or unsuccessful attempt to climb up; **2 = 'normal';** 3 = hyperactive.

Lab 1 2

8. **Trunk Curl:** (0-3) Suspend mouse by tail for 15 seconds and monitor for curling of trunk. **'Normal' response is curling up laterally to at least horizontal.** 0 = zero or abnormal response e.g. hindlimb clasping; 1 < 90º curl; **2 = curls to 90º or more;** 3 = climbs up tail.

9. **Righting:** (0-3) Hold mouse by tail base. Hold your other hand flat with thumb up and little finger down to provide a vertical surface. Bring the dorsum (back) of the mouse to the back of your hand. '**Normal' response is to quickly flip over to climb up the hand**.
0 = does not right itself; 1 = struggles to right itself; **2 = rights itself promptly;** 3 = hyperactive.

10. **Forelimb placing (Proprioceptive positioning):** (0-3) Hold the mouse by the tail on a hard surface. Using the wooden applicator, gently move a forelimb out to the side. **Normal response is to rapidly return leg to normal position.** 0 = Leg stays where placed; 1 = slow or incomplete return; **2 = Prompt return to normal position**; 3 = hyperactive response.

11. **Rear Limb Withdrawal:** (0-3) Hold mouse by the tail on a hard surface. Pick up the hindfoot and pull the limb out at a 45º angle until it is stretched then let go. **Normal response is to rapidly return leg to 'normal' position.** 0 = Leg drops and does not return to normal position; 1 = slow to return; **2 = Prompt return to normal position**; 3 = hyperactive response.

12. **Ear/Pinna Response:** (0-3) Using a teased-out cotton tipped applicator, gently touch the ear pinna. Watch closely! **A 'normal response is a rapid ear twitch.** 0 = no response; 1 = slow/difficult to elicit response; **2 = obvious response;** 3 = hyper repetitive response.

13. **'Whisk' response:** (0-3) The vibrissae are stimulated using a 'teased out' cotton tipped applicator. This test can be difficult to elicit/assess because vibrissae are difficult to see, or mouse may see the approaching stimulus. **Touching vibrissae should elicit a response:** either a cessation of "whisking" (continual movement of whiskers), or a responsive nose quiver, which may be subtle.
0 = no response; 1 = difficult to elicit response; **2 = normal response;** 3= hyperactive response

14. **Palpebral reflex:** (0-3) Using a teased-out cotton tipped applicator, gently touch the cornea.
0 = no reaction; 1 = slow blink; **2 = quick blink;** 3 = hyper repetitive blinking.

15. **Visual Placing/Reach/Touch:** (Y or N) Hold the mouse by the tail and lower it slowly, steadily, toward the wire bar lid on top of the cage**. A visual mouse will start to reach or struggle** down towards the surface well in advance of touching it. A blind mouse will not reach out until forelimbs or whiskers touch. This test can be difficult to assess, as long vibrissae may touch without you seeing them, in which case you could interpret a blind mouse as sighted.

16. **Clicker (hearing test)**: (0-3) Hold mouse by tail base on a hard surface. After a moment of calm silence, use the clicker once, observing closely for a Preyer response (ear flick), or 'stop' response (head motionless briefly). Be careful not to allow the mouse to see you activate the clicker. Repeated clicks are often ineffective. 0 = no response; 1 = slow/difficult to elicit response; **2 = immediate response;** 3 = abnormal response (seizures, hyperactive escape, etc)

17. **Grip Strength:** (# sec) Place mouse on wire bar lid 1-2 feet above surface. Start the timer for 60 secs, shake grid gently then rapidly flip over the wire bar lid. <60 secs is abnormal; **normally mice hold on upside down easily for 60 secs.**

Lab 1 2

LAB 2 Specimen Collection

Facial Blood Collection

Aims: Humane collection of good quality blood specimens from facial vessels.

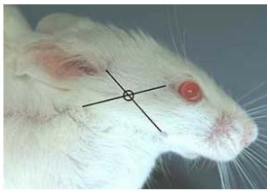
- ☐ Minimal trauma (for all) GOOD RESTRAINT IS CRITICAL!!
- ☐ Sufficient anticoagulated blood for tests, NO clots, NO hemolysis.

Materials (Supplies):

- ☐ 3-4 mm lancet usually (26G needle, short (1/2 inch or less)
- ☐ Small blood collecting tube (e.g. microvette or microtainer type)
- ☐ Clean work surface.
- ☐ Dry clean Kimwipe or gauze pad.
- ☐ Mouse
- ☐ [Anesthesia you may request anesthesia for your mouse]

Methods (Procedure):

1. With your dominant hand, lift the mouse, holding tail near base.
2. Place the mouse on the wire bars of the cage, so it can grab the bars.
3. Cup your free (non dominant) hand over the mouse, and scruff it firmly with thumb and first finger.
   a. **NOTE: It is critical to hold a lot of skin near the ears; to immobilize head** (without suffocating mouse). The tongue may protrude slightly.
   b. Tuck tail between your last two fingers to restrict body movement.
4. Mouse should be gently securely restrained in your non-dominant hand.
5. Lift the mouse, and locate the hairless 'cowlick' on the side of the jaw.
   The puncture site is just caudal to hairless cowlick.
   If you do not see the cowlick, try imagining a line along the lateral face at the level of the nose; intersected by a perpendicular line originating between the eye and ear. Their intersection is the puncture site.



(www.medipoint.com)

6. Pick up the lancet or needle with your free (dominant) hand.
7. Prick. [If using a needle, pierce the skin NO deeper than the bevel]
8. Quickly discard the sharp into the sharps container and
9. Collect 4-7 drops of blood into collection tube.
10. Press the collection site gently/firmly with dry clean paper or gauze to stop bleeding.
11. Release the mouse into cage when bleeding has stopped.

Terminal Blood Collection by Cardiocentesis.

Aims: Terminal blood collection by Cardiocentesis.

- ☐ Minimal tissue trauma, maximum blood collection, NO clots, NO hemolysis.
- ☐ Exsanguination as secondary Euthanasia method.

Materials (supplies):

- ☐ Deeply anesthetized, (or recently deceased) mouse
- ☐ 1cc syringe/22-25g needle **(primed to loosen syringe plunger)**
  Note: shorter needles /syringes are easier to handle.
- ☐ Blood tubes (e.g. microvette or microtainer type for small specimens)
  - o With anticoagulant for CBC (usually EDTA = lavender top)
  - o Gel separator tubes (GST) preferred for serum, plasma
  - o Eppendorf, for practice…

Lab 1 2

Terminal Blood Collection by cardiocentesis (cont)

Methods (Procedure):

1. Anesthetize mouse, confirm deep anesthesia by lack of response to toe pinch.
2. Position animal ventrum (belly) so that your dominant hand can easily hold syringe and needle parallel to mouse, with needle pointing to head.
3. Abduct (spread) forelimbs to expose ventral thorax completely.
4. Palpate and identify the xiphoid process between the lowest ribs.
5. With dominant hand, hold syringe, needle bevel up, and fingers positioned to pull plunger easily.
   a. Place non dominant thumb on abdomen, pressing gently, stabilize syringe by resting it gently on opposite thumb,
   b. [OR try: hold arms abducted, pushing down with non dominant thumb and 1st finger.
6. Insert needle/syringe under xiphoid at ~30o angle, aiming slightly down toward dorsal neck.
7. Pull back slightly on plunger. If blood does not 'flash' in hub, rotate/reposition needle/ syringe slightly (in/out) WITHOUT pulling it completely out of chest.
8. As blood enters syringe, continue pulling back gently. Don't pull hard.
9. Depending on mouse size, you should be able to collect 500-1000ul of blood, sometimes more.
10. Remove needle and gently eject blood into tube.

NOTE Different mouse/hand positions work for different people – figure out what works for you.
NOTE Mouse blood clots quickly (15-20 seconds), so anticoagulant tube is the usually the priority.

Blood Glucose

Aim: Measure blood glucose using Glucometers.

- ☐ Minimize trauma for animal welfare and better results. Trauma/stress → ☊Glycemia!

Materials (Supplies):

- ☐ Small weigh dish with 1-2 drops mouse blood (anticoagulated for practice)
- ☐ Glucose strip (check expiration dates – expired OK for practice only)
- ☐ Glucometers (check batteries)

Methods (Procedure):

OneTouch® Ultra https://support.onetouch.com/s/article/OneTouchUltra2ApplyingBloodReadingResults?language=en_US

1. Insert strip - Press the white bar end firmly into the meter.
2. Wait 8-10 seconds until display shows "Apply Blood".
3. Touch strip tip (with channel) to blood drop.
4. blood is drawn into narrow channel and fills confirmation window.
5. Wait for confirmation window to fill completely.
6. Within a few seconds blood glucose level appears on the display, with unit of measure.
7. Record results.
8. Remove/discard used strip. Meter should turn off automatically.

Accu-Chek Compact https://www.youtube.com/watch?v=HJDRLF0Yq10

1. Pull down on test button (between "M" and "S") to advance test strip.
2. Touch front edge of strip (black notch) against blood drop.
3. Test strip will draw up the blood.
4. When strip has enough blood, "Analyzing" (or 000) appears on display.
5. Results should display in ~4 seconds. Record results.
6. Pull down on test button again to release the used strip.

Lab 1 2

Fecal Occult Blood aka: guaiac test. https://www.hemocue.us/hemoccult/
Aim: Detect blood in feces. (e.g. IBD, or GI tumor studies)
[How it works: When hydrogen peroxide (developer) is dripped on to guaiac paper, it oxidizes alpha-**guaiac**onic acid to a blue colored quinone. Heme catalyzes reaction for rapid change].
Materials (Supplies)

- ☐ SOFT Feces (+ blood drop to demo 'positive' reaction)
- ☐ Test Slide (Envelope) for Fecal Occult Blood
- ☐ Smearing Stick
- ☐ Developer

Methods (Procedure):

1. Retrieve 2 soft fecal pellets from cage.
2. Touch 1 pellet to blood in weigh dish (= 'positive' control for this demonstration).
3. Open Test envelope.
4. Use wooden applicator to smear both pellets onto circles I and II.
5. Close envelope, Wait ~5 minutes.
6. Open the back of the envelope and apply 2 drops developer on each smear.
7. Rapid change to Blue is a positive result ● indicating presence of blood (heme).
8. No or very slow change indicates absence of blood/heme.

Urinalysis
Aims: Collect mouse urine, measure urine specific gravity, test with dipstick (chemstrip).
Materials (Supplies):

- ☐ 96 well microtiter plate and or collection 'substrate' in a mouse container/cage.
- ☐ Small pipette
- ☐ Veterinary refractometer
- ☐ Multistix or similar urine dipstick

Methods (Procedure):

1. Place plate(s) (or sand or foil) in container/cage to cover the bottom.
2. Place mouse in container undisturbed 20-30 minutes.
3. Mouse may have urinated, or may urinate as it is lifted from the cage –hold mouse over the collection substrate/plate while lifting…
4. **Refractometer**
    a. Pipette 2 drops on the glass plate and close top
    b. Read **Specific Gravity SG** through the viewer, angled toward a good light source
    c. [Urine Osmolality ~last 2 digits x~30 = #particles of solute per Kg solvent]
    d. Read **SP TP TS** (serum protein, total protein, total solids) g/dl - may? Give a reasonable measurement of urine protein? Usually used on serum plasma or effusions)
    e. Record results
5. **Dipstick**: Specific gravity, protein, glucose
    a. Place 1 drop on each test pad,
    b. Compare pad color with the guide on container.
    c. Record results
    d. Compare SGs: refractometer vs dipstick.
    e. Compare TPs: refractometer vs dipstick.

NOTE: the important concerning protein in urine is albumin or microalbuminuria.
NEITHER refractometer nor dipstick values are expected to measure these.

LAB 3-4 Practical Pathology

Pathology Protocol/Procedures (ref Brayton, McKerlie, Brown 2014[1]; Brayton 2001[2])

1. **Necropsy: PLAN; Materials & Methods**
2. **Materials: Equipment/Supplies**
3. **Methods: Necropsy Procedure** - External exam, Dissection
   - **Figure 1: Body Condition (BC) assessment**
   - **Figure 2,3: Mouse Lymph Nodes; Mouse Mammary Glands**
   - Figure 4: 10 Slide Tissue List
4. **Methods: Tissue Trimming**
   - **Figures 5,6: Mouse head, landmarks and trimming**
   - **Table 1: Trim Suggestions by Cassette/Slide number**
   - **Table 2: Histopathology evaluation, by slide number and tissues**
5. **General References & Links**
6. **Methods: Perfusion procedure**

PLAN for PATHOLOGY to:

1. Assess Morbidity, Mortality, unexpected findings in colonies;
2. Assess Morbidity, Mortality, unexpected findings during studies;
3. Confirm and characterize phenotypes etc research endpoints/outcomes;
4. Characterize and validate translational research models.

Project Management: Plan for Specimens, Documentation, Data, Reports:

- Strategies vary with resources and aims of programs / projects; etc.

Consider

- **Specimen** management: identification, preservation, storage/ retrieval (e.g. fluids, wet /frozen tissues, paraffin blocks, glass and digital slides)
- **Data** management: integrity/ security AND accessibility for comparisons within/ between studies.
- **Project** management: e.g. software for specimens, data, reporting etc.

Identify Redundant labelling/identification/documentation can save a study…

Weigh/ Measure/ Quantify Quantitative data rules, subjective impressions, not so much…

Document documentation = essential. Consider scans, photo back ups etc…

- **Paper necropsy reports / checklists** – useful but get wet/dirty → photodocument?
- **Animal/Specimen images/photos** facilitate comparisons within/ between studies;
  - **Confirm that photos / documentation comply with institutional rules/ requirements**
  - **Include identification/ruler** (can crop out for publication);
  - **Orient** animals/specimens similarly, and to make sense anatomically.
  - Images should **illustrate findings / comparisons**, not horrify viewers.
  - Pathology images require **robust data handling capabilities**. Still photomicrographs can exceed 5MB, scanned slides can exceed 1GB.
- **ALSO Be flexible** able to adjust procedures to new information, aims, findings…

Necropsy (dead + view), also autopsy (self + view) refer to *post mortem* examination, should:

- Should systematically evaluate tissues and identify lesions.
- Standardize (documented) procedures to improve comparisons within and between studies, even with examinations months /years apart. [1] [2] [3] [4] [5] [6]
- Modify procedures to achieve diagnostic and/or research aims.

LAB 3-4 Practical Pathology

MATERIALS: NECROPSY Plan, Equipment/Supplies

1. **PLAN (SOP, Protocol….)**
   a. **PLAN Procedures** e.g. Euthanasia, Collections, Weights
      - Euthanasia method to suit aims, and must be APPROVED in your protocol.
        E.g. cervical dislocation interferes with blood collection etc evaluations.
      - BODY weight before/after euthanasia? Blood Collection? BE consistent…
      - Tiny tissue weights before/ after fixation? Expect evaporation effects on fresh tissue...
      - **Consider Checklists -** ensure all procedures are done, order can be important…

   b. **PLAN Identification:** Redundant accurate simple
      - System for animal/specimen ID – consider photodocumenting animal/specimen ID
      - Specimens/ containers Labels ON+IN container e.g. labelled tags/ cassettes in container; Prepare/label containers cassettes in advance when possible;
      - Consider Photodocumenting identification, findings, procedures, cassetting etc.

   c. **PLAN Documentation** to achieve aims, e.g. procedures, findings etc data;
   d. **PLAN Personnel** e.g. team for procedures/recording (dirty/clean) etc;

2. **Protective Equipment (Including PPE personal protective equipment)**
   a. **Workstation**: ventilated workstation e.g. down-draft table or fume hood to protect prosector (person performing necropsy) from fixative fumes etc.
   b. **PPE: Face /respiratory protection**: to protect from splash/ droplets;
      Fitted respiratory protection (e.g. N95) for allergy concerns.
   c. **PPE: Eye protection:** Glasses / goggles to protect from allergens, splashes, etc.
      Magnifying glasses /goggles facilitate dissection / examination of small specimens.
   d. **PPE: Gloves**: For multiple animals, consider hand lotion and double gloving.
      When top gloves are damaged / soiled, replace only the top gloves.
   e. **PPE: Lab coats** etc to protect skin and clothes.

3. **Equipment/supplies Record relevant specifications, RRID etc**
   a. **Camera:** Photodocument identification, findings, procedures.
   b. **Weigh machine (0.001g -100g): Body weight, organ weights, mass lesions.**
   c. **Metric ruler** (or ruled label). Note: Record 3 dimensions as 2x3x2mm, or 2-3mm diam.
      Consistent location in photos (e.g. lower right) can be cropped out for publication.
   d. **Calipers:** for more precise measurements e.g. tumor/wound size at study endpoints.
   e. **Cutting board:** e.g. plastic cutting boards can be cleaned, re used, trimmed to size.
      Porous cutting boards e.g. cork are difficult to clean.
   f. **Paper towels**: many tissues (e.g. skin and reproductive tract) can be laid flat and will adhere to a dry paper towel for fixation, and towel can be labelled in pencil.
   g. **Forceps**: **blunt-ended, serrated, toothed cause less damage** vs pointy or smooth **forceps.**
      Straight forceps may be easier to handle (vs curved).
   h. **Scissors**: **Much of mouse dissection can be done with minimal use of scissors/blades.**
      Small cheap sturdy for skin, hair, paper etc.
      Small fine $$ blunt end for fine dissections. Straight scissors may stay sharper.
   i. **Blades**: **usually NOT for mouse dissections** (may be useful to split/ mince specimens for other tests). Essential to trim fixed tissues (See **Trimming** below).

LAB 3-4 Practical Pathology

j. **Syringes/ needles:** To infuse lung and GIT with fixative: 3ml syringe / 20-22G needle.
For cardiac blood collection: 1 or 3ml syringe, short 22-25G needle –what works for you?
Shorter needles (<1inch/<2cm long) are easier to handle.
BEWARE: Smaller gauge needles penetrate deeper than expected, missing lumens.

4. **Fixative**: 10% Neutral buffered formalin (**10%NBF**) for most situations, including IHC. [3 7 8 9 10]
Submerge tissues promptly ~1:10 V:V, i.e. ~1ml specimen:10ml fix. Rocking can improve fixation.
5. **Decalcifying (demineralizing):** boney tissues (e.g. head, spine, legs) for routine histology processing.
REMOVE SKIN so decal solutions can penetrate to/through bone. Tooth enamel = hardest tissue.
Remove tongue per protocol for head decal, + oral exam, +tongue histology.
Consider formic acid solutions that fix AND decalcify, so hard tissues can be trimmed within 24 hours along with soft tissues, and also may be suitable for IHC.
6. **Tissue cassettes** for paraffin processing: 3×2.5×0.4cm, + options for special needs e.g. extra deep or large, compartmented, finer grid etc. Consult histo lab for their preferences!

'Methods': Necropsy Procedure

- **Begin ASAP** after death. Refrigeration can usefully delay decomposition to some extent...
- **Consider perfusion/fixation** → dissect/weigh/collect tissues later, when you have more time…
- **DO NOT FREEZE** mice / specimens intended for Formalin Fixed Paraffin Embedded (FFPE) histology.

BEFORE YOU CUT: EXAMINE, RECORD

1. **IDENTIFICATION: confirm/record tattoos/tags…. Sex, color** (albino, agouti, black, other);
2. Euthanasia & blood collection – per approved protocol;
3. **BODY WEIGHT BEFORE ANY DISSECTIONS**
4. **External lesions 'dysmorphology'** e.g. domed head, microphthalmia, reduced/ extra/ abnormal digits, nipples, genitalia; masses, wounds, ulcers, alopecia. Record size location.
5. **PALPATE** for subjective **body condition score (BCS)** 1-5 (Figure 1), also for pregnancy etc abdominal masses or fluid. Record size (volume for fluid), color, consistency e.g. soft or fluctuant, firm or hard. 'Hard' should be reserved for boney or mineralized masses.

DISSECT, EXAMINE, RECORD, COLLECT (After external exam)

Orient animals in the same direction (e.g. head up, or right side up) to recall findings accurately, and document similarly between cases. Record size/weight, color, consistency of findings.
Avoid fruit / vegetable descriptors.:

1. **Remove pelt:** to assess subcutis, fat (scant, adequate, ample, excessive), mammary tissue, subcutaneous lesions and abdominal organs *in situ*, and to facilitate decalcification:
Pinch and separate OR incise ventral abdominal skin (pubic/inguinal area has thinnest skin), exert gentle pressure cranially and caudally until pelt is removed. Examine skin and animal.
Aseptic collections of effusions or mass lesions, abscesses, can be done at this point.
Fix skin flat on paper– see ppt: Cut 'cranial' skin on Ventral midline (so ears are up), cut caudal skin on dorsal midline (preserving preputia/clitoral/mammary glands); Lay skin flat on paper.
2. **Remove "chain" of salivary glands:** from ear to ear under the chin: parotid, sublingual, submandibular salivary glands, with lymph nodes. Save in **cassette 4;** Table 1, Fig 4.
3. **Open abdomen,** incise peritoneum xiphoid to pubis, examine contents *in situ*. Record abnormalities.

LAB 3-4 Practical Pathology

4. **Remove sternum/ Open thorax:** lift sternum by xiphoid process and cut rostrally (toward head) through ribs / clavicles, to open thorax. Spread ribs to examine contents, note fluid or masses. Note size or absence of thymus. Save, fix **sternum**, laid flat on paper, or in **cassette 1**.
5. **Remove measure thymus, Fix in Cassette 1, OR note as ABSENT or NT (no tissue) …**
6. **Expose cervical trachea** by blunt dissection; **infuse lungs** with fixative via 3ml syringe/20-25g needle. **TIP: Bending needle** ~45o may facilitate insertion into trachea lumen.
   Lungs should expand, and excess fixative will reflux up trachea. **NOT necessary** to clamp or tie trachea, but do not squish lungs after infusion. Infusion difficulty may be due to pneumonia or neoplasia, more often is due to dissection damage or misplaced needle.
7. **Remove tongue with larynx, trachea, esophagus from the head and neck:**
   Cut/separate mandibular symphysis with scissors, between incisors, to separate mandibular rami and expose tongue. Grasp tongue and retract caudally to remove thoracic viscera en bloc.
8. **Remove trachea, heart, lungs, aorta, esophagus from thorax en bloc.**
   Use blunt dissection (fingers>scissors) to free these tissues.
9. **Examine oral cavity** for abnormal dentition, impacted food, abscesses, tumors etc
10. **Split pelvis to complete removal of viscera**: push abdominal contents laterally,
    insert **closed scissors** into pelvic canal, open scissors gently to open pelvis along pubic symphysis, cut symphysis/midline soft tissue, to expose pelvic viscera.
11. **Remove abdominal-pelvic viscera:** Grasp diaphragm, retract or excise it from dorsal body wall, gently lift out all viscera, include adrenal glands, kidneys, gonads, pelvic contents, to anus/ urogenital orifices. Nick/incise right kidney for identification (scissors).
12. **Examine viscera** *ex situ*.
13. **Heart** Collect/weigh, Fix intact.
14. **Lungs** intact in **cassette #2** (per Table 1) dorsum down, **with Larynx/trachea thyroids**.
    Thyroid glands should be small, immediately caudal to larynx on left + right trachea.
15. **Dissect off liver and spleen**, **examine and weigh them**. Include small liver lobe attached to stomach lesser curvature to ensure accurate liver weights. Handle liver gently by diaphragm or hilus. The **median and left lateral lobes** are largest. **Gall bladder** lies between right and left 'halves' of median lobe. Separate lobes for immersion fixation.
    Free **spleen**, remove fat etc for an accurate weight. Fix spleen intact (unless huge e.g. >3g).
16. **Dissect off kidneys and adrenals**, **examine and weigh kidneys.** Right kidney should be rostral to left. **Adrenals** can be saved/ fixed intact in **cassette #3** (per Table 1), and weighed post fix if necessary. Female adrenal glands normally are larger than males'.
17. **Separate reproductive tract from pluck.** Tissue caudal to kidneys that is not bowel, is mostly reproductive tract and fat. Lay it flat on paper, spread tissues into anatomic orientation to examine (and fix) on paper. Or tiny tissues can be fixed intact in **cassette #8** (per Table1).
18. **Infuse and extend GI tract:** Infuse several segments with fixative via 3ml syringe/20-25g needle, usually <2ml fixative total, to preserve content/ mucosa (or can infuse earlier in necropsy).
    Extend GI tract by grasping stomach in one hand, and rectum (fecal balls) in the other and separating/ extending gently, **TIP**: keep fingers close together for precision.
    TIP: Wetting GIT with fixative makes it less sticky/fragile, easier to handle.
    Fix GIT intact/submerged or try Swiss roll etc options.
19. **Mesentery, lymph nodes and pancreas** As you extend GI tract, gently pull/dissect these pale soft fatty tissues from the bowel, and save/fix in **cassette #5**.
20. **Submerge saved tissues in at least 10x their volume of fixative**. Small fixed tissues usually can be trimmed for histology after ~24h fixation
21. **Head:** Sever separate from cervical spine at mobile Occipito-Atlantal (OA) joint; NO CRUNCH!
22. **Legs with pelvis**: Separate hemipelvis from spine, cut caudal to rostral near spine; NO CRUNCH!

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23. **Arms with scapula:** Separate from thorax; may cut/crunch thru tiny clavicles).
24. **Spine:** trim off ribs /tail - OK to fix/decal after removing head/limbs/
25. **Decalcify head, leg etc boney tissues**, **with skin removed.** Brain, eyes, muscle etc remain in situ.

- **Sternum (slide #**1) may not require decal, and marrow histomorphology may be preferred to decalcified marrow (e.g. leg/femur).
- Acid 'digests' tissue, Overexposure compromises staining and evaluation. With new or unfamiliar solutions, evaluate exposure periods to optimize protocol.
- Tissue:solution ratio should be ~ 1:10, and gentle agitation or rocking may improve results.

Figure 1. Body Condition (BC) score/ assessment from Ullman & Foltz 1999.[13]

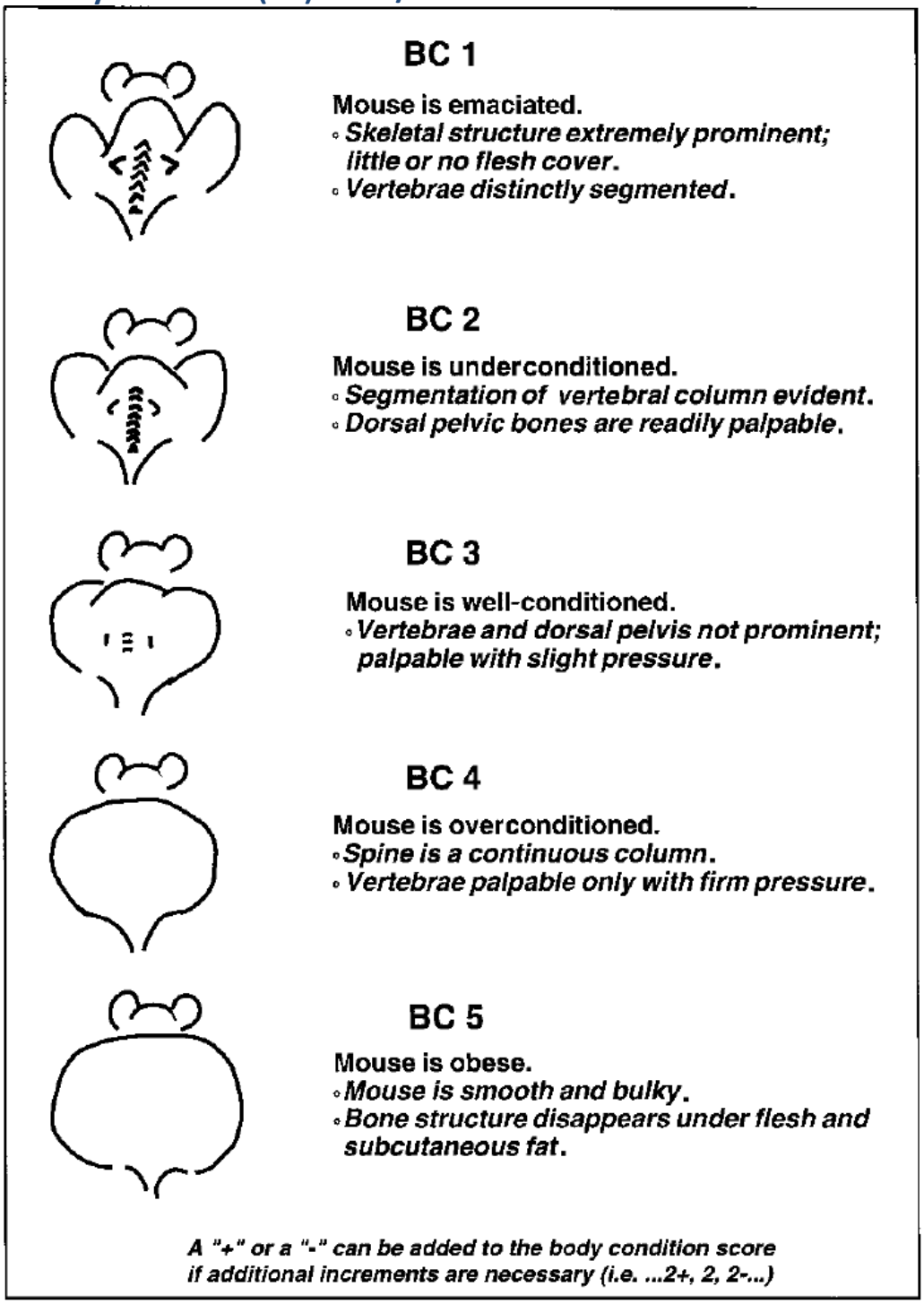

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Figure 2. Mouse Lymph Nodes (adapted from Van den Broeck, et al. (2006).[16]

#	English name	Nomina Veterinaria
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	Tracheobronchal 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

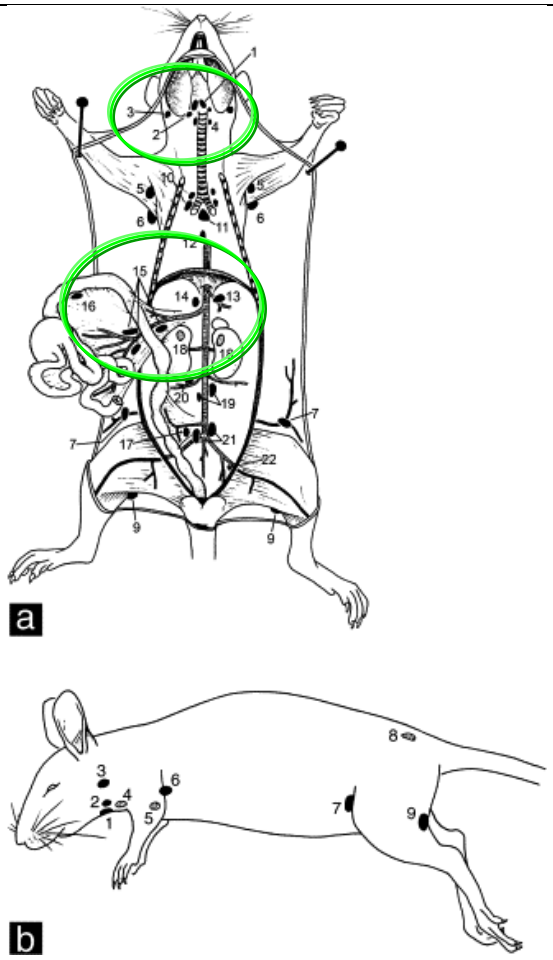


Figure 3. Mouse Mammary glands (adapted from Dunn 1951[17] &/or Cloudman 1936,[18] 1941[19])

A (lateral view)
1. Mammary Gland-Left Cervical
2. Mammary Gland-Left Thoracic
3. Mammary Gland-Left Thoracic
4. Mammary Gland-Left Abdominal

B (ventral view)
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

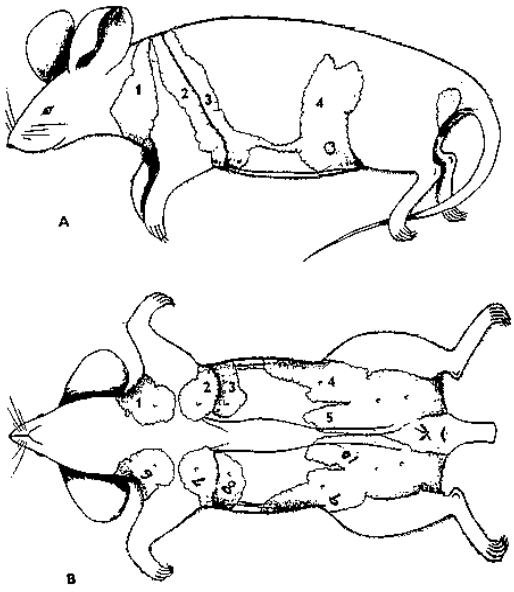
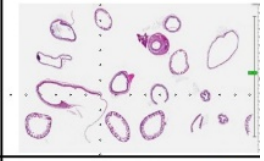
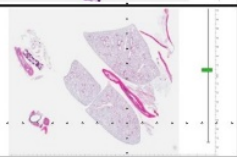
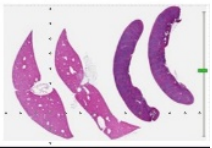
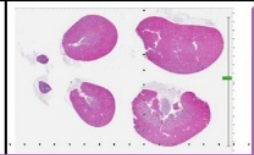
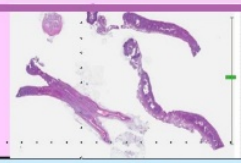
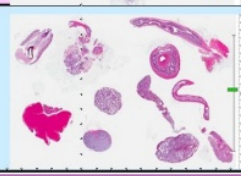
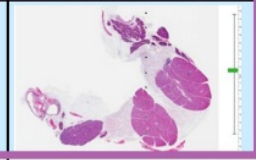
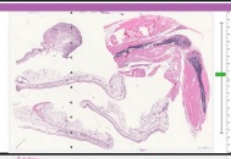
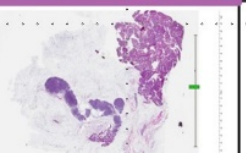
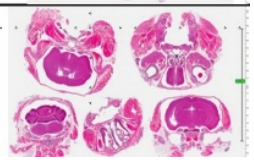


Figure 4: 10 Slide Tissue List /Slides

Cassette/Slide Numbers/Tissues

1. **Heart, thymus, sternum**, tongue
2. **Lungs, trachea**, thyroid/parathyroid, esophagus etc
3. **Kidneys, adrenals**
4. **Salivary glands,** lymph nodes
5. **Pancreas, mesentery,** lymph nodes
6. **GI tract**
7. **Liver, spleen**
8. **Repro, urinary bladder etc**
9. **Skin,** clitoral/preputial gland, etc
   Leg/knee – decalcified
10. **Head** – decalcified

Etc….

1. Heart, Sternum, Thymus, Tongue		6. GI – Cross sections	
2. Lung, Trachea, Thyroid, Esophagus		7. Liver, Gall bladder, Spleen	
3. Kidneys (R cross, L Long), Adrenal		8. Repro **(Female)**	
4. Salivary glands, L nodes **(Female)**		8. Repro **(Male)**	
4. Salivary glands, L nodes **(Male)**		9. Skin + leg/knee	
5. Pancreas, Mesentery, Nodes etc		10. Head (post decal)	

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LAB 3-4 Practical Pathology

Methods: Tissue Trimming

LAB 4 Trim fixed tissues for histology

Mouse tissues cassetted during dissection can include: **(1)** thymus sternum; **(2)** lung, thyroid, trachea; **(3)** adrenal glands; **(4) salivary** glands; **(5)** pancreas; **(8)** reproductive tract (if small). These may not require further trimming. Other tissues must be "trimmed" and submitted for processing in labelled cassettes.

MATERIALS Equipment/supplies **Record relevant specifications, RRID etc**

(Per above) Well ventilated area, PPE, Camera; Weigh machine; Metric ruler; **Calipers; Cutting board; forceps, scissors +**

a) **Necropsy reports**;
b) **Fixed tissues**;
c) **Cassettes** – Label in advance for tissues you plan to trim/submit.

- **ID/Number Cassettes** to facilitate retrieval of specific tissues/blocks/slides, e.g. Case/project#-slide# per protocol.
- **Pencil labels are more reliable** than markers (due to alcohols in processing). Unless automated Bar/QR coding management is available,
- **(Table 1)** summarizes a 10 slide protocol, with trim recommendations by tissue and cassette #; Additional cassettes can include lesions etc .
  d) **Blades**: inexpensive 1.5in single-edge blades to trim most tissues more expensive 2.25in Weck or similar blades to brains or decalcified heads,
  e) **Fresh fixative** for submission to histo lab, or storage of saved tissues.

METHODs (PROCEDURE):

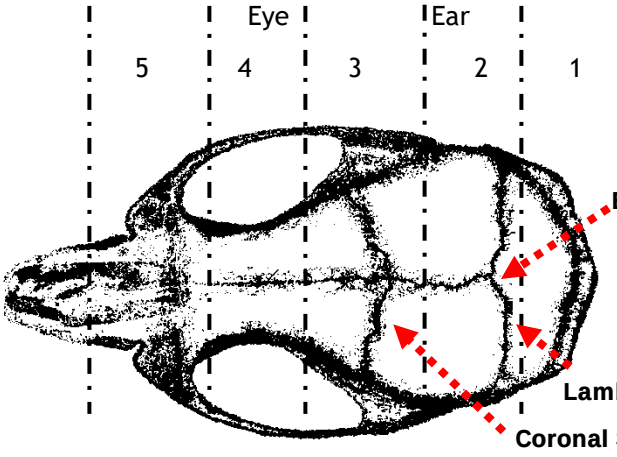
a) **CHECK NECROPSY Report for tissues and LESIONS to be trimmed**.
b) TRIM TISSUES per plan/protocol to achieve aims, facilitate comparisons, (e.g. **Table 1)**
c) TRIM TISSUES in a well-ventilated area or hood, wearing appropriate PPE.
d) **Cut/slice tissues** with a single clean swipe of a sharp blade. **NO sawing, NO squishing.**
   a. Trim tissues to ~3-4mm thick to fit in cassettes without grid marks or "squish artifact."
   b. **DO NOT stuff cassettes.** Tissues need adequate exposure for processing to paraffin.
   c. Down side of tissue in cassette becomes TOP of block, sectioned FIRST.
e) Decalcified tissue should cut easily. Crunchy tissue requires additional decalcification.
f) Rinse Decalcified tissues before trimming. Submit cassettes in NBF for processing (or PBS for short periods). Beware EtOH artifacts in brain/CNS. Consult Histo lab for preferences.
g) Submit properly labeled cassettes to histology lab in clean fixative.
h) Store fixed tissues in sufficient clean fixative to keep them moist, in sealable bags or other suitable containers, with identification in the container as well as on it.
i) Clean up, and Discard used fixative, unwanted tissues properly as hazardous waste.

Histopathology

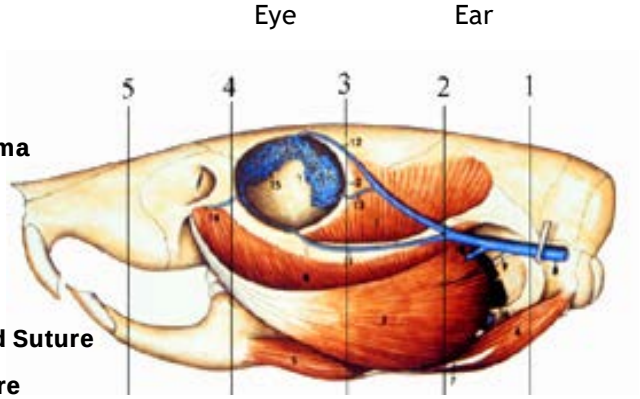
Table 2 summarizes common histology findings and lesions by slide number and tissue.
Diagnostic criteria and terminology for gross and histopathology findings should be standardized to facilitate comparisons within and between studies.
Anatomy and pathology terminology in reporting should align with published systems[5 6 11 12]e.g. Figures 2-3 include referenced nomenclature for lymph nodes and mammary glands respectively.

LAB 3-4 Practical Pathology

Figure 5: Mouse head, anatomic landmarks, and sectioning decalcified heads



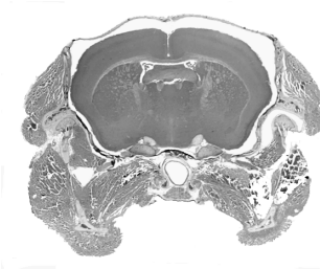
Adapted from Paxinos & Franklin 2001 [14]



Adapted from Popesko et al. 1992 [15].

Figure 6. Decalcified Mouse head sections – 5 Coronal/ Frontal sections. Numbered in order of cut, caudal to rostral (Large heads or specific needs may require additional cassette, or additional trimming). Sections 1-3 are similar to RENI/RITA recommendations https://reni.item.fraunhofer.de/reni/trimming/manus.php?mno=005

1. Cerebellum (section placed in cassette **front**/rostral/anterior side **down**);
2. Ears/hippocampus/Pituitary (section placed in cassette **front**/rostral/anterior side **down**);
3. Cerebrum (section placed in cassette **back**/caudal/posterior side **down**);
4. Eyes, oral cavity (section placed in cassette **back**/caudal/posterior side **down**);
5. Nose, vomeronasal, incisors etc (section placed in cassette **back**/caudal/posterior side **down**); long noses may require additional trimming to fit in cassette.



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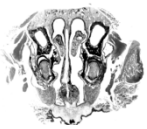

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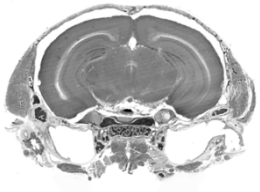

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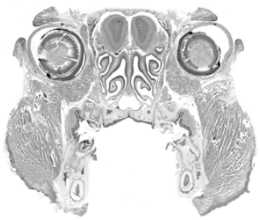

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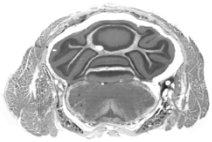

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LAB 3-4 Practical Pathology

Table 1: Cassette (slide) numbering, tissues, trimming suggestions [+options, additional tissues]

Cassette #	Tissues	Table 1: Trimming suggestions
1	♥Heart	Heart: Hemisect longitudinally to expose all chambers and valves. Right ventricle has a thinner wall than left and may wrinkle slightly when pressed. For some purposes, multiple transverse (cross)-sections may be preferred.
	Thymus	Thymus: both lobes usually can be included intact; or section and place flat slide down. **Note absence if thymus is NOT found.**
	Tongue	Cross section or longitudinal section flat side down.
	Sternum	Place intact, deep or internal side down /flat in cassette, to section to marrow easily; Trim off excess ribs to facilitate sectioning.
	[Diaphragm	Short strips can be sectioned on edge]
2	**Lung - entire**	Dorsal side down - in cassette during initial dissection
	Trachea	Cross section at thyroid, or include intact for longitudinal section
	Esophagus	Usually with trachea in long section or Xsection.
	Thyroid, parathyroid	Transect trachea at level of thyroid, or trim to evaluate lesions. OR Special dissection (dissecting microscope) + special cassettes for more specific evaluation of small tissues.
	[Aorta	Attached to thoracic viscera, or dissected off and included separately.]
	[Lymph nodes	Mediastinal nodes or small pieces of thymus may be attached but not seen at dissection]
3	Kidneys	Usually 2 cross sections of right kidney + 2 sagittal sections of left kidney
	Adrenal glands	In cassette. Small adrenals may require special cassettes (smaller holes), sponges, or tea bags; consult histo lab regarding preferences for tiny bits.
	[Lymph nodes	Frequently included with pararenal fat and adrenals. See Fig 4.]
4	Salivary glands with lymph nodes	In cassette: paired parotid, sublingual, submandibular salivary glands (with attached lymph nodes - Fig 4.). Removed *in toto* by dissecting from neck, from one ear canal to the other. Measure /record abnormalities (e.g. large Lnodes, tumors).
	Exorbital lacrimal glands	Sometimes included when salivary glands are dissected off *in toto* (+ likely to be found in head section (10).)
	Auditory sebaceous gl.	Sometimes included when salivary glands are dissected off *in toto.* (+ likely to be found in head section (10).)
	Lymph nodes	Usually included when salivary glands are dissected off *in toto.* See Fig 4.
	Mammary gl.	Usually included here from female mice (smaller in male mice) See Fig 4,5.
5	Pancreas	In cassette: During dissection, pancreas, fat, mesentery, lymph nodes, were stripped from GI tract to include in this cassette. These tissues can be difficult to distinguish grossly but readily identified microscopically.
	Lymph nodes, fat, vasculature	Usually included in this section – See Fig 4
6 GIT	Stomach	Section to include forestomach and glandular stomach
	Small intestine Cecum, colon	Include cross sections or segments according to your needs, or histo lab preference s
[6 a, b, c	Swiss roll Open]	Immediately after euthanasia, open SI LI (incised longitudinally), examine, fix, roll into 1 or 2 deep cassettes; or photodocument/measure lesions and select lesions for histo
[6 a, b, c	Swiss roll Closed]	At dissection, after fixative infusion with, **before fixation,** intact (closed) small intestine can be rolled into 1 cassette, Large intestine rolled into 2nd cassette; cecum and stomach sectioned into 3rd cassette. (fixed intestines do not roll well into cassettes)

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Cassette #	Tissues	Table 1: Trimming suggestions
7	**Liver**	1 Section thru left lateral lobe, hilus to periphery; 1 Section through median lobe to include gall bladder; include lesions in any lobes. Measure /record abnormalities.
	Gall bladder	Include with median lobe; or can be sectioned separately if enlarged.
	Spleen	Small spleens hemisected on long axis (best half in cassette), or intact if tiny. Cross sections for large spleens (and tox etc protocols)
8 Urogen	Urinary bladder	Include (small) bladder with entire tract in cassette; or section/separate at trimming.
8 Female	Uterus Ovaries Vagina	Reproductive tract can be fixed flat, intact on paper. A small reproductive tract can be included intact in cassette before or after fixation. For larger tracts or lesions, cross sections or segments should be included in cassette. Measure /record abnormalities.
8 Male	Testes Epididymis Seminal vesicle + coagulating glands	Entire tract can be fixed flat, intact on paper. A small reproductive tract can be put intact in cassette before or after fixation. For larger tracts or lesions, cross sections or segments of different regions should be included in cassette. Large seminal vesicles are common in older males; 1 or 2 cross sections can be assessed. Measure /record abnormalities.
	Prostate	Parts are included with entire tract in cassette; [Alternatively cross section at bladder neck; OR special dissection of each lobe.]
9	**Skin**	Cut ~3mm diameter ribbons of flat, fixed skin, parallel to hair growth and to long axis of mouse, including facial skin, clitoral or preputial glands. BE SURE TO INCLUDE any abnormalities/lesions noted in the report.
	Mammary glands	Usually found in female skin sections; or mammary pads can be harvested and evaluated specifically.
	Preputial or clitoral glands	Sebaceous glands (2 lobes) in inguinal subcutis, near genital openings.
	[+/- Decalcified LEG – SKIN REMOVED]	May include in this cassette (consult histo lab, do not crowd cassette). Medial (flat/boney) side down for best section of femur knee; Remove XS and poorly decalcified tissue e.g. feet. USE separate cassette/slide for specific assessments or lesions.
10	**Decalcified Head** In cassette: Front Down - Front Down - Back Down - Back Down - Back Down -	Use consistent orientation and anatomic landmarks Fig 2,3, to achieve comparable sections between cases. External ear canal openings and eyes are useful landmarks. Hold nose in one hand, using clean, single strokes, make 4 or 5 sections, from caudal to rostral, that usually fit in one cassette. 1) Cut just caudal to ear canal for section to include cerebellum, medulla; 2) Cut just rostral to ear canal for section to include middle ear, inner ear, or both; pituitary, thalamus, hippocampus; 3) Cut just caudal to eyes for cerebrum, usually hippocampus, thalamus; 4) Cut just rostral to eyes for eyes, Harderian glands, oral cavity, molars; 5) Nose with nasal cavity, sinuses, vomeronasal organ, incisor roots etc Large heads may require 2 cassettes OR trim off mandible for 1 cassette.
[11 a b]	Decalcified spine	Cervicothoracic and lumbosacral spine segments (with muscle, vertebrae, spinal cord) usually can be represented well in **2 cassettes.** **3 Cross-sections:** 1) rostral spine; 2) at last rib (~thoracolumbar junction); 3) sacral area; **2 para sagittal (long) sections**: 1) cervicothoracic, 2) lumbar, obtained by sectioning tissue cleanly from one side to level of vertebral bone/canal, for a flat surface on paraffin block, for sections of deeper tissues including spinal canal and cord
[12 etc]	Lesions:	**CHECK NECROPSY REPORT TO ENSURE TRIMMING OF ANY RECORDED FINDINGS:** Trim lesions to include adjacent normal issue for context, to reflect gross measurements and photographs, and correlate to gross findings.

LAB 3-4 Practical Pathology

Table 2: Histopathology evaluation, by slide number and tissue. [20] [21]

Slide #	Tissues	Table 2: Common features and lesions to look for on histology:
1	♥**Heart**	Enlarged chambers, thickened walls, myofiber degeneration loss or hypertrophy, Inflammation, fibrosis, mineralization, thrombi, amyloid.
	Thymus See also slide 2, 4	2 lobes? Distinct medulla and cortex? Approx. size? Apoptosis ? necrosis? Large: neoplasia? Hyperplasia or inflammation; Small: lymphoid depletion or hypoplasia (as in nude scid etc ID) ; Cysts, ectopic thyroid or parathyroid?
	Lymph nodes	Lymphadenomegaly due to inflammation, hyperplasia (reactive), vs neoplasia; Small nodes e.g. depletion, or hypoplasia (as in nude scid etc ID mice) .
	Tongue	Inflammation, mineralization, myofiber changes.
	Diaphragm	Myofiber changes as in muscular dystrophy or other myopathy
	Sternum	**Marrow**: Cellularity? Trilineage hematopoiesis; Myeloid: erythroid M:E ratio (approx.); Neoplasia; fibroosseous lesion/ proliferation. Muscle – myofiber degeneration regeneration, atrophy, fibrosis, fat, mineral.
2	**Lung - entire**	Inflammation/infiltrates mononuclear etc, XS BALT? Acidophilic crystals/macrophages; neoplasia – lung, hematopoietic embolic/metastatic?
	Trachea	Inflammation/infiltrates mononuclear etc.
	Esophagus	Inflammation/infiltrates mononuclear etc; hyperkeratosis, bacteria etc…
	Thyroid, parathyroid	Hyperplasia, neoplasia, ectopic thymus, inflammation, amyloid;
	Aorta	Inflammation/ mineralization in susceptible mice; Atheroma, Aneurysm in models?
3	Kidneys – right/cross, left /long	Hydronephrosis; Tubule degen/regen; glomerulopathy; amyloid (glomerular, interstitial); inflammation/infiltrates mononuclear etc; mineralization…
	Adrenal glands	Inflammation/infiltrates mononuclear etc. X zone vacuolation in females; subcapsular cell hyperplasia; pigment (ceroid) laden cells; cortical nodules; neoplasia,
4	Submandibular glands	Cw male (+ acidophilic ductules?); cw female (smaller - acidophilic ductules?); Inflammation/infiltrates mononuclear etc; degeneration/atrophy; neoplasia.
	Sublingual salivary glands (mucous)	Inflammation/infiltrates mononuclear etc; degeneration/atrophy; neoplasia.
	Parotid salivary glands	Inflammation/infiltrates mononuclear etc; degeneration/atrophy; neoplasia; **amyloid**.
	Exorbital lacrimal glands	Inflammation/infiltrates mononuclear etc; degeneration/atrophy; neoplasia. see also head slide (10).
	[Auditory sebaceous glands]	Inflammation/infiltrates mononuclear etc; neoplasia – see also head slide (10).
	Lymph nodes	**As above**
	[Mammary glands]	In females: Inflammation, hyperplasia, secretion (milk production), neoplasia.
5	Pancreas exocrine	Adequate / uniform zymogen distribution, zymogen depletion, exocrine atrophy or loss, neoplasia (rare unless GM); Inflammation/infiltrates mononuclear etc.
	Pancreas endocrine	Islet inflammation (insulitis), degeneration (as in some diabetes models), hyperplasia (especially in fat mice); neoplasia (rare).
	Lymph nodes	**As above**
	Mesentery	Infiltrates/inflammation mononuclear etc; Vasculature: Arteritis, periarteritis;
	Fat white and or brown	Adequate, excessive, scant; Fat necrosis, mineralization, inflammation; Inflammation with or without bacteria, associated with injection?

LAB 3-4 Practical Pathology

Slide #	Tissues	Table 2: Common features and lesions to look for on histology:
6 GI	Forestomach	Scant ingesta ? hyperkeratosis ? Inflammation/infiltrates; erosion, ulcer; hyperplasia, neoplasia; Expected (friendly) bacteria Lactobacillus-like? Cocci? Trimorphic yeasts? Cw candidiasis? Blood/pigment in lumen?
	Glandular stomach	Inflammation/infiltrates; erosion, ulcer; hyperplasia, neoplasia; Superficial yeasts (*Candida/Kazachstania* sp)? *Cryptosporidium muris* (in glands)?
	CONTENT	Adequate ingesta in stomach? Expected (scant) micro flora ? blood or blood pigment?
	Duodenum	Inflammation/infiltrates; erosion, ulcer; hyperplasia, neoplasia; *Giardia muris, Spironucleus muris*; Blood/pigment in lumen?
	Jejunum	Inflammation/infiltrates; erosion, ulcer; hyperplasia, neoplasia Cestodes, coccidia; Blood/pigment in lumen?
	Ileum,	Inflammation/infiltrates; erosion, ulcer; hyperplasia, neoplasia, **amyloid**: *Cryptosporidium parvum*; (SFB segmented filamentous bacteria ~ Normal flora)
	Cecum	Inflammation/infiltrates; erosion, ulcer; hyperplasia, neoplasia; Nematodes: pinworms v other?; flagellates, entamoebae.
	Colon	Inflammation/infiltrates; erosion, ulcer; hyperplasia, neoplasia; Nematodes: pinworms v other?; flagellates, entamoebae.
	Rectum	Prolapse? Inflammation, erosion, ulcer;, hyperplasia, neoplasia; Nematode larvae in crypts?
	CONTENT	Adequate ingesta /digesta? Expected micro flora ? blood pigment?
7	**Liver** (L Lateral+ Median Lobe/)	Inflammation/infiltrates; hepatocyte vacuolation (lipid, glycogen, etc.), atrophy, anisocytosis, anisokaryosis; pigment (usually hemosiderin or bile), degeneration, or necrosis, with syncytia or inclusion bodies; biliary hyperplasia, cholestasis; neoplasia hematopoietic, hepatocellular, hemangiosarcoma (vs peliosis) etc
	Gall bladder	Inflammation, erosion, ulceration, hyperplasia, neoplasia; Hyalinosis / crystals in mucosa.
	Spleen	Splenomegaly (by weight): Inflammation, hyperplasia, neoplasia, pigment hemosiderin or melanin in pigmented strains, amyloid; Small spleen (by weight): depletion or hypoplasia as in nude or scid.
8 Urogen	**Urinary bladder**	Distension size? Calculi? Birefringent? Inflammation/infiltrates mononuclear etc; erosion/ulcer; neoplasia.
8 Female	Uterus	Inflammation (pyometra or metritis), hyperplasia – cystic?, neoplasia; Embryos, Implantation sites, atrophy: pigment, mineralization.
	Ovaries	Normally developing follicles, corpora lutes, atretic follicles, oviduct; Neoplasia, cysts, atrophy, pigment, amyloid.
	Vagina	Inflammation, erosion, ulceration, hyperplasia, neoplasia; Hyperkeratosis, mucification; granulocytes compatible with cycle ?
8 Male	Testes	Spermatogenesis Active? Reduced? Inflammation or sperm granulomas? Seminiferous epithelium degeneration/loss; neoplasia, Interstitial cell loss/hyperplasia/neoplasia;
	Epididymis	Mature sperm? Degenerate or giant cells; stasis; inflammation;
	Seminal vesicle + coagulating glands	Distension, inflammation/infiltrates mononuclear etc; hyperplasia, neoplasia.
	Prostate	Inflammation, hyperplasia, neoplasia.

LAB 3-4 Practical Pathology

Slide #	Tissues	Table 2: Common features and lesions to look for on histology:
9	Skin	Inflammation, erosion, ulceration, bacteria, hyperplasia, neoplasia; Acanthosis, hyperkeratosis, bacteria compatible with *C bovis*? Mites?
	Subcutis	Adequate fat? Inflammation, neoplasia.
	Mammary glands	Inflammation, hyperplasia, neoplasia.
	Preputial / clitoral glands	Inflammation, abscesses, bacteria, hyperplasia, neoplasia.
	+/- leg decalcified	Muscle – myofiber degen/regen, atrophy, fat, mineral, rhabdomyosarcoma?. Bone/knee Degenerative joint disease (OA/DJD)? Inflammation /arthritis; Marrow: Cellularity? Trilineage hematopoiesis; Myeloid: erythroid M:E ratio (approx); fibroosseous lesion/ proliferation – Neoplasia hematopoietic
10	**Decal Head:** Brain	Dilated ventricles (thin? cortex?); corpus callosum present/absent? Inflammation; neuron necrosis/pyknosis; neuropil loss/rarefaction; neoplasia; Artifacts Hemorrhage (cervical dislocation?); white matter vacuolation?
	Pituitary	Hyperplasia, neoplasia, cysts;
	Ears	Inner, middle (bulla) Inflammation, bacteria, neoplasia.
	Eyes	Retina folds, loss (degeneration); lens globules/cataracts; Cornea inflammation (keratitis), mineralization
	Harderian glands	Pigment (porphyrin), acinar atrophy; Inflammation/infiltrates; neoplasia (esp adenomas)
	Bone, marrow	Hematopoiesis M:E; depletion hypoplasia hyperplasia neoplasia etc
	TM joint	Degenerative joint disease (OA/DJD)? Inflammation /arthritis;
	Oral cavity	Inflammation, neoplasia;
	Incisor teeth	Dysplasia, fracture, inflammation; Ameloblast/odontoblast necrosis/loss.
	Molar teeth	Periodontal hairs / inflammation, alveolar bone loss, hypercementosis.
[11	Spine decal]	Cord degeneration inflammation neoplasia Bone: hematopoietic marrow vs neoplasia, etc; Osteosarcoma etc neoplasia?
[12 etc	Lesions]	

Online Resources for Pathology, Terminology, Diagnostic criteria

1. NORECOPA PREPARE Guidelines on Necropsy https://norecopa.no/prepare/15-necropsy with links to JOVE videos etc resources.
2. Pathbase: http://www.pathbase.net/Necropsy_of_the_Mouse/printable.php
3. Tox regulatory (RITA NACAD) trim recommendations from Ruehl-Fehlert et al 2003; Kittel et al 2004; Morawietz et al 2004 (up to ~40 slides per mouse… $$$) https://www.niehs.nih.gov/research/resources/visual-guides/guides
4. goRENI https://www.goreni.org/ for INHAND International Harmonization of Nomenclature and Diagnostic criteria – searchable by diagnoses organs etc.
5. NTP National Toxicology Program. Non Neoplastic Lesion Atlas https://ntp.niehs.nih.gov/nnl/
6. Mouse Tumor Biology (MTB) Database http://tumor.informatics.jax.org/mtbwi/index.do
7. Allen Brain Atlas https://mouse.brain-map.org/static/atlas
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LAB 3-4 Practical Pathology

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LAB 3-4 Practical Pathology

Methods; Perfusion Transcardiac Perfusion (via left ventricle)

Note: See Necropsy/ Perfusion PPT for more images.

Aims:

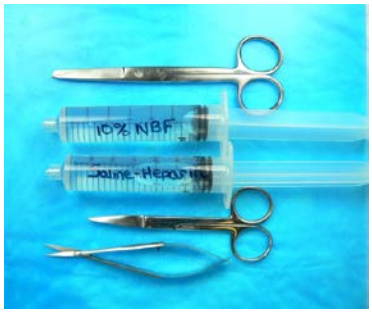
- Uniformly perfuse all tissues with fixative delivered via the vascular system;
- Achieve excellent tissue preservation;
- Minimize artifacts of decomposition and handling that interfere with analysis.

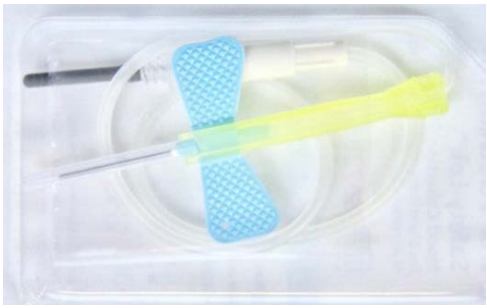
Overview:

- Blood is flushed from vasculature (exsanguination), as Perfusate/fixative is Injected slowly into left ventricle, Driven through systemic circulation;
- Blood and perfusate(s) drain from the incised right atrium.

Success indicators

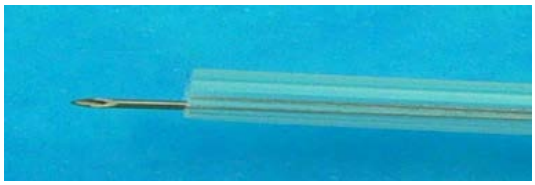
- Pallor, blanching of liver etc tissues;
- Muscle contraction;
- Excellent reproducible histology ☺





Materials (Supplies):

1. Approved protocol for procedures;
2. Down draft table or fume hood;
3. Collection Method for XS/waste fixative;
4. PPE (gloves, mask, eye protection);
5. Two 20ml syringes LABELLED
   - Saline-Heparin flush (~10units/ml)
   - 10% Neutral Buffered Formalin
   - **[for practice 1 syringe of ~80% EtOH]**
6. Vacutainer Butterfly collection set 25G x ¾ x 12in
   - **Clip/cut ~3mm from needle guard so 'cuff' prevents needle from piercing through heart**
7. Dissecting tools: Scissors + fine/iris scissors, forceps, hemostat.



Methods (Procedure):

1. Prepare supplies, syringes, butterfly infusion set;
2. Attach infusion set to syringe and 'prime it' by filling with perfusate (so no air bubbles);
3. Euthanize mouse, weigh & record weight in grams;
4. Promptly so blood does not clot: Remove pelt to expose thorax, Cut Right and left ribs to open thorax; Reflect/remove sternum ribs to fully expose heart
5. Incise (nick) right atrium using fine scissors. (before or shortly after starting perfusion (step 7))
6. Carefully insert cuffed needle into left ventricle.
7. Slowly inject perfusate **[for practice ~10ml EtOH is sufficient]**
8. After ~1min/25ml hep/sal, Without moving needle, switch syringe to fixative ~1min/25-50ml
   - Expect increasing pallor with exsanguination;
   - Expect stiffening/extension with formalin/PFA infusion.

Lab 1 2

JH Phenotyping Core Mouse Hematology (+References, Resources)

MOUSE HEMATOLOGY (CBC)

IDEXX ProCyte Dx® Hematology Analyzer; IDEXX Laboratories Inc; Westbook, ME

NOT reference ranges: Mice Strain, sex, age, immune status, disease conditions not known.

7 July 2016 to 21 August 2017 (N Forbes McBean);
Exclusion criteria: Any Error Message.

	RBC (M/µL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW.SD (fL)	RET (K/µL)
Mean	8.13	13.63	43.78	56.18	17.55	31.22	33.1	8.13
Low	3.57	6.1	16.7	39	12.6	27	24.2	3.57
High	15.2	21.7	69.8	90.8	31	37.6	63.1	15.2
SD	2.13	2.01	6.93	10.74	3.57	1.51	5.09	2.13
n	1119	1119	1119	1119	1119	1119	1119	1119

	PLT (K/µL)	PDW (fL)	MPV (fL)	WBC (K/µL)	NEUT (K/µL)	LYMPH (K/µL)	MONO (K/µL)	EO (K/µL)	BASO (K/µL)
Mean	675.09	8.97	7.34	7.52	2.79	4.12	0.45	0.11	0.05
Low	59	5.7	5.2	1.06	0.03	0.12	0	0	0
High	2633	23.9	13.1	56.08	32.03	23.46	5.08	2.03	2.33
SD	369.3	2.29	1.65	4.81	3.25	2.87	0.59	0.14	0.18
n	1122	1122	1122	1122	1122	1122	1122	1122	1122

Concurrent relevant controls are critical to obtaining useful information from clinical pathology tests.

References, resources for mouse CBC (and clinical chemistry):

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Lab 1 2

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14. Mazzaccara et al 2008. Age-Related Reference Intervals of the Main Biochemical and Hematological Parameters in C57BL/6J, 129SV/EV and C3H/HeJ Mouse Strains https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2582346/ **Isoflurane, retroorbital; EDTA; ABX Pentra 60C; pooled blood [serum], dry chemistry Vitros 250**
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23. Serfilippi et al 2003. Serum Clinical Chemistry and Hematology Reference Values in Outbred Stocks of Albino Mice from Three Commonly Used Vendors and Two Inbred Strains of Albino Mice https://pubmed.ncbi.nlm.nih.gov/19760836/ **Crl:CFW(SW) BR, Tac:(SW)fBR, HsdWin:CFW1, Crl:CD-1(ICR) BR, Tac:Icr:Ha(ICR)fBR, Hsd:ICR (CD-1), Crl:CF-1, Hsd:NSA(CF-1), FVB/NCrlBR, C57BL/6J-Tyrc-2J/+; CO2 cardiocentesis; EDTA, Bayer Technicon H1; SST, Hitachi 704**
24. Kile et al. 2003. Sex and strain-related differences in the peripheral blood cell values of inbred mouse strains https://pubmed.ncbi.nlm.nih.gov/12532271/ **Retro orbital, EDTA; ABBOT CELL DYN 3500R**

BOLD Font: Strain, collection site, anesthesia, anticoagulant, instrument
NOS = Not otherwise specified
Rev 2021 CB

‘Ball park’ organ weights… 8wo 16wo

	8wo	16wo	8wo	16wo	8wo	16wo	8wo	16wo	8wo	16wo	8wo	8wo	8wo	8wo	16wo	16wo
wt g	**BW g**	**BW g**	**Brain g**	**Brain g**	**Heart g**	**Heart g**	**KidL g**	**KidL g**	**KidR g**	**KidRg**	**LIV g**	**LIV g**	**Spleen**	**NODscid**	**Spleen**	**NODscid**
Fe g mean	20.05	24.10	0.42	0.42	0.12	0.12	0.12	0.14	0.13	0.15	1.04	1.21	0.09	0.03	0.09	0.04
M g Mean	24.69	29.91	0.42	0.41	0.14	0.16	0.18	0.23	0.18	0.22	1.31	1.45	0.09	0.03	0.08	0.03
Ind* loest g	**16.20**	**16.90**	**0.31**	**0.34**	**0.08**	**0.08**	**0.08**	**0.10**	**0.09**	**0.11**	**0.77**	**0.10**	**0.04**	0.02	**0.03**	0.03
Strain Sex	NOD F	NOD F	D2 F	C F	C F	C3 F	B6 F	B6F	B6 F	B6 F	D2 F	C3F	D2 F	F	**NOD F**	M
Ind* HIEST g	**30.80**	**39.30**	**0.48**	**0.51**	**0.20**	**0.24**	**0.23**	**0.33**	**0.23**	**0.35**	**1.75**	**1.98**	**0.12**	0.04	**0.14**	0.05
Strain Sex	NOD M	B6D2 M	NOD M	NOD F	B6 M	B6D2 M	D2 M	NOD M	NOD M	NOD M	C F	NOD M	C3 F	F	C F	F
OUTLIERS g			>.505		>.8			0.01	0.95	<.027						
			NOD F		C D2			CB F	B6 F	B6D2 M						
			8wo	**16wo**	8wo	**16wo**	8wo	**16wo**	8wo	**16wo**	8wo	**16wo**	**8wo**	**8wo**	**16wo**	**16wo**
% BW			**Brain**	**Brain**	**Heart**	**Heart**	**Lkid**	**Lkid**	**Rkid**	**Rkid**	**LIV**	**LIV**	**Spleen**	**NODscid**	**Spleen**	**NODscid**
Fe Mean %			2.09	1.76	0.58	0.57	0.59	0.58	0.65	0.61	5.20	6.41	0.42	0.15	0.38	
M Mean%			1.70	1.40	0.59	0.53	0.74	0.76	0.75	0.76	5.29	4.87	0.31	0.13	0.28	
Ind* loest %			**1.08**	**1.07**	**0.40**	**0.37**	**0.41**	**0.41**	**0.41**	**0.47**	**3.92**	**0.50**	**0.19**	0.12	**0.11**	0.11
Strain Sex			mult	CB M	C3F CBM	CB M	CB M	B6 F M	B6 F	B6 F	B6 F	C3 F	CB M	F	?	F
Ind* HIEST %			**2.53**	**2.21**	**0.79**	**0.79**	**1.04 M**	**1.12**	**1.03**	**1.15**	**8.75**	**6.74**	**0.64**	0.15	**0.54**	0.16
Strain Sex			B6F	CB F	NOD F	B6 F	CB M	NOD M	CB M	NOD FM	C F	NOD F	C3 F	M	C F	F
OUTLIERS g					<1% >4%	>.1%	<.1%	<.1%	<.1%	<.1%						
					mult	B6 M	mult	mult	mult	mult						

Adapted from https://phenome.jax.org/projects/Jaxpheno2 B6 C D2 C3 CB FVB NOD

*Ind = individual animals low & high values.

2025 JHU ME 680.712 Mouse Short Course
LAB SESSION 1

Wednesday Laboratory Sessions 1 – Start in Public Health WB506

Welcome + Instructions

Lab WB506 will have gowns, gloves, masks and tools, and worksheets.
Bring your printed lab manual or device to refer to while you are doing procedures.
Please leave food, personal items, etc. in designated area of lab room and hallway.

1. Select first mouse, and bring it to your work station.
2. As a team, conduct the full SHIRPA/CSD, then one person attempts the facial blood collection.
3. Before attempting cardiocentesis, ask a lab instructor to anesthetize the mouse. You may request anesthesia for your mouse before attempting facial bleed as well.
4. AFTER cardiocentesis, confirm death with cervical dislocation, weigh mouse, compare to your guestimate, and bring deceased mouse to designated cage/site.
5. Obtain a 2nd mouse, repeat steps 2-4.
6. Practice glucometry, fecal occult blood test, urinalysis, before or after 2nd mouse, depending on availability of equipment/space.

For Glucometry: Use anticoagulated whole blood (purple top tube)
For Fecal Occult blood test: Save few fecal pellets in Eppendorf tube or weigh-boat
For URINE Refractometry & Dipstick: Save urine in Eppendorf tube. Compare to tap water.

GRADUATE STUDENTS TAKING THE COURSE FOR CREDIT MUST complete and turn in work sheets for each lab session, Write your name clearly on EACH worksheet.
Please turn in 2 work sheets for this lab
Finally Clean up your work area, properly discarding biomaterials and sharps.

Again

1) Pair up
2) With first mouse, do SHIRPA exam, specimen collections, confirm death, weigh mouse
3) Repeat with 2nd mouse
4) Practice glucometry, fecal occult blood test, urinalysis BEFORE or AFTER 2nd mouse
5) Complete and turn in worksheets (they will be returned on Friday if you want them)
6) Clean up.

Check Box if you are a Graduate student taking ME 680.712 for credit ☐

YOUR NAME: _____

LAB 1 (Wednesday)

JWJH Modified SHIRPA Scoring Chart

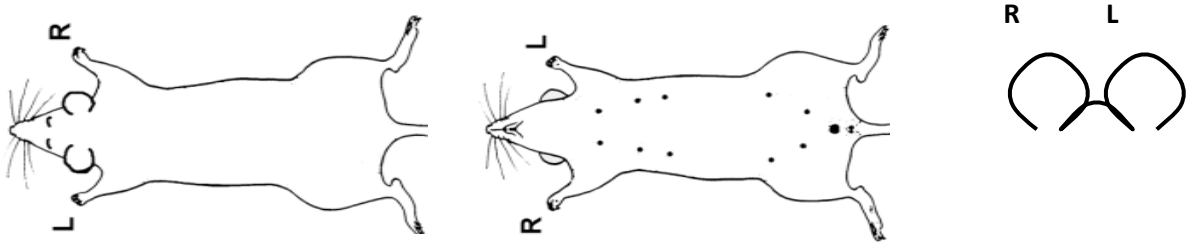
Modified from Julie Watson 2019

Scoring Key: 0=zero 1=slow or reduced **2- normal** 3=hyper

*Body Condition score: 1=emaciated 2= low body fat **3=normal** 4= excessive body fat 5=grossly obese

Animal ID/#:		Genotype	
DOB/Age:		Sex:	M F
Weight (g):		Color: Albino Black Agouti Other:	
Body Condition Score*:			
EMPTY CAGE (2 min)		Escape	Y…….**N**
Gait abnormal	Y…….**N**	Exploring	0….1…..**2**…….3
Posture abnormal	Y…….**N**		0= <1 side 1=< 1 circuit 2=multiple circuits 3=frantic
Freezing	Y…….**N**	Digging	0…..1…..**2**……3
Wild Running	Y…….**N**	Grooming	0…..1…..**2**……3
Stereotypies	Y…….**N**	Rearing	0…..1…..**2**……3

PHYSICAL EXAM **Dorsum** **Ventrum** **Tail** **Ears**



DRAW Bald patches/abnormalities ID

Bald patches	Y……..**N**	Piloerection	Y……..**N**
Physical abnormality	Y……..**N**	Whisker damage	Y……..**N**
4. Aggression	**Y**…….N	11. Rear limb withdrawal	0….1….**2**….3
5. Body Tone	0….1….**2**….3	12. Ear twitch	0….1….**2**….3
6. Petting Escape	0….1….**2**….3	13. 'Whisk' response	0….1….**2**….3
7. Passivity	0….1….**2**….3	14. Palpebral reflex	0….1….**2**….3
8. Trunk Curl	0….1….**2**….3	15. Visual Placing	**Y**…….N
9. Righting	0….1….**2**….3	16. Clicker	0….1….**2**….3
10. Forelimb placing	0….1….**2**….3	17. Grip strength	< 60 sec. **> 60 sec**

Notes: _____

Check Box if you are a Graduate student taking ME 680.712 for credit ☐

YOUR NAME: _____

LAB 2 (Wednesday) Specimen Collections

Facial Blood Collection

(www.medipoint.com)

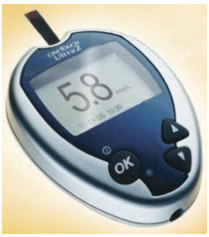
Blood Volume: _____ul

☐ Clotted 🙁 ☐ NOT clotted 😊

Post bleed mouse activity:

☐ normal
☐ slow
☐ dead

Terminal Blood Collection (Cardiocentesis)



☐ Successful ☐ Unsuccessful

Blood Volume: _____ul

☐ Clotted 🙁 ☐ NOT clotted 😊

Glucose:

☐ Accuchek _____mg/dL

☐ One Touch _____mg/dL

Which do you prefer ? ☐ Accuchek ☐ One Touch

Why ?

Fecal Occult Blood:

Control (feces + blood) turned blue ☐ ,**or** did not turn blue ☐

Test specimen (just feces) turned blue ☐**, or** did not turn blue ☐

Urine Refractometry

Specific Gravity: Tap water: _____ Urine:

Mouse Weight _____ **Guestimate** _____ **Weighing Machine** _____

Blood: _____g Total: _____g

Note 1ml of blood weighs ~1.06g)

Check Box if you are a Graduate student taking ME 680.712 for credit ☐

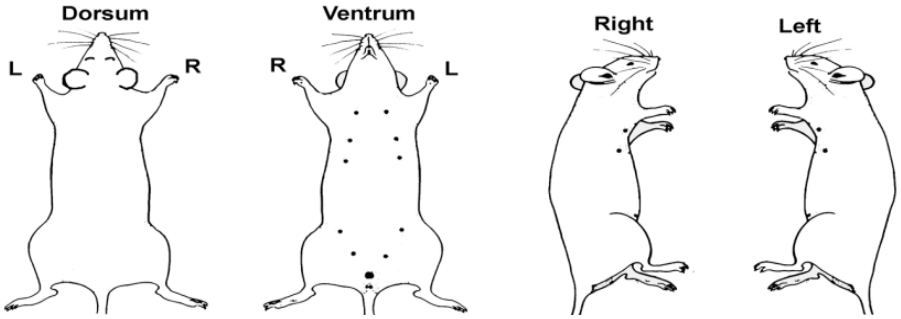
YOUR NAME: _____

Lab 3 Thursday Gross Examination (Necropsy)

Please write your name on Container (70%EtOH - not formalin)

Terminal Bleed (Cardiocentesis) Blood Volume: _____ul

Age: _____
Sex: _____
Coat color: _____
Eye color: _____
Body Weight: _____ **g**
Body Condition: _____



Gross Findings: Draw, describe; indicate size/weight when possible.

WNL	X	Wt g	Tissue / Disposition – describe abnormalities (back of page)
			Pelt on paper towel (cranial-ventral; inguinal-dorsal) - in container
			Salivary glands in cassette 4
			Thymus in **cassette 1** – note **size:** _____ (weigh if it seems large)
			Lungs infused
			Pluck removed **(mandible & pelvis split)**
			Tongue in container
			Heart* in container
			Lungs (dorsal side down), trachea, esophagus in **cassette 2**
			Adrenals in **cassette 3**
			Right kidney* (nicked) and **left kidney*** intact
			Spleen* in container (Hemisect if larger than 0.5g)
			Liver* (lobes separated) in container
			Reproductive tract - spread on paper towel - in container
			G.I. elongated, infused - in container
			Pancreas & mesentery in **cassette 5**
			Head (+/- vertebral column, **right leg**) in DECAL.
			***** **Normally heart, kidneys, spleen, liver are weighed**

'X' - Abnormalities, number the box 1,2,3,… and describe below, continue on back.

1. _____

_____

_____

_____

_____

Check Box if you are a Graduate student taking ME 680.712 for credit ☐

YOUR NAME: _____

Lab 4 Friday Tissue Trimming

Cassette	Tissue, trim procedure – Check Gross Report – Ensure trimming of any lesions
☐ 1	**Heart** – Hemisect on long axis to expose all four chambers – include both halves usually **Tongue** – X or long section ****Sternum** - Ensure inside (marrow side) down - remove XS bone/ribs ****Thymus** – already in cassette
☐ 2	****Trachea /larynx-** X or long section include **thyroid / parathyroid** ****Lungs** – Ensure Dorsal side down
☐ 3	**Right kidne**y - cross section ~midline. Include both halves when there is room; **Left Kidney** – long section Include better half (OR X section for kidney study) **Adrenals** - on foam (use special cassettes if necessary)
☐ 4	****Salivary glands** with **lymph nodes** – already in cassette
☐ 5	****Pancreas** / **mesentery** (+ Lymph nodes) – already in cassette
☐ 6	**GI Tract -** representative cross sections of duodenum, jejunum, ileum, colon U section tip of cecum with P patch; stomach section to include glandular + forestomach ☐ **Swiss rolled into cassettes at collection
☐ 7	**Liver** - **Median lobe** section to include gallbladder between left and right parts. **Left lateral lobe** section from hilus to edge **Spleen**- longitudinal section→ both halves or better half; OR intact for tiny spleen <.0.02g OR X-section(s) for giant spleen >~1g
☐ 8	Reproductive: intact OR Trim representative sections of all structures or
☐ 9	**Skin**: 3-4mm strips section sectioned parallel to hair growth. ☐ Cranial skin to include muzzle eyelid- neck ☐ Inguinal skin- to include clitoral or preputial gland, perineum **LEG Decal:** femur/knee remove non decalcified tissue e.g. foot
☐ 10	**Decal head** - Cut on caudal and rostral side of ear canal -→ section 1,2 ☐ Cut on caudal and rostral side of eyes→ sections 3,4 ☐ Cut off and discard very tip of nose; you should have five sections total ☐ First two back sections laid in cassette rostral (front) side down ☐ Final sections in cassettes caudal (rear) side down
☐ 11	**<u>Lesions (any abnormality 'X' noted in gross examination should be trimmed)</u>**