Mouse Pathobiology & Phenotyping

Short Course

2015 Lab Manual
4th edition

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* **ALL participants** and instructors should arrive promptly or early.
* **ALL participants** and instructors are expected to clean up after themselves AND to assist in clean up of their work area.

+ **Graduate students** taking this course for credit must complete worksheets & hand them in for laboratory credit. (4 completed worksheets will receive full credit)

http://www.hopkinsmedicine.org/mcp/PHENOCORE/courseCURRENT.html
How to Give a Mouse a Physical

Julie Watson
Dipl. ACLAM
Associate Professor, Molecular and Comparative Pathobiology
Director of Rodent Clinical Programs
Johns Hopkins

Background

- 1968 - Samuel Irwin - First Phenotyping Screen
  - Psychopharmacologia (Berl.) 13 222-257

- 1997 - SHIRPA stage I
  - Mamm Genome. 1997 Oct;8(10):711-

- 1997 - Jacqueline Crawley

- Current - 2015 - Websites:
  - http://empress.har.mrc.ac.uk
  - http://www.europhenome.org

EMPRESS RECOMMENDATIONS FOR OPEN FIELD

- Noise and light standardized in husbandry room (upper rack 10x lux)
- Test in first half of light cycle
- Stabilize mice 30 mins before testing in anteroom
- Standardize light levels 150 -200 lux in testing chamber

- REF: Fentrop N, Wotjak CT (2000) Fiat lux! Spotting a common experimental problem,
  Poster presented at Measuring Behavior The Netherlands 2000, 3rd international
  Conference on Methods and Techniques

SHIRPA

- SmithKline Beecham Pharmaceuticals
- Harwell, MRC Mouse Genome Centre and Mammalian Genetics Unit
- Imperial College School of Medicine at St Mary's
- Royal London Hospital, St Bartholomew's and the Royal London School of Medicine
- Phenotype
- Assessment

Goals

1. Detect abnormalities likely to affect future phenotyping tests
   - Blindness
   - Physical defects
   - Deafness

http://www.hopkinsmedicine.org/mcp/PHENOCORE/courseCURRENT.html
Some “Abnormalities” are Expected!

Age-related hearing loss & vestibular defects (<3m) Blindness (rd1 gene)
- Many 129 strains
- A/J
- C57BR, C57L
- DBA
- I, LP, NOD
- C57BL/6J @12-18m OR EARLIER!
- FVB
- C3H
- BUB
- CBA
- SJL
- SWR
- NON
- P, PL

Environmental Variation May Affect Phenotype
- Husbandry methods (see John Crabbe, Science 1999)
- Noise (e.g. on age-related hearing loss)
- Group or single housing (e.g. on aggression)
- Diet (e.g. on cancer phenotypes)
- Medications (e.g. on cancer phenotypes and gene expression)
- Light intensity (particularly for albino mice)
- Time of day related to light or dark cycle

Other Pitfalls
- How big is the gene effect compared to the background variability?
  - Background strain not inbred F1’s, N2’s, chimaeras (!) ... 2.5 years to congeneric
  - Don’t have comparable controls
- Do you have enough animals? (12 ea. WT het -/- M&F)
- Statistical analysis ? - ordinal data – non parametric
- Genotyping reliability – Transnetyx estimates HH 10% gene not present.

Assuming the phenotype is real ...
- Presence of abnormal behaviors, e.g. motor or neurological deficits
- Absence of normal behaviors
- Suggests further testing

Water Maze
(Note water not yet opaque)

Open Field

http://www.hopkinsmedicine.org/mcp/PHENOCORE/courseCURRENT.html
Behavioral Phenotyping Level 1 Screen

Accession #_________

Date________Investigator ____________________Genotype___________________________________

Background strain(s)_____________Inbred / N#___ Tg /TM  KO/KI/Cond    Gene Name_____________

Key:  0 = zero;  1 = slow or reduced;  2 = normal;  3 = hyper

Animal # WT       Hemi        -/-    Animal # WT       Hemi        -/-

DOB/Age Sex                  M     F DOB/Age Sex                   M    F

Weight (g) Condition Score

Fur color Weight (g) Condition Score

Fur color

Empty Cage  2 mins: Gait abnormal         Y  N

Posture abnormal    Y  N

Freezing                  Y  N

Wild running           Y  N

Stereotypies            Y  N

Escape                     Y  N

Exploring        0..1..

2 ..3

0= <1 side; 1 =< 1 circuit; 2= multiple circuits; 3= frantic

Digging           0..1..

2 ..3

Grooming        0..1..

2..3

Rearing           0..1..

2..3

Empty Cage  2 mins:

Gait abnormal         Y  N

Posture abnormal    Y  N

Freezing                  Y  N

Wild running           Y  N

Stereotypies            Y  N

Escape                     Y  N

Exploring        0..1..

2 ..3

0= <1 side; 1 =< 1 circuit; 2= multiple circuits; 3= frantic

Digging           0..1..

2 ..3

Grooming        0..1..

2..3

Rearing           0..1..

2..3

Notes: _______________________________________________________________________________

____________________________________________________________________________________

This is for 2 mice

SIMPLE EQUIPMENT

WEIGHT

PERTINENT INFORMATION

TESTER BLINDED TO GENOTYPE

Observation in Cage

– Gait, posture, general appearance: do the mice look as expected?

– Are normal behaviors present?

  • Exploring, thigmotaxis, digging, grooming, rearing

Observation in Cage

http://www.hopkinsmedicine.org/mcp/PHENOCORE/courseCURRENT.html
Rearing/Escape/Thigmotaxis

Digging

Subtle Deficits
Limited Rearing

Observation in Cage

– Gait, posture, general appearance: do the mice look as expected?

– Are normal behaviors present?
  • Exploring, thigmotaxis, digging, grooming, rearing

– Are abnormal behaviors present?
  • seizures, pruritus, motor deficits, stereotypies

Obvious Motor Deficits

• Picture: Nadine Forbes

Abnormal Behavior
Ulcerative Dermatitis

• Video: Nadine Forbes
Abnormal Motor Behavior: Dermatitis

Physical Exam

- Pick up, record abnormal physical features
  - Whisker loss, bald patches
    - barbering, fighting, dermatitis, parasitism
  - Unkempt haircoat, piloerection
    - sick mouse
  - Eyes, legs, tail
    - Genetic/congenital defects, fighting, parasitism

Whisker loss

Effects of Whisker Removal

- Aggression tests – decreased early withdrawal
- Decrease in flight
- Decrease in freezing

- Effect of whisker removal on defensive behavior in rats during early ontogenesis
  - Shishelova,
  - Neuroscience and Behavioral Physiology, 36 (8) Oct. 06, 883-888

Open Field
Barbering

• Usual: barbering by socially dominant mouse
  – Requires cooperation

• All mice in cage may barber if e.g. overcrowding stress

• More social barbering = less physical aggression

• Whiskers are important
  – Bitten off, not pulled out
  – Used for object & texture discrimination
  – Exploration, balance and orienting

Tests of General Reactivity

Four Tests

• Response to approach
• Body tone
• Petting escape
• Passivity

Normal Response to Approach

Normal Body Tone

High Body Tone

Petting Escape
Exacerbated Escape Attempts

Tests of Postural Reactions and Reflexes

- Trunk curl
- Righting reflex
- Forelimb proprioceptive positioning
- Rearlimb withdrawal

Passivity

Trunk Curl

Righting Reflex

Proprioceptive Positioning
Withdrawal – Slow (129)

Tests of Facial Nerve: Sensory & Motor

- Ear twitch
- Whisker response
- Palpebral reflex (V, VII)

Ear Twitch

Whisker Response

Palpebral Response

Sight: Visual Placing
Blind Mouse – Tactile Placing

Virtual Sight Test By Optokinetic Tracking

Optokinetic Tracking

http://www.cerebralmechanics.com

Visual cues

Opaque water bath

Elevated Maze

Light Dark Box

Visual Cliff

Caroline Thaung, Karen Arnold alan J. Jacksemb and Peter J. Coffey. Present visual field tracking differentiates normal sighted from retinal degenerate mice


Sight Needed for Morris Water Maze Test for Learning & Memory

Visual cues

Opaque water bath

Sight Needed for Tests of Anxiety

Elevated Maze

Light Dark Box

Visual Cliff


Sight Even Needed for Motor Performance

• C3H/HeJ mice (with retinal degeneration) compared with (Pdeb+) mice (without retinal degeneration) on the rotarod

• The sight-impaired C3H mice stayed on the rotarod longer than did their sighted Pdeb+ partners
Hearing Test

Clicker

Tests That May be Affected by Hearing Loss

Prepulse Inhibition
For sensorimotor gating

Cued Fear Conditioning
For learning and memory

Other Tests

- Provoked Aggression
- Grip Strength

Test for Provoked Aggression

Aggression

Resident intruder test

Grip Strength Normal
Grip Strength Abnormal

Grip Strength Apparatus

Images from EMPRESS

“DigGait” by MouseSpecifics

By Jacqueline Crawley

Questions?

Acknowledgements

• Janice Clements
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• Jacqueline Crawley
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• Cory Brayton
• Nadine Forbes

http://www.hopkinsmedicine.org/mcp/PHENOCORE/courseCURRENT.html
**JHJW Modified SHIRPA Summary**

This screen should take about 10 minutes per mouse and provides a basic evaluation for abnormal behaviors, and absence or reduction in normal behaviors or reflex responses. Abnormalities in this basic screen can direct more in-depth testing.

1. **Condition Score: (1-5)**
   1 = **Emaciated** vertebra distinctly segmented. Little or no flesh cover
   2 = **Thin** – segmentation of vertebra evident. Skeletal structure: dorsal pelvic bones are readily palpable
   3 = **Normal.** Mouse is well-conditioned. Vertebrae and dorsal pelvis not prominent; palpable with slight pressure
   4 = Mouse **over conditioned.** Vertebrae palpable only with firm pressure
   5 = Mouse is **obese.** Bone structure disappears under flesh and subcutaneous fat

2. **Gait abnormal (Y or N).** What is 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, loss of balance

3. **Posture abnormal? (Y or N).** What is abnormal? Body rounded or hunched, Head tilt or other head or body asymmetry, tail dragging or held rigid. Picture shows a rounded (abnormal) position.

4. **Body Tone: (0-3)** Hold the mouse by the tail base on a hard surface. With 2 fingers gently press down over the mid dorsum. Normal tone: will resist 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

5. **Petting Escape: (0-3)** Hold the mice by the tail base on a hard surface. With finger and thumb stroke down the flanks (sides) of the mouse 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

6. **Passivity: (0-3)** Hold the mouse by the tail and place front paws on the edge of the cage top. Normal mice will promptly climb up to the top of the cage. Falling off 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.

7. **Trunk Curl: (0-3)** Suspend mouse from 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 clasiing; 1 < 90\(^\circ\) curl; 2 = **curls to 90\(^\circ\) or more;** 3 = climbs up tail.

8. **Righting: (0-3)** – Hold mouse by tailbase. 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. Once the normal mouse feels your hand, it will quickly flip over so as to climb up the hand. 0 = does not right itself; 1 = struggles to right itself; 2 = **rights itself;** 3 = hyperactive.
JHJW Modified SHIRPA Summary

9. **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 is difficult test to perform, principally because the whiskers of most mice are very long and may touch without you seeing them in which case you could interpret a blind mouse as sighted.

10. **Whisker response:** *(0-3)* The vibrissae are stimulated using a ‘teased out’ cotton tipped applicator. This test can be difficult to elicit consistently because vibrissae difficult for you 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 a response; 2 = normal response; 3 = hyperactive response.

11. **Ear Twitch:** *(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 = difficult to elicit response; 2 = an obvious response; 3 = hyper repetitive response.

12. **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.

13. **Forelimb place:** *(0-3)* Hold the mouse by the tail on a hard surface. Using the wooden applicator, gently move a forelimb out to the side. The normal mouse will immediately return the limb under the body. 0 = Leg stays where placed; 1 = slow or incomplete return; 2 = Promptly returns leg to normal position; 3 = hyperactive response.

14. **Withdrawal:** *(0-3)* Hold the mouse by the tail on a hard surface. Pick up the hindfoot and pull the limb out at a 45° angle until it is stretched then let go. A normal mouse will rapidly return the hindlimb to normal position. 0 = Leg drops to ground and doesn’t return to normal position; 1 = slow to return; 2 = rapid return; 3 = hyperactive response.

15. **Biting:** *(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. This should be scored as no biting, or biting.

16. **Clicker (hearing test):** *(0-3)* Hold mouse by the tail base on a hard surface. After a moment of silence & calm, 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 = difficulty in eliciting response; 2 = immediate response; 3 = abnormal response (seizures, hyperactive escape, etc)

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

Aim: obtain blood samples from the facial vessels of a mouse.

- **Required:**
  1. 26 gauge needle, short (1/2 inch or less), or 4-5 mm lancet,
  2. Small blood collecting tube,
  3. Clean work surface,
  4. Alcohol pad,
  5. Mouse.

  ✧ **Not required:** Anesthetics.

**SURVIVAL FACIAL BLEED (CONTINUED)**

- Pick up the mouse, holding tail near its base.
- Place the mouse on the wire bars of the cage.
- Cup the free hand over the mouse, and scruff it **firmly** using the thumb and first finger.
  - **NOTE:** It is critical to hold a lot of skin; so much so that it looks like the mouse's tongue sticks out. Not so tight as to kill the mouse.
- You can tuck the tail between your last two fingers.
- You should now have the mouse gently and securely restrained in your non-dominant hand, and be able to pick the mouse up.

**SURVIVAL FACIAL BLEED (CONTINUED)**

- Locate the hairless freckle on the side of the jaw.
  - If the freckle cannot be found, draw a mental line along the lateral face at the level of the nose. Draw another mental perpendicular line down from between the eyes and ears. Where those lines intersect is the “sweet spot.”
  - Pick up the lancet or needle with your free hand.
  - **Prick.** If using a needle, go in **only** to the depth of the bevel.
  - Quickly discard the sharp into the sharps container, and pick up your collection tube.
  - Collect 4-7 drops of blood.
  - Press gently/firmly with alcohol pad to stop bleeding.
  - Release the mouse into its cage when bleeding has stopped.
**BLOOD GLUCOSE MEASUREMENT**

- **Aim:** Measure whole blood glucose using One Touch or Accuchek Glucometers
- **Required:**
  1. Small pipette,
  2. Small weigh dish,
  3. Glucose strip, (check expiration dates)
  4. Glucometers,
  5. Mouse.

**BLOOD GLUCOSE (ONE TOUCH)**

- With small pipette, place **1-2 drops** of un-clotted blood into the small weigh dish.
- Orient the glucose strip so the white bars face you.
- Press the white bar end firmly into the top slot of the meter.
- Wait 8-10 seconds until the LSD screen shows "Apply Blood".
- Touch the free end of the strip to the blood in the weigh dish.
- Record results.
- Pull strip out of slot and discard.
- The meter will turn off automatically.

**BLOOD GLUCOSE (ACCUCHEK)**

- With small pipette, place 1-2 drops un-clotted blood into the small weigh dish.
- Slide and release strip ejector (between “M” and “S”)
- Test strip will appear
- Touch free end of strip to drop of blood in weigh dish.
- Record results

**FECAL OCCULT BLOOD**

- **Aim:** detect blood in feces
- **Required:**
  1. Test Slide (Envelope) for Fecal Occult Blood,
  2. Smearing Stick,
  3. Developer,
  4. Feces.

**AKA: guaiac test**

- When hydrogen peroxide (developer) is dripped on to guaiac paper, it oxidizes alpha guaiaconic acid to a blue colored quinone.
- Heme catalyzes, accelerates reaction → rapid change

- Retrieve 2 soft fecal pellets from cage.
- Touch 1 pellet to blood in the weigh dish (= ‘positive’ for this demonstration).
- Open Hemoccult envelope (side that reads “For in vitro Diagnostic Use”).
- Use wooden applicator to smear both pellets onto circles I and II.
- Close envelope, Wait 2 minutes.
- Open the back of the envelope and apply 2 drops developer on each smear.
- Rapid change to Blue is a positive result, indicating presence of blood (heme).
- No or very slow change indicates absence of blood/heme.
**Fecal Occult Blood**

<table>
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<tr>
<th>Positive</th>
<th>Negative</th>
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<tbody>
<tr>
<td>Blue</td>
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</table>

**Terminal Bleed**

**AIM:** Terminal maximal blood collection by cardiocentesis

- **Required:**
  1. Anesthetic,
  2. 1cc 26G syringe/needle,
  3. Blood tubes
    - With anticoagulant for CBC, plasma etc,
    - Eppendorf, plain tube, or gel separator tube for serum
  4. Mouse.

**Terminal Bleed**

- Anesthetize mouse.
- Orient animal ventrum (belly) up with nose toward your non-dominant side.
- With non-dominant hand, brace the animal; abduct (spread) forelimbs for maximum exposure of thorax.
- With dominant hand, feel xiphoid process; keep eyes fixed on that spot.
- Insert 26G 1cc needle/syringe just left of xiphoid at about 30° angle, aiming toward the dorsal neck.
- Pull back slightly on the plunger. If blood is absent, reposition needle/ syringe slightly (in/out, side to side) WITHOUT pulling completely out of chest.
- As blood enters syringe continue pulling back gently.
- Depending on the size of the mouse, you should be able to remove 500-1000ul of blood, sometimes more.
  - Mouse blood clots quickly (within 15-20 seconds).
- Remove needle and gently eject blood into Eppendorf tube.

**Urinalysis**

**Aims:**

1. Collect mouse urine.
2. Measure urine specific gravity.
3. Test with dipstick (chemstrip).

- **Required:**
  1. Veterinary refractometer,
  2. 96 well microtiter plate, clean,
  4. Mouse in container (opaque & small is good).
IN CONCLUSION 1

- You can get good data from inexpensive tests.
- Which glucometer do you prefer?
  - Accuchek? Your result: 
  - One touch? Your result: 
- Why? ________________.
- Which method do you prefer for urine SG?
  - Refractometer Your result: ___.
  - Chemstrip Your result: ___.
- Why? ________________.

IN CONCLUSION 2

- **Weighing** was not included in this lab for practical reasons, but is another source of good inexpensive data.
- Which procedures are useful to your studies?
  - Growth curves (from body weights )
  - Bleeding
  - CBC
  - Chemistry
  - Urinalysis
  - Fecal Occult blood
  - Other: _____
Mouse Necropsy / Autopsy

1. Equipment and materials ................................................. Page 1
2. Necropsy Procedure .................................................. Page 3
3. Tissue trimming ........................................................ Page 5 + Table 1
4. Histopathology .......................................................... Page 5 + Table 2
5. Reporting and Archiving Data and Specimens .................. Page 5
6. General References & Links ........................................... Page 6

Figure 1: Body Condition (BC) assessment
Table 1: Cassette (slide) numbering, tissues and trimming suggestions
Figures 2,3: Mouse head, landmarks for trimming
Figure 4: Mouse Lymph Nodes
Figure 5: Mouse Mammary Glands
Table 2: Histopathology evaluation, by slide number and tissues
References

Necropsy (viewing the dead) refers to the post mortem examination of organs and tissues (autopsy = self + view). A useful technique should efficiently evaluate all organs and lesions, and be teachable and reproducible. A checklist can help to ensure that all procedures are performed (e.g., photography, radiography, and gross necropsy), and that all tissues are examined, weighed or measured. There are many necropsy techniques. Variations can be justified by specific aims or requirements of a diagnostic procedure or research study. However a standardized systematic procedure, to which minor variations can be made, can improve comparisons within and between studies, even when initial examinations are months or years apart. [1] [2]

Strategic use of pathology in research settings can include clinical and anatomic pathology to:

1. Assess disease problems, including decreased production, in breeding colonies;
2. Assess disease problems in research animals during studies;
3. Confirm and characterize phenotypes or other research endpoints;
4. Characterize and validate translational research models

Equipment and Materials

1. Protective Equipment
   a. Workstation – a ventilated workstation such as a down-draft table or a fume hood to protect the prossector (person performing the necropsy) from fixative fumes or other hazardous materials.
   b. Eye protection - glasses or goggles to protect the eyes from splashes with fixative, or other potentially hazardous materials. Magnifying reading glasses may facilitate dissection and examination of small specimens.
   c. Face protection - face masks or respirators to protect sensitive individuals from mouse allergens. An appropriately ventilated downdraft table or hood provides good protection also.
   d. Gloves – Essential - when multiple animals will be examined, hand lotion and double gloving may work better than frequent glove changes. When the top gloves are damaged or soiled, replace only the top gloves, to minimize exposure of hands to drying or contaminating materials. Latex gloves are common. Vinyl gloves are useful for individuals with latex allergies.
   e. Lab coats or other apparel to protect skin and clothes from fixatives and contaminating materials.
2. **Cutting board** - An inexpensive, plastic cutting board is adequate for most purposes. It should be relatively easy to clean and able to withstand frequent use. Some necropsy methods use pins and soft or porous cutting boards. Usually these cannot be cleaned or decontaminated as easily as plastic, and can be quite expensive.

3. **Paper towels** - Many tissues (e.g., skin and reproductive tract) can be laid flat on a paper towel, and will adhere to it to facilitate examination and ensure uniform fixation.

4. **Small metric ruler** - A ruler (or ruled label) should be included in photographs to facilitate subsequent measurements. It should be located so it can be cropped out of the image, should that be necessary for publication. When a finding or organ is reported as being small or large, it should be measured and/or weighed. Mass lesions and organs with three dimensions should have measurements recorded for three dimensions. For example, a spot may be 2x3 mm, a mass may be 2x3x2 mm.

5. **Forceps** - Blunt-ended, serrated, or toothed forceps seem to cause less damage to delicate mouse tissues. Fine-pointed forceps can create artifactual holes or tears. Smooth forceps can require extreme (and damaging) pressure to grip slippery tissue.

6. **Scissors** - Fine, blunt-ended scissors seem to cause less damage. Sharp-tipped corneal scissors are common but can make holes or tears, especially in inexperienced hands.

7. **Scalpel blades, single edge blades** - Blades usually are not necessary for initial mouse dissections. But sharp, fresh blades are essential for trimming tissues for histology processing, to ensure a flat tissue surface in the paraffin block, to facilitate sectioning (See trimming section below). Inexpensive single-edge blades may work as well or better than scalpel blades and handles.

8. **Syringe and needle** - A 3-ml syringe with 20-22-gauge needle to infuse the lungs and gastrointestinal tract with formalin. Smaller needles can be used, but may penetrate more deeply than expected, injecting material outside the targeted lumen. Shorter needles (less than 1 inch or 2 cm long) are easier to handle.

9. **Fixative** - For immersion fixation for paraffin processing, dissected tissues should be submerged promptly in fixative, at 1:10 V:V proportions, i.e., 1 ml of specimen per 10 ml of fixative. Rocking or agitation of specimens in the container can improve exposure of tissue to fixative. 10% Neutral buffered formalin (10%NBF) is suitable for soft tissues in most situations. Other options are discussed elsewhere.[1] [3] [4]

10. **Decalcifying (demineralizing) solution** - Boney tissues (e.g., head, spine, legs) can be decalcified quite easily for histology processing and evaluation. Some formic acid based solutions fix and demineralize simultaneously, so that mouse tissues are fully fixed and adequately demineralized for paraffin processing within 24 hours. Bouin's solution also fixes and demineralizes but does not penetrate very deeply. Specimen size, exposure time, or perfusion methods may need to be modified to optimize fixation and demineralization of different types of specimens. Once adequately demineralized, tissues should be processed promptly because overexposure to acids or chelators will compromise tissues for evaluation or other tests. Immunohistochemical techniques may or may not work on demineralized tissues.

11. **Tissue cassettes** - For paraffin processing. Cassettes can be compartmented, extra deep, extra large, or have extra fine grid for special needs.

12. **Specimen containers with labels**.

13. **Identification** - Redundant identification IN and ON containers is recommended.

14. **Camera** - To document findings.
Necropsy Procedure
Ideally, the animal should be evaluated immediately after death. If delay is unavoidable, refrigeration (not freezing) can slow tissue decomposition. Carcasses and other research materials should be held in designated refrigerators (i.e. not refrigerators that are used for food for animals or personnel). Freezing is discouraged, because histopathology will be compromised. Various mouse necropsy techniques are published. A systematic reproducible technique that serves the needs of the project or program should be developed. Following is a protocol for external and internal examination, *en bloc* removal of viscera, followed by further dissection, examination, organ weights.

External examination
1. **Sex** (presence, absence or abnormalities of nipples, external genitalia);
2. **Weight** (body weight in grams, note if before or after bleeding, record the bleed site and volume of blood removed);
3. **Coat color** (albino, agouti, black, other); and condition (alopecia, ulcers, note distribution and approximate area of involvement);
4. **Eye color** (albino, pink, black; also note size and symmetry);
5. **Body condition**, (e.g., thin, adequate or good body condition, or obese, or semiquantitative scoring as in Figure 1)
6. **External lesions ‘dysmorphology’** (e.g., domed head, microphthalmia, masses, open wounds, reduced or extra digits) should be described and measured.
7. **Palpate** for pups or other abdominal masses, or abdominal fluid. A sterile fluid sample can be obtained with a needle and syringe for chemistry (BUN, protein), cytology, or microbiology evaluation. The consistency of any palpated masses should be described as soft or fluctuant, firm or hard. ‘Hard’ should be reserved for boney or mineralized masses.

Dissection
Begin the examination by always orienting animals in the same direction (e.g., head up or head right) so that the side of the lesion can be recalled accurately. Preference may be influenced by handedness of the prosector. Weigh organs. Record lesions or unusual findings. Note color, consistency, size and or weight. Avoid fruit or vegetable descriptors. Specific steps in dissection follow:
1. **Remove the pelt**: this facilitates assessment of subcutaneous fat (minimal, adequate, or abundant), and reveals subcutaneous lesions and abdominal organs *in situ*: Incise the ventral abdominal skin and exert gentle pressure cranially and caudally until the pelt has been removed. Examine skin and animal.
2. **Remove the “chain” of salivary glands**: parotid, sublingual, submandibular salivary glands, and lymph nodes that extend from ear to ear under the chin. These usually fit, *in toto*, in one cassette. See Table 1, Fig 4.
3. **Open the abdomen**, xiphoid to pubis, and examine the contents *in situ*.
4. **Remove the sternum**: lift it by the xiphoid process and cut anteriorly through ribs and clavicles, to expose the thoracic cavity. Examine the contents, noting fluid or masses, and absence or enlargement of the thymus. Save and fix the sternum, laid flat, for histology.
5. **Expose the trachea** by blunt dissection and use the 3-ml syringe/21-gauge needle to *infuse the lung* with fixative. The lungs should expand fully, and excess fixative will reflux up the trachea. Difficulty in infusion may be due to inflammatory or neoplastic processes. It is not necessary to clamp or tie the trachea. After infusion, it is important not to compress the lungs during subsequent dissection.
6. **Cut the mandibular symphysis** with a scissors to separate mandibular rami and expose the tongue. Grasp the tongue with forceps or fingers, and gently retract caudally to remove the tongue, larynx, trachea, and esophagus from the head and neck. Continue retracting to remove the heart, thymus,
and lungs, with aorta, esophagus and trachea, from the thorax. Use blunt dissection with the scissors to free these tissues. Examine the oral cavity.

7. **Examine the removed viscera (tongue to diaphragm).** The thyroid glands are immediately caudal to the larynx on either side of the trachea. They may be difficult to see without magnification, but thyroid glands usually are included if 2-4 mm of trachea immediately caudal to the larynx is examined by histology.

8. **Split the pelvis** to facilitate complete removal of abdominal contents: insert closed scissors into the pelvic canal and open them gently to separate the halves of the pelvis, usually along the pubic symphysis.

9. **Remove and examine the abdominal contents:** Grasp the diaphragm with the forceps, cut at its deepest extent, and retract gently to lift out all of the abdominal contents together. The adrenal glands and kidneys tend to remain, deep in the retroperitoneal space; blunt dissection (scissors) may facilitate removal. Abdominal contents can be examined individually *ex situ*, organs dissected weighed, and any abnormalities recorded.

10. **Dissect off liver and spleen** for examination and weighing. A small liver lobe usually is attached to the lesser curvature of the stomach. Be certain to capture and include this lobe to ensure accurate liver weights. When manipulating the liver, lift it gently, or grasp parts that will not be submitted for histology (e.g., diaphragm or smaller lobes). The median and left lateral lobes are the largest, and usually selected for histology, unless lesions are evident in other lobes, or if experimental protocol dictates otherwise. The gall bladder lies between the 2 'halves' of the median lobe. Lobes should be separated for fixation. The spleen should be dissected free and weighed intact, less fat and attached tissues. Usually it is fixed intact and trimmed after fixation.

11. **Dissect off kidneys and adrenals** for examination and weighing. The right kidney and adrenal should be anterior to the left. Female adrenal glands normally are larger than male adrenal glands

12. **Dissect off reproductive tract.** Tissue caudal to the kidneys that is not gastrointestinal tract, is mostly reproductive tract and fat. Remove all of this. Lay it flat on a dry piece of paper and spread the tissues into anatomic orientation to facilitate examination, fixation and histology. The paper with attached tissue can be submerged in fixative.

13. **Infuse and extend the gastrointestinal tract:** Infuse different segments with 0.5–1.0 ml of formalin fixative via 3-ml syringe/21-gauge needle, 0.5–1.0 ml of formalin, aiming to fix and preserve the contents and mucosa *in situ*. This can be done earlier in the dissection procedure also. Extend the GI tract gently by grasping the stomach in one hand and the rectum (fecal balls) in the other, and separate gently to break the mesenteric attachments. Dissect off the mesentery, lymph nodes, and pancreas. These pale, soft tissues may be difficult to distinguish grossly, but can be placed into a cassette and fixed for histology examination (Table 1).

14. **Submerge all tissues to be saved in at least 10x their volume of fixative.** Soft tissues and bones can be trimmed for histology processing as early as 20 hours after dissection.

15. **Bone** must be decalcified (demineralized) for routine histology processing in paraffin. Skin and other soft tissues should be removed so that the solution can penetrate the bone.  Sternum amy not require decalcification to permit evaluation of marrow. Morphology of fixed but not decalcified marrow usually is far better than decalcified marrow. Formic acid based fixing/decalciifying solutions can demineralize most mouse tissue satisfactorily within 24 hours. Overexposure to the acid will digest the tissue and compromise evaluation. With new formulations, evaluate different periods of exposure and handling to determine the best protocol. Similar to fixation, the ratio of tissue to solution should be approximately 1:10, the tissue covered completely by the solution, and gentle agitation or rocking may improve results.

16. **Photography** – Digital images become part of the data, and can be a key part of the publication. Inexpensive digital cameras can produce publication quality images. Even camera phones or flat
bed scanners can be useful for documentation. Short video clips of abnormal movements or behaviors can be very useful. Images should be planned and composed to facilitate comparisons within and between studies, permit measurements (include a ruler), and include identification for documentation. Orientation of the animal or specimens should be consistent, and should make sense anatomically. Images should illustrate the lesion and not horrify viewers. Identification or labeling is important for images, as it is for any other data.

**Trimming fixed tissues for histology (Table 1)**

During dissection, several tissues can be placed directly in cassettes and submitted for histology processing as is. These can include lung, thyroid, trachea, salivary glands, lymph nodes, pancreas, reproductive tract (if small). Other tissues must be “trimmed” further and placed into histology cassettes for processing. Trimming should be performed in a well-ventilated area or hood. Used fixative should be discarded as a hazardous waste. After trimming, labeled cassettes should be submitted in clean formalin. Once fixed, tissues to be saved can be stored in clean fixative sufficient to keep them moist, in sealable bags or other suitable containers.

During trimming, tissues should be cut with a single clean swipe of a sharp blade, not squished or sawed. Inexpensive single-edge blades are suitable for trimming most tissues, and they should be replaced as soon as they become dull so that tissue is not damaged. Trimmed specimens should be less than 3mm deep to fit into standard cassettes without generating grid marks and “squish artifact.” For decalcified skulls, trimming may be facilitated by use of Weck blades, which have a longer sharper cutting. Decalcified tissue should be soft and easily cut. Crunchy tissue requires additional decalcification. Decalcified specimens should be rinsed in water, and cassettes can be kept for short periods in buffered saline until histology processing.

Numbering of cassettes should be systematic to facilitate retrieval of specific tissues from archived material. A regular graphite pencil remains the usual marker of choice because many inks are removed by alcohols in paraffin processing. (Table 1) summarizes a numbering system in a 10 slide protocol, and summarizes trimming recommendations for each tissue and cassette. Additional cassettes can be prepared to include lesions identified during dissection.

**Histopathology**

Table 2 summarizes common histology findings and lesions by slide number and tissue. Diagnostic criteria and terminology for gross findings and for histopathology findings should be standardized to facilitate comparisons within and between studies. Anatomy and pathology terminology should be consistent with widely used and published systems to facilitate communication and publication. [1] [2] Figures 4 and 5 include referenced nomenclature for lymph nodes and mammary glands respectively.

**Reporting and Archiving Data and Specimens**

Data handling and reporting strategies vary with the resources and goals of the program or project. Paper copies of reports with a checklist for each system or tissue may be sufficient for some projects. Databases, servers and specialized hardware and software may be key to the success of large projects and multidisciplinary and collaborative efforts. Systems and strategies to handle and report pathology data and specimens should aim to preserve and protect data and specimens, to make them accessible for further evaluation, and to facilitate comparisons within and between studies. Pathology data frequently includes many large images that require significant data handling capabilities, and server space. Still images can exceed 5MB, virtual microscopy images can exceed 400MB. Archiving of specimens, including fluids, wet tissues, frozen tissues, paraffin blocks, glass slides, and virtual (digital) slides also can require significant planning for preservation methods, identification, storage methods and capacity, and retrieval. [1]
General References for Mouse Biology, Pathology and Histology


On line resources


8. Treuting’s virtual / digital slides corresponding to Treuting & Dintzis 2012. [http://repository.aperio.com/c/2012/](http://repository.aperio.com/c/2012/)


Figure 1. Body Condition (BC) assessment from Ullman & Foltz 1999.[9]

BC 1
Mouse is emaciated.
- **Skeletal structure extremely prominent; little or no flesh cover.**
- **Vertebrae distinctly segmented.**

BC 2
Mouse is underconditioned.
- **Segmentation of vertebral column evident.**
- **Dorsal pelvic bones are readily palpable.**

BC 3
Mouse is well-conditioned.
- **Vertebrae and dorsal pelvis not prominent; palpable with slight pressure.**

BC 4
Mouse is overconditioned.
- **Spine is a continuous column.**
- **Vertebrae palpable only with firm pressure.**

BC 5
Mouse is obese.
- **Mouse is smooth and bulky.**
- **Bone structure disappears under flesh and subcutaneous fat.**

* A "+" or a "-" can be added to the body condition score if additional increments are necessary (i.e. ...2+, 2-, ...)*
<table>
<thead>
<tr>
<th>Cassette #</th>
<th>Tissues</th>
<th>Trimming suggestions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>♥Heart</td>
<td>Heart: Hemisect (cut in half, longitudinally) to expose all the chambers and valves. The right ventricle has a thinner wall than the left ventricle and may wrinkle slightly when pressed. For some purposes, multiple transverse (cross)-sections may be preferred.</td>
</tr>
<tr>
<td>Thymus</td>
<td>Thymus: both lobes usually can be included intact; or section and place flat slide down. Note its absence if it is not found.</td>
<td></td>
</tr>
<tr>
<td>Tongue</td>
<td>Cross section or longitudinal section flat side down</td>
<td></td>
</tr>
<tr>
<td>Diaphragm</td>
<td>Short strips can be sectioned on edge</td>
<td></td>
</tr>
<tr>
<td>Sternum</td>
<td>Place intact, deep or internal side down in cassette, to section to narrow easily; trim off boney parts of ribs to facilitate sectioning</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Lung - entire</td>
<td>Dorsal side down – can be put in cassette during initial dissection</td>
</tr>
<tr>
<td>Trachea</td>
<td>Cross section at thyroid, or include intact for longitudinal section</td>
<td></td>
</tr>
<tr>
<td>Esophagus</td>
<td>Include intact for longitudinal section</td>
<td></td>
</tr>
<tr>
<td>Thyroid, parathyroid</td>
<td>Transect trachea at level of thyroid, or trim to evaluate lesions. Special dissection (with dissecting microscope) and use of special cassettes are necessary for more specific evaluation of small tissues.</td>
<td></td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>Mediastinal nodes or small pieces of thymus may be attached to thoracic viscera</td>
<td></td>
</tr>
<tr>
<td>Aorta</td>
<td>Can be included attached to thoracic viscera, or dissected off and included separately</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Kidneys</td>
<td>Usually can include 2 sagittal sections of left kidney plus 2 cross sections of right kidney</td>
</tr>
<tr>
<td>Adrenal glands</td>
<td>Small or important adrenals can be preserved by special cassettes (with smaller holes), sponges, or tea bags.</td>
<td></td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>Frequently included with pararenal fat and adrenals. See Fig 4.</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Salivary glands with lymph nodes</td>
<td>Paired parotid, sublingual and submandibular salivary glands (with attached lymph node See Fig 4.) can be removed in toto by dissecting them off underlying tissue, from one ear canal to the other. They usually fit intact in one cassette.</td>
</tr>
<tr>
<td>Exorbital lacrimal glands</td>
<td>Sometimes included when salivary glands are dissected off in toto</td>
<td></td>
</tr>
<tr>
<td>Auditory sebaceous glands</td>
<td>Sometimes included when salivary glands are dissected off in toto</td>
<td></td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>Usually included when salivary glands are dissected off in toto. See Fig 4.</td>
<td></td>
</tr>
<tr>
<td>Mammary glands</td>
<td>Often included in female mice when salivary glands are dissected off. See Fig 4,5.</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Pancreas</td>
<td>During initial dissection, strip fat, pancreas, mesentery and lymph nodes, from the GI tract to include in this cassette. These tissues can be difficult to distinguish grossly but are readily identified microscopically.</td>
</tr>
<tr>
<td>Lymph nodes, fat, vasculature</td>
<td>Usually included in this section – See Fig 4</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Stomach</td>
<td>Section to include forestomach and glandular stomach</td>
</tr>
<tr>
<td>Small intestine</td>
<td>Include cross sections or segments according to your needs or histologist’s preferences</td>
<td></td>
</tr>
<tr>
<td>Cecum, colon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 a, b, c</td>
<td>Swiss roll Open</td>
<td>The entire intestine can be opened, examined, fixed, and rolled into 1 or 2 deep cassettes</td>
</tr>
<tr>
<td>6 a, b, c</td>
<td>Swiss roll Closed</td>
<td>Alternatively intact (closed) small intestine can be rolled into 1 cassette, Large intestine can be rolled into 2nd cassette; cecum and stomach can be sectioned into a 3rd cassette.</td>
</tr>
<tr>
<td>Cassette #</td>
<td>Tissues</td>
<td>Table 1: Trimming suggestions</td>
</tr>
<tr>
<td>-----------</td>
<td>---------</td>
<td>------------------------------</td>
</tr>
<tr>
<td>7</td>
<td>Liver</td>
<td>Section through left lateral lobe, hilus to periphery; section through median lobe to include gall bladder; include lesions in any lobes.</td>
</tr>
<tr>
<td></td>
<td>Gall bladder</td>
<td>Should be included in the section of median lobe; can be sectioned separately if enlarged.</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>A small spleen may be included intact; a larger spleen can be hemisected along its long axis, and one or both halves evaluated.</td>
</tr>
<tr>
<td>8 Female</td>
<td>Uterus Ovaries Vagina</td>
<td>Reproductive tract can be fixed flat and intact on a piece of paper. A small reproductive tract can be included intact in the cassette after fixation. For larger tracts or for lesions, cross sections or segments of different regions should be included in the cassette.</td>
</tr>
<tr>
<td></td>
<td>Urinary bladder</td>
<td>Often included in sections when the entire tract is included in the cassette; or can be included separately.</td>
</tr>
<tr>
<td>8 Male</td>
<td>Testes Epididymis Seminal vesicle + coagulating glands</td>
<td>Reproductive tract can be fixed flat and intact on a piece of paper. A small reproductive tract can be included intact in the cassette after fixation. For larger tracts or for lesions, cross sections or segments of different regions should be included in the cassette.</td>
</tr>
<tr>
<td></td>
<td>Prostate</td>
<td>Often included in sections when the entire tract is included in the cassette; or lobes can be dissected and fixed separately.</td>
</tr>
<tr>
<td></td>
<td>Urinary bladder</td>
<td>Often included in sections when the entire tract is included in the cassette; or can be included separately.</td>
</tr>
<tr>
<td>9</td>
<td>Skin</td>
<td>Cut 4mm diameter ribbons of flat, fixed skin, parallel to hair growth and to long axis of mouse, to include any lesions, or areas of interest, including facial skin, clitoral or preputial glands.</td>
</tr>
<tr>
<td></td>
<td>Mammary glands</td>
<td>Usually included with female skin sections; or mammary pads can be harvested and evaluated specifically.</td>
</tr>
<tr>
<td></td>
<td>Preputial or clitoral glands</td>
<td>Sebaceous glands (2 lobes) in inguinal subcutis, near genital orifices.</td>
</tr>
<tr>
<td></td>
<td>+/- Decalcified leg</td>
<td>May be included in this cassette also. Trim tissue from medial aspect so that the femur can be seen on the flat (cut) surface.</td>
</tr>
<tr>
<td>10</td>
<td>Decalcified Head</td>
<td>Section systematically, using similar anatomic landmarks Fig 2,3, and consistent orientation to achieve consistent comparable sections between cases. External ear canal openings and the eyes are useful landmarks. Holding the 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 a section that includes cerebellum, medulla 2) cut just rostral to ear canal for section to include middle ear, internal ear, or both; pituitary, thalamus, hippocampus. 3) cut just caudal to eyes for section with cerebrum, usually hippocampus, thalamus 4) cut just rostral to eyes for section with eyes, Harderian glands, oral cavity, molars 5) Nose section should be included also. In long nosed mice, the tip of the nose may need to be removed to fit into the cassette.</td>
</tr>
<tr>
<td>11</td>
<td>Decalcified spine</td>
<td>Cervicothoracic and lumbosacral spine segments (with muscle, vertebrae, spinal cord) usually can be accommodated in a total of two cassettes. Cross-sections can be cut at anterior (or both) ends of each segment, then from the intervening segment, tissue can be cut cleanly from one side to the level of vertebral bone, to provide a flat surface on the paraffin block, for sectioning into deeper tissues including spinal canal and cord</td>
</tr>
<tr>
<td>12 etc</td>
<td>Lesions:</td>
<td>Trim lesions to include adjacent normal issue for perspective and context, to reflect gross measurements and photographs, and correlate to gross findings.</td>
</tr>
</tbody>
</table>
**Figure 2:** Mouse head, anatomic landmarks, and sectioning decalcified specimens

Adapted from Paxinos & Franklin 2001 [10]  

**Figure 3.** 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.
**Figure 4.** Mouse Lymph Nodes (adapted from Van den Broeck, et al. (2006).[12]

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

**Figure 5.** Mouse Mammary glands (adapted from Dunn 1951[13] &/or Cloudman 1936,[14] 1941[15])

**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
Table 2: Histopathology evaluation, by slide number and tissue.

<table>
<thead>
<tr>
<th>Slide #</th>
<th>Tissues</th>
<th>Common features and lesions to look for on histology:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><strong>Heart</strong></td>
<td>Enlarged chambers, thickened walls, myofiber degeneration loss or hypertrophy, inflammation, fibrosis, mineralization, thrombi</td>
</tr>
<tr>
<td></td>
<td>Thymus</td>
<td>Two lobes? Distinct medulla and cortex, apoptosis or necrosis. Large: neoplasia? Hyperplasia or inflammation. Small: lymphoid depletion or hypoplasia (as in nude or scid mice). Cysts, ectopic thyroid or parathyroid.</td>
</tr>
<tr>
<td></td>
<td>Tongue</td>
<td>Inflammation, mineralization, myofiber changes in muscular dystrophy or other myopathy.</td>
</tr>
<tr>
<td></td>
<td>Diaphragm</td>
<td>Myofiber changes in muscular dystrophy or other myopathy.</td>
</tr>
<tr>
<td></td>
<td>Sternum</td>
<td>Marrow: cellularity, relative myeloid and erythroid contributions, neoplasia. Muscle: myofiber changes.</td>
</tr>
<tr>
<td>2</td>
<td><strong>Lung - entire</strong></td>
<td>Inflammation (pneumonia), infection, neoplasia.</td>
</tr>
<tr>
<td></td>
<td>Trachea</td>
<td>Inflammation primarily.</td>
</tr>
<tr>
<td></td>
<td>Esophagus</td>
<td>Inflammation primarily.</td>
</tr>
<tr>
<td></td>
<td>Thyroid, parathyroid</td>
<td>Hyperplasia, neoplasia, ectopic thymus, inflammation.</td>
</tr>
<tr>
<td></td>
<td>Aorta</td>
<td>Atheromatous lesions, aneurysms, inflammation or mineralization in susceptible mice.</td>
</tr>
<tr>
<td>3</td>
<td>Kidneys – right/cross, left /long</td>
<td>Hydronephrosis, nephropathy, amyloid, inflammation, mineralization.</td>
</tr>
<tr>
<td></td>
<td>Adrenal glands</td>
<td>Inflammation, neoplasia, cortical nodules, X zone vacuolation in females, subcapsular cell hyperplasia and pigment (ceroid) laden cells in old mice</td>
</tr>
<tr>
<td></td>
<td>Lymph nodes</td>
<td>Inflammation, hyperplasia (reactive), hypoplasia, atrophy or depletion, neoplasia.</td>
</tr>
<tr>
<td>4</td>
<td>Submandibular glands</td>
<td>Larger in male with more acidophilic ductules, Inflammation, neoplasia, atrophy, cysts.</td>
</tr>
<tr>
<td></td>
<td>Sublingual salivary glands</td>
<td>Inflammation, neoplasia,</td>
</tr>
<tr>
<td></td>
<td>Parotid salivary glands</td>
<td>Inflammation, neoplasia, amyloid.</td>
</tr>
<tr>
<td></td>
<td>Exorbital lacrimal glands</td>
<td>Inflammation, neoplasia,</td>
</tr>
<tr>
<td></td>
<td>Auditory sebaceous glands</td>
<td>Inflammation, neoplasia,</td>
</tr>
<tr>
<td></td>
<td>Lymph nodes</td>
<td>Lymphadenomegaly due to inflammation, hyperplasia, neoplasia; Small lymph nodes e.g. due to atrophy or depletion, or hypoplasia (as in nude or scid mice).</td>
</tr>
<tr>
<td></td>
<td>Mammary glands</td>
<td>In females: Inflammation, hyperplasia, galactorrhea (milk production), neoplasia.</td>
</tr>
<tr>
<td>5</td>
<td>Pancreas exocrine</td>
<td>Adequate and uniform zymogen distribution, zymogen depletion, exocrine atrophy or loss, neoplasia (rare)</td>
</tr>
<tr>
<td></td>
<td>Pancreas endocrine</td>
<td>Islet inflammation (insulitis), degeneration (as in some diabetes models), hyperplasia (especially in fat mice), neoplasia (rare)</td>
</tr>
<tr>
<td></td>
<td>Lymph nodes</td>
<td>As above.</td>
</tr>
<tr>
<td></td>
<td>Mesentery vasculature</td>
<td>Arteritis, periarteritis.</td>
</tr>
<tr>
<td>Slide #</td>
<td>Tissues</td>
<td>Table 2: Common features and lesions to look for on histology:</td>
</tr>
<tr>
<td>--------</td>
<td>---------</td>
<td>---------------------------------------------------------------</td>
</tr>
</tbody>
</table>
| 5 cont | Fat white and or brown | Adequate, atrophy small cells, hypertrophy large cells (as in fat mice)  
Fat necrosis, mineralization, inflammation,  
Inflammation with or without bacteria, often associated with injection |
| 6 | Forestomach  
Glandular stomach | Inflammation, erosion, ulceration, hyperplasia, neoplasia  
Expected (friendly) bacteria Enterococcus or Lactobacillus-like,  
Superficial yeasts (Kazachstania sp) vs Cryptosporidium muris (in glands) |
| | Duodenum | Inflammation, erosion, ulceration, hyperplasia, neoplasia  
Giardia muris, Spironucleus muris |
| | Jejunum | Inflammation, erosion, ulceration, hyperplasia, neoplasia  
Cestodes, coccidia |
| | Ileum, | Inflammation, erosion, ulceration, hyperplasia, neoplasia  
Cryptosporidium parvum |
| | Cecum | Inflammation, erosion, ulceration, hyperplasia, neoplasia  
Nematodes: pinworms, flagellates, entamoebae |
| | Colon | Inflammation, erosion, ulceration, hyperplasia, neoplasia  
Nematodes: pinworms, flagellates, entamoebae |
| | Rectum | Prolapse? Inflammation, erosion, ulceration, hyperplasia, neoplasia  
Nematode larvae in crypts? |
| 7 | Liver (L Lateral+ Median Lobe/) | Inflammation, neoplasia, hepatocyte vacuolation (lipid, glycogen, etc.) degeneration, pigment (usually hemosiderin or bile), hepatocyte degeneration or necrosis, with syncytia or inclusion bodies, hepatocyte hypertrophy, anisocytosis, anisokaryosis, biliary hyperplasia, cholestasis |
| | Gall bladder | Inflammation, erosion, ulceration, hyperplasia, neoplasia  
Hyalinosis or 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 | Uterus | Inflammation (pyometra or metritis), hyperplasia – cystic?, neoplasia  
Embryos, Implantation sites, atrophy: pigment, mineralization |
| Female | Ovaries | Normally Developing follicles, corpora lutea, atretic follicles, oviduct  
Neoplasia, cysts, Atrophy, pigment, amyloid |
| | Vagina | Inflammation, erosion, ulceration, hyperplasia, neoplasia  
Hyperkeratosis, granulocytes compatible with cycle? |
| | Urinary bladder | Inflammation, erosion, ulceration, neoplasia |
| 8 | Testes | Active spermatogenesis? reduced spermatogenesis, degeneration or loss of seminiferous epithelium, neoplasia, Interstitial cell hyperplasia or neoplasia,  
Inflammation or sperm granulomas |
<p>| Male | Epididymis | Mature sperm? Degenerate cells or giant cells, inflammation |
| | Seminal vesicle + coagulating glands | Distention, inflammation, hyperplasia, neoplasia |
| | Prostate | Inflammation, hyperplasia, neoplasia |
| | Urinary bladder | Inflammation, erosion, ulceration, neoplasia, submucosal lymphoid infiltrates or nodules |</p>
<table>
<thead>
<tr>
<th>Slide #</th>
<th>Tissues</th>
<th>Table 2: Common features and lesions to look for on histology:</th>
</tr>
</thead>
</table>
| 9      | Skin                     | Inflammation, erosion, ulceration, bacterial colonies, hyperplasia, neoplasia  
Acanthosis, hyperkeratosis, and bacterial colonies compatible with C bovis |
|        | Subcutis                 | Adequate fat? Inflammation, neoplasia                                                                                       |
|        | Mammary glands           | Inflammation, hyperplasia, galactorrhea, neoplasia                                                                           |
|        | Preputial or clitoral    | Inflammation, abscesses, bacterial colonies, hyperplasia, neoplasia                                                          |
| +/− leg decalciﬁed | Muscle changes – myofiber degeneration regeneration, and atrophy  
Bone marrow: Marrow: cellularity, relative myeloid and erythroid contributions, bone or  
fibroosseous proliferation |
| 10     | Decal Head               |                                                                                                                             |
|        | Brain                    | Inflammation, Neoplasia  
Dilated ventricles, corpus callosum, neuron necrosis                                                                          |
|        | Pituitary                | Hyperplasia, neoplasia                                                                                            |
|        | Ears                     | Inflammation, bacteria, neoplasia                                                                                          |
|        | Eyes                     | Retina, retinal degeneration, cataracts, cornea inflammation (keratitis), mineralization, neoplasia                       |
|        | Harderian glands         | Inflammation, neoplasia, atrophy, pigment (porphyrin)                                                                          |
|        | Bone, marrow             | As above                                                                                                                   |
|        | TM joint                 | Arthritis                                                                                                                  |
|        | Oral cavity              | Inflammation, neoplasia                                                                                                      |
|        | Incisor teeth            | Dysplasia, inflammation, fracture                                                                                           |
|        | Molar teeth              | Periodontal hairs and inflammation, alveolar bone loss, hypercementosis,                                                   |
| 11     | Spine decal              | Neoplasia, hematopoietic involving marrow and adjacent tissue, Osteosarcoma or other tumors                                 |
| 12 etc | Lesions:                 |                                                                                                                             |
References in order of appearance


PROCEDURE SUMMARY

I. Materials
II. Cassette Numbering
III. External Examination
IV. Dissection, Collection
V. Decalcification
VI. Trimming

I. MATERIALS

1. Prosector (recorder, photographer are nice too)
2. Relevant records & report forms
3. Ventilated work station
4. PPE
   - Eye protection
   - Gloves
   - Lab coat or other protective uniform
5. Measuring tools
   - Small metric ruler
   - Weighing scale (0.001g)
6. Fixative and decalcifying solutions

I. MATERIALS (CONTINUED)

7. Cutting Board
8. Paper Towels
9. Instruments
10. Scissors (fine/blunt & student grade for skin, paper)
11. Blades
12. Syringes and needles
13. Decalcifying solution
14. Specimen containers
15. Labeled cassettes (1-10)
16. Pencils/markers
17. Fixatives:
   - NBF = 10% neutral buffered Formalin
     - Formalin (37-40% formaldehyde) 100ml
     - Sodium phosphate, monobasic, monohydrate 4.0 g
     - Sodium phosphate, dibasic, anhydrous 6.5 g
     - dH2O to 1 liter
     - pH
   - Other options
     - Bouin’s, Fekete’s, Telly’s etc acid alcohol
     - PFA – paraformaldehyde – for ISH etc

II. CASSETTE NUMBERING

1. Heart, thymus, tongue, sternum
2. Lungs, trachea, thyroid/parathyroid, esophagus
3. Kidneys, adrenals
4. Salivary glands, cervical lymph nodes
5. Pancreas, mesentery, mesenteric lymph nodes
6. G.I. tract
7. Liver, spleen
8. Repro. tract, urinary bladder, (+/- rectum)
9. Skin, clitoral/preputial gland (+/- Decal leg)
10. Head – decalcified

II. CASSETTE OPTIONS

- Size, depth, compartments, hole size
- Disposable vs stainless

Fixatives:

- NBF = 10% neutral buffered Formalin
  - Formalin (37-40% formaldehyde) 100ml
  - Sodium phosphate, monobasic, monohydrate 4.0 g
  - Sodium phosphate, dibasic, anhydrous 6.5 g
  - dH2O to 1 liter
  - pH

- Other options
  - Bouin’s, Fekete’s, Telly’s etc acid alcohol
  - PFA – paraformaldehyde – for ISH etc

Decalcifying Solutions (Decal)

- Nitrical ® – Not a fixative – strong, fast
  - Nitric acid, water
- Decal Stat ® – Not a fixative, strong < 1hr!
  - HCL, EDTA, water
- Decal ® – Not a fixative, not as fast - overnight.
  - HCL, EDTA, water
- Immunocal ® – Not a fixative, gentler? overnight
  - Formic acid, water – better for immuno-histochemistry?
- Formalcal 4 ® – fixative /decalifier, gentler ~ 24hr.
  - Formic acid, EDTA, Formaldehyde, water
  - www.leicabiosystems.com

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  - Formalin (37-40% formaldehyde) 100ml
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  - Sodium phosphate, dibasic, anhydrous 6.5 g
  - dH2O to 1 liter
  - pH

- Other options
  - Bouin’s, Fekete’s, Telly’s etc acid alcohol
  - PFA – paraformaldehyde – for ISH etc
### III. EXTERNAL EXAMINATION

1. Check records, identification – correct animal?
2. Weigh
3. Observe - measure, quantify whenever possible
   - Identifying tags, marks, ear punches, amputations
   - Color of coat and eyes
   - Wounds, lesions, masses, alopecia, etc – presence/absence of whiskers, nipples, etc
4. Palpate - measure, quantify whenever possible
   - Body condition
   - Abdominal mass (soft, fluctuant, firm, hard)
   - Abdominal fluid (obtain sterile sample)
   - Skin - Subcutaneous mass

### IV. DISSECTION (ANIMAL ORIENTATION)

- Orient animal in the same direction
  - e.g. head to the right or head to the left
  - Are you right or left handed?
  - If always in same direction, prossector will better recall where lesions were located

### IV. DISSECTION (PELT REMOVAL A)

- Incise ventral abdominal skin
- Pull skin cranially and caudally widening the incision until tear is complete

### IV. DISSECTION (PELT REMOVAL B)

- Pull anterior skin up to forelimbs
- Pull right and left forelimbs out of pelt by holding each elbow
- Pull anterior skin up to ears
- Cut through external ear canals (between ears and skull) to facilitate pulling more rostrally
- Pull skin up to eyes, Cut around eyes
- Pull skin off completely over nose
- Cut anterior skin (hoody) along ventral midline to open and lay flat on paper towel
- Examine for mammary, lymph node or other masses
- Record abnormalities, including location & size

### IV. DISSECTION (PELT REMOVAL C)

- Pull posterior skin down to hind limbs
- Pull right and left hind limbs separately out of skin by holding femoral-tibial joint (knee)
- Pull posterior skin down to tail/perineum
- Cut through anus-perineal skin
- Pull off completely over tail
- Cut posterior skin (pants) along dorsal midline to lay flat on paper towel
- Note if clitoral/preputial gland came off with skin
  - If not, find it on the animal, remove and place in cassette 9
- Examine for mammary, lymph node or other masses
- Examine posterior skin, including location & size

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http://www.hopkinsmedicine.org/mcp/PHENOCORE/courseCURRENT.html
IV. DISSECTION (SALIVARY GLANDS)

- Around ventral neck, from ear to ear, find ‘chain’ of salivary gland and lymph nodes
- Remove entire chain
  - Start from either ear
  - Use blunt fine scissors
  - Lift and cut from ear canal to ear canal, under glands
- Note, weigh, measure, any abnormalities
- Place in cassette #4
  - Deep (dorsal) side down
  - Close cassette
  - Submerge in specimen container

IV. DISSECTION: OBSERVE, EXAMINE, RECORD

- What do you see?
  - Normal (WNL): ample body fat, ample ingesta, fecal balls
  - Abnormal: Prolapse, Splenomegaly; Pale?? Probably exsanguination

IV. DISSECTION (THORAX - A)

- Incise Peritoneum
  - Open to expose/examine abdominal contents
- Remove Sternum
  - Hold xiphoid process w/ forceps
  - Cut ribs on right and left sides along sternum
  - Remove sternum and attached ribs
  - Trim ribs and extra tissue from sternum
  - Lay sternum flat, internal/marrow side down, on paper towel
  - Spread remaining ribs to open thorax and expose thoracic contents
- Remove Thymus
  - White soft tissue covering top of heart – 2 lobes
  - Place in cassette #1, close, fix
  - Note/record size or absence of thymus

IV. DISSECTION: STERNUM

IV. DISSECTION: THYMUS IN SITU

IV. DISSECTION (LUNG INFUSION, THORAX - B)

- Expose cervical trachea
  - With forceps, reflect small/thin cervical muscles around trachea
  - Observe tracheal rings
- Infuse lungs
  - Fill 3ml syringe w/ 10% NBF
  - Attach 20-25G needle
  - Insert needle (bevel side up) along the same plane of the tracheal route
  - Slowly inject 10% NBF; note lung inflation

IV. DISSECTION: TRACHEA EXPOSED
IV. DISSECTION (EN BLOC REMOVAL)

- Split mandible symphysis with scissors and open rami laterally
- Grasp tongue w/ forceps, reflect caudally, and cut attachments to lift out tongue, larynx, trachea, esophagus
- Remove thoracic organs en bloc by lifting plus blunt dissection along spine, until tongue to diaphragm is free
- Cut diaphragm from body wall to free attached esophagus
- Holding cut diaphragm, lift/retract abdominal contents caudally and pull/cut dorsal attachments along spine
  - Identify and include small adrenals and ovaries
- Split pelvis by inserting closed scissors into pelvic inlet then opening them gently
- Completely remove viscera by pulling further caudally
- Tongue to anus should come off in one piece (en bloc)

IV. DISSECTION: SPLIT PELVIS

- Look for AORTA too
- Nice to include it with lungs/esophagus
**IV. DISSECTION: VISCERA EN-BLOC**

- **Tongue**: Hold the organ mass by the tongue, find and nick the right kidney with blade or scissors.
  - Right kidney is higher (more cranial)
  - This facilitates identification later.
- **Heart**: Remove, weigh, fix (in toto in container).
- **Tongue**: Cut from larynx, fix (in toto in container).
- **Lungs**: Hold trachea, cut it free from diaphragm.
  - Lay lungs and attached larynx, trachea and esophagus dorsal side down in cassette #2.
  - Close cassette #2 and submerge in specimen container.

**IV: DISSECTION (BY ORGAN - A)**

- **Heart** – WEIGH IT
- **Tongue**
- **Lungs with**
  - trachea larynx esophagus lymph nodes aorta etc

**IV: DISSECTION: CASSETTE 2**

- **Lungs**
- **Trachea**
- **Larynx (+ thyroids)**
- **Esophagus**
  - Mediastinal lymph nodes
  - Thymus remnants
  - Aorta

**IV: DISSECTION (BY ORGAN - B)**

- **Kidneys**: Remove with adrenals attached.
  - Remove adrenals etc and place in cassette #3.
  - Clean extraneous tissue from kidneys.
  - Weigh, fix (in toto in container).
- **Spleen**: remove and clean off extraneous tissue.
  - Weigh, fix (in toto in container).
- **Liver**: Remove all lobes.
  - Confirm all lobes (check for small quadrat with stomach).
  - Remove diaphragm etc from median lobe.
  - Weigh all lobes together.
  - Separate lobes, fix (in toto in container).

**IV: DISSECTION (BY ORGAN - C)**

- **Liver**
  - Left lateral Lobe
  - Median lobe
  - Gall bladder
- **Spleen**
- **Kidneys**
  - Adrenals still attached
- **Reproductive Tract**: separate from GI tract
  - Check for missing pieces, ovary, bladder, etc.
  - Also check animal shell or GI tissue mass.
  - Trim off fat etc.
  - Spread out flat on paper towel; Fix in toto in container.
- **Gastrointestinal tract**
  - Starting at the distal colon (fecal balls), gently elongate by stretching/pulling on the tract itself.
  - Continue back to the stomach.
- **Pancreas/mesentery**: trim from GI tract.
  - Place pancreas, mesentery, fat, nodes in cassette #5, close and submerge in specimen container.

**IV: DISSECTION: FEMALE REPRODUCTIVE TRACT**

- **Ovaries**
- **Uterine horns**
- **Urinary Bladder**
  - Ventral to cervix, vagina et
IV: DISSECTION: MALE REPRODUCTIVE TRACT

- Seminal vesicles
- Coagulating gland
- Bladder
- Prostate
- Vas deferens
- Epididymis
- Testes
- Urethra
- Penis

IV: DISSECTION: GI TRACT

Identify
1. Stomach
2. Cecum
3. Fecal Balls

IV: DISSECTION (GI TRACT)

- GI tract infusion, fixation
  - With 3ml syringe, infuse entire gut with 10% NBF at multiple sites from stomach to colon
  - Fix (in toto, in container)
  - Envelope skin and submerge in specimen container

- GI tract: Swiss roll options
  - Closed: roll closed intestine into 2-3 cassettes
  - Open: open intestine, flush clean with fixative, examine, count/measure lesions, roll into cassettes

IV: DISSECTION (PARTS ON PAPER)

1. Skin
2. Sternum
3. Reproductive Tract
   - Examine, measure lesions, abnormalities
   - Envelope/roll gently (still on paper) and submerge in fixative
   - CLEAN UP

IV-V: DISSECTION-DECAL (BONEY PARTS)

1. Head: Sever from cervical spine
   - submerge in Formaldehyde-4® ~ 24 hr

2. Legs, spine – Save in fix, or decalcify
   - Hind legs with pelvis
     - Separate from spine, cutting caudal to rostral
     - Should not crunch if separating cleanly from spine
   - Arms with scapula
     - separate from thorax – should not crunch (except clavicle)
   - Spine – Cervical, thoracic, lumbar, sacral
     - trim off ribs, usually tail

V. DECALCIFY / DEMINERALIZE

- Sever Head
  - Cut at flexion = atlanto occipital joint
  - Should not crunch much through the joint
END OF NECROPSY

1. Labeled container of saved fixed tissues +
   • Labeled Cassettes
     • 2,4,5 (or more) contain tissues
     • May be useful to include lesions in labeled cassettes
   1. On paper – skin, sternum, repro
      • May be useful to include lesions, and label in pencil
2. Weights: Body, heart, spleen, liver, kidneys
   • Additional tissues – before or after fixation?
3. Report: with weights, measurements

VI. TRIMMING TISSUES INTO CASSETTES

Aims

➢ For diagnostic, baseline or comprehensive evaluations:
   • Assess as much as possible on relatively few slides
   • Tissues dissected and trimmed reproducibly to facilitate comparisons between animals & studies

➢ For tox evaluations
   • http://reni.item.fraunhofer.de/reni/trimming/

Cassette 1

- Thymus - already in cassette – both lobes
- Sternum - Marrow (in)side down in cassette
- Tongue - Section Longitudinally - half in cassette
- Heart - Hemi-sect sagittally to see all chambers
  Both halves in cassette

- Close cassette and re-submerge

Cassette 2

- Trachea, larynx, thyroid
  • Remove excess tissue with forceps
  • Identify thyroids below larynx
  • Transect trachea below (distal to) thyroid
  • Place larynx/thyroid into cassette
  ➢ Hoping for cross section
- Lungs - Entire, dorsal side down in cassette

JH#1 EXAMPLE

JH#2 EXAMPLE

http://www.hopkinsmedicine.org/mcp/PHENOCORE/courseCURRENT.html
VI. TRIMMING TISSUES INTO CASSETTES

Cassette 3
- Adrenals – trim off fat etc (or retain it)
  - Use biopsy foam if < 2mm
  - Males’ usually are smaller than females’
  - Right kidney → 1 or 2 cross sections
  - Left kidney → 1 or 2 longitudinal sections
    - Both halves of both kidneys, Cut side down
- If using biopsy foam, sandwich sections between pads
- Close cassette and re-submerge

Cassette 4
- Salivary glands and cervical – submandibular lymph nodes
  - Should be in cassette already
    - Submandibular Glands
    - Sublingual Glands
    - Parotid Glands
    - Exorbital Lacrimal Glands
    - Mammary glands

SALIVARY GLANDS, ASSOCIATED NODES
Which is Male? A or B?
1. Submandibular
2. Sublingual
3. Parotid
  - (exorbital lacrimal)
4. Lymph nodes

Cassette 5
- Pancreas, mesentery and lymph nodes
  - Should be in cassette already

Cassette 6: GI Tract
- Stomach – separate (cut) from duodenum
  - Section (hemisection) to include squamous and glandular portions
- Small intestine: duodenum, jejunum and ileum
  - 1-2 sections 4-5mm long → O on cross section - from each region
- Cecum
  - Cut U-like section from tip
- Proximal Colon (note diagonal stripes = mucosal folds)
  - 2 sections 4-5mm long → O on cross section
- Distal Colon (fecal balls usually)
  - 2 sections 4-5mm long → O on cross section
- Close and submerge cassette
VI. TRIMMING TISSUES INTO CASSETTES

Cassette 7

- Liver – median and lateral lobe sections
  - Median lobe -> 4mm wide section, include gall bladder
  - Cut above "crease" & just below the gall bladder
  - Left lateral lobe -> 4mm wide section
    - Cut diagonally from hilus to edge to get nice long section
  - Lesions – include any lesions in these or other lobes
- Spleen - Hemisect longitudinally/sagittally
  - Put 1 or both sections in cassette
  - 2 liver sections + 1-2 spleen sections normally fit in one cassette

Cassette 8 – Reproductive Tract

- When small, male or female reproductive tract fit intact into the cassette
  - Ensure that all organs are in one plane – foam can help
- If necessary, separate and trim representative sections of gonads accessory sex glands, urinary bladder and rectum
- Prostate or other protocols (MMHCC or Simons & al, 2010) may require special dissections

Cassette 9

- Skin - Cut strips 4mm wide, parallel to hair growth, of Representative areas e.g.
  - Craniofacial- dorsal neck - to include periocular, periauricular, perioral skin
  - Ventral- inguinal – to include preputial or clitoral gland, external orifices
- Lesions
  - Leg - decalcified, sometimes included here
  - Close cassette & submerge

- Haired Skin sections best when block is sectioned parallel to section (roughly parallel to hairs too)
VI. TRIMMING TISSUES INTO CASSETTES

Cassette 10 - Head – Decalcified

- External ear openings & eyes are primary landmarks.
- Lambdoid & coronal sutures also are useful
- 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 sections and progressing anteriorly/rostrally.

1st section ➔ Cerebellum – brainstem
- Cut transversely caudal to ear canals, or at the bregma & lambdoid suture
- Place FRONT/ROSTRAL side down in the cassette

2nd section ➔ Ears; hippocampus; midbrain; pituitary
- Next cut is rostral to ear canals; between coronal and lambdoidal sutures (closer to coronal suture)
- Place FRONT/ROSTRAL side down in the cassette

3rd section ➔ Forebrain, cortex, (hippocampus)
- TMJ, molars
  - Next cut is caudal to eyes, near coronal suture
  - Place BACK/CAUDAL side down in the cassette

4th section ➔ Eyes, Harderian glands, olfactory lobes, molar teeth
- Next cut is just rostral to eyes
- Place BACK/CAUDAL side down in the cassette

5th section: Nose turbinates, incisors
- Remaining nose may fit in cassette OR
- Cut 3-5mm rostral to last cut
- Place BACK/CAUDAL side down in the cassette

JH#10 EXAMPLE

1. Cerebellum medulla ➔ Front down
2. Ears hippocampus, midbrain, pituitary ➔ Front down
3. Forebrain, molars ➔ Back down
4. Eyes, olfactory bulbs ➔ Back down
5. Nose ➔ Back down

A 10 SLIDE MOUSE PROTOCOL

- Perfusion & Dissections by Nadine Forbes;
- Slides by Bonnie Gambicher
- Slide scanning courtesy of Flagship Biosciences

http://www.hopkinsmedicine.org/mcp/PHENOCORE/courseCURRENT.html
Aims of perfusion:

- To use the vascular system to deliver fixative to tissues;
- Achieve excellent tissue preservation;
- Prevent/preclude post mortem cell/tissue degeneration (autolysis), and dissection artifacts that interfere with analysis of histology specimens.

Overview

- Blood is flushed from vasculature
- Fixative is delivered
  - Injected slowly through left ventricle
  - Driven through the systemic circulation and allowed to drain from the incised right atrium
- Success indicators
  - Muscle contraction
  - Blanching of liver
  - Mouse should be stiff
  - Excellent histology 😊

Materials

1. Down draft table or fume hood
2. PPE (gloves, mask, eye protection)
3. Two 20ml syringes
4. Saline-Heparin flush (10units/ml)
5. 10% Neutral Buffered Formalin
6. Vacutainer Butterfly collection set 25G x ¾ x 12in
7. Absorbent paper towels
8. Razor blade
9. Dissecting tools
  - Iris scissors, forceps, fine spring scissors

Saline Heparin flush

- Saline heparin lock flush
- Or
  - 1000 unit heparin vial
    - 1ml heparin diluted in 100ml 0.9% saline
  - 5000 unit heparin vial
    - 1ml heparin diluted in 500ml 0.9% saline

Materials

1. Scissors
  - Big coarse
  - Fine for RA
2. Hep/saline
  - 20ml syringe
3. Fixative
  - 20ml syringe
4. Blade
5. Butterfly infusion set
  - 25g
  - 3/4 inch
Method

1. Fill 20ml syringe with saline-heparin
2. Fill 20ml syringe with 10% NBF
3. Open Butterfly pack, cut ~3mm off of plastic needle guard
   ◦ ‘cuff’ prevents needle from piercing through heart
4. Attach blood collecting set to saline-heparin syringe and prime

Method (continued)

8. Incise (nick) right atrium using the fine spring scissors

Method (continued)

9. Carefully insert needle (attached to saline heparin syringe) into left ventricle
10. Slowly inject saline heparin
    ◦ Usually ~5-10ml is sufficient
    ◦ Blood & tissues should become pink or pale

Method (continued)

11. Without retracting needle, remove the Hep/saline syringe and attach fixative (10%NBF) syringe
12. Slowly, over > 1 minute, inject fixative
    ◦ Watch for muscle tremors (chemical)
    ◦ Total body stiffening indicates good perfusion
    ◦ Usually ~5-10ml is sufficient

10% NBF Perfusion (video)
LAB 1  (Wednesday)

Behavioral Phenotyping Level 1 Screening
(from JW 1/06)

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

<table>
<thead>
<tr>
<th>Animal #:</th>
<th>N/A</th>
<th>Wild Type</th>
<th>Hemi</th>
<th>-/-</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOB/Age:</td>
<td>Adult</td>
<td>Sex</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>Weight (g):</td>
<td></td>
<td>Coat Color:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body Condition (BC) Score*:</td>
<td></td>
<td>Eye Color:</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**EMPTY CAGE (2 mins.)**

| Gait abnormal? | Y | N |
| Posture abnormal | Y | N |
| Freezing | Y | N |
| Wild Running | Y | N |
| Stereotypies | Y | N |

**PHYSICAL EXAM**

Dorsal

Ventral

**DRAW**

Bald patches/abnormalities

| Bald patches | Y.......N |
| Physical abnormality | Y.......N |
| Body Tone | 0....1....2....3 |
| Petting Escape | 0....1....2....3 |
| Passivity | 0....1....2....3 |
| Trunk Curl | 0....1....2....3 |
| Righting | 0....1....2....3 |
| Visual Placing | Y | N |
| Reach touch | Y | N |

| Piloerection | Y.......N |
| Physical abnormality | Y.......N |
| Body Tone | 0....1....2....3 |
| Petting Escape | 0....1....2....3 |
| Passivity | 0....1....2....3 |
| Trunk Curl | 0....1....2....3 |
| Righting | 0....1....2....3 |
| Visual Placing | Y | N |
| Reach touch | Y | N |

| Whisker response | 0....1....2....3 |
| Ear twitch | 0....1....2....3 |
| Palpebral reflex | 0....1....2....3 |
| Forelimb Place | 0....1....2....3 |
| Right leg withdraw | 0....1....2....3 |
| Biting | 0....1....2....3 |
| Clicker | 0....1....2....3 |

| Grip strength | < 60 sec. | > 60 sec. |

Notes: ___________________________
LAB 2 (Wednesday)
Specimen Collection

Survival Bleed
Blood Volume: _______________ul

Post bleed mouse activity:
☐ normal
☐ slow
☐ dead

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 ☐

Terminal Bleed (Cardiocentesis)
☐ Successful ☐ Unsuccessful
Blood Volume: _______________ul

Mouse Weight
Guestimate ______ Weighing Machine ______
Blood: ___________ g Total: ___________ g

Note 1ml of blood weighs ~1.06g)
### Lab 3 Thursday

#### Gross Examination (Necropsy)

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

<table>
<thead>
<tr>
<th>Terminal Bleed (Cardiocentesis)</th>
<th>Blood Volume:</th>
<th>ul</th>
</tr>
</thead>
</table>

**Age:**

**Sex:**

**Coat color:**

**Eye color:**

**Body Weight:** __________ g

**Body Condition:**

---

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

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

---

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

1. 

2. 

3. 

4. 

5. 

6. 

7. 

8. 

9. 

10. 

11. 

12. 

13. 

14. 

15. 

---
### Lab 4 Friday

**Tissue Trimming**

<table>
<thead>
<tr>
<th>Cassette</th>
<th>Tissue, trim procedure – Check Gross Report – Ensure trimming of any lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>☐ 1</td>
<td><strong>Heart</strong> - cut in half to expose all four chambers (coronal section) – include both halves usually&lt;br&gt;<strong>Tongue</strong> - longitudinally section to produce a left and right side&lt;br&gt;<strong>Sternum</strong> position inside(marrow side) down&lt;br&gt;<strong>Thymus</strong> – already in cassette</td>
</tr>
<tr>
<td>☐ 2</td>
<td><strong>Trachea / larynx</strong> – 3mm length should include thyroid / parathyroid glands&lt;br&gt;<strong>Lungs</strong> - Make sure lungs are positioned dorsal side down</td>
</tr>
<tr>
<td>☐ 3</td>
<td><strong>Right kidney</strong> - is cross sectioned. Include both halves when there is room&lt;br&gt;<strong>Left Kidney</strong> - is sectioned longitudinally - Include both halves when there is room&lt;br&gt;<strong>Adrenals</strong> - are secured on foam</td>
</tr>
<tr>
<td>☐ 4</td>
<td><strong>Salivary glands</strong> with lymph nodes – already in cassette</td>
</tr>
<tr>
<td>☐ 5</td>
<td><strong>Pancreas and mesentery</strong> (+ Lymph nodes)– already in cassette</td>
</tr>
<tr>
<td>☐ 6</td>
<td><strong>G.I. Tract</strong> - several cross representative sections of duodenum, jejunum, ileum, cross section tip of cecum, several cross sections of proximal and distal colon. Stomach sectioned to represent non-glandular and glandular portions&lt;br&gt;<strong>G. I. Tract</strong> - Nothing done if Swiss rolled</td>
</tr>
<tr>
<td>☐ 7</td>
<td><strong>Liver</strong> - Median lobe section to include gallbladder between left and right parts.&lt;br&gt;<strong>Left lateral lobe</strong> section from hilus to edge&lt;br&gt;<strong>Spleen</strong>- longitudinally sectioned - Include both halves when there is room</td>
</tr>
<tr>
<td>☐ 8</td>
<td>Reproductive Organs: Trim to provide representative sections of all structures or include intact</td>
</tr>
<tr>
<td>☐ 9</td>
<td><strong>Skin</strong>: 3-4mm strips section parallel to hair growth.&lt;br&gt;<strong>Cranial skin</strong> to include muzzle eyelid- neck&lt;br&gt;<strong>Inguinal skin</strong>- to include clitoral or preputial gland, perineum</td>
</tr>
<tr>
<td>☐ 10</td>
<td><strong>Decal head</strong> - Cut on caudal and rostral side of ear canal – section 1,2&lt;br&gt;Cut on caudal and rostral side of eyes – sections 3,4&lt;br&gt;Cut off and discard very tip of nose; you should have five sections total&lt;br&gt;First two back sections laid in cassette rostral (front) side down&lt;br&gt;Final sections in cassettes caudal (rear) side down</td>
</tr>
<tr>
<td></td>
<td><strong>Lesions (any abnormality ‘X’ noted in gross examination should be trimmed)</strong></td>
</tr>
</tbody>
</table>