Mouse Pathobiology & Phenotyping

Short Course 2012

Lab Manual

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# Lab Manual

## Practical Mouse Evaluation & Pathology

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How to Give a Mouse a Physical

Julie Watson
Dir. Rodent Programs, JHU

Background

- 1968 - Samuel Irwin - First Phenotyping Screen
  - Psychopharmacologia (Berl.) 12 222-257
- 1997 - SHIRPA stage I
  - Mamm Genome. 1997 Oct;8(10):711
- 1997 - Jacqueline Crawley
  - "What's Wrong with my Mouse?" Wiley-Liss 2000.
- Current - Websites:
  - EMPReSS (European Mouse Phenotyping Resource of Standardized Screens at Eumorphia) http://www.eumorphia.org/EMPReSS/servlet/EMPReSS.Frameset
  - SHIRPA at MRC ENU Mutagenesis Program http://www.mgu.har.mrc.ac.uk/facilities/mutagenesis/mutbase/

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


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
Some “Abnormalities” are Expected!

Age-related hearing loss & vestibular defects (<3m)

- Many 129 strains
- A/J
- C57BR, C57L
- DBA
- I, LP, NOD

Blindness (rd1 gene)

- FVB
- C3H
- BUB
- CBA
- SI/L
- SWR
- NON
- P, PL

* Errijgers, V. (2006) FVB.129P2-Pde6b Tyr/Ant, a sighted variant of the FVB/N mouse strain suitable for behavioral analysis. Genes Brain and Behavior 0(0)

Environmentally Induced 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 (I) ... 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
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 -/

DOB/Age Sex                  M     F

Weight (g) Condition Score 1..2..3..4

Fur color

Empty Cage 2 mins:

Gait abn normal         Y  N

Posture abnormal    Y  N

Freezing Y

Wild running           Y  N

Stereotypies            Y  N

Escape                     Y  N

Grooming 0… 1 - 2 3 -6…>6

Digging 0 …1 - 2 3 -6…>6

Escape

Exploring 0..1..2..3

Digging 0..1..2

Grooming 0..1..2.

Rearing 0..1..2

Exploring 0..1..2..3

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

Physical abnormality Y..N

Whisker damage Y..N

Body tone 0..1..2..3

Whisker response NA   0..1..2

Ear twitch 0..1..2

Passivity 0..1..2..3

Palpebral reflex 0..1..2

Trunk curl 0..1..2

Forelimb place 0..1..2

Righting 0..1..2..3

RL withdraw 0..1..2

Visual placing 0..1..2

Biting 0..1..2..3

Reach c touch 0..1..2..3

Clicker 0..1..2..3

Grip: >60 <60 time _______

Notes: __________________________________________

DORSAL DORSAL VENTRAL VENTRAL

Biosafety Cabinet

SIMPLE EQUIPMENT

Initial Information

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

JHU Pheno 2012 Lab Manual P 4
Rearing/Escape/Thigmotaxis

Digging

Subtle Deficits
Limited Rearing

Obvious Motor Deficits

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

Abnormal Behavior
Ulcerative Dermatitis

• Picture: Nadine Forbes

• 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 Physiolog.*, 36 (4) Oct. 06, 883-888

Open Field

Normal Whiskers

Stereotypy

Video: Dawn Ruben
Barbering
Ref. Kalueff et al. Behavioral Processes 2005

• 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

JHU Pheno 2012 Lab Manual P 8
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

Sight Needed for Tests of Anxiety

Sight Even Needed for Motor Performance

- C3H/HeJ mice (with retinal degeneration) compared with (Pde6b+) mice (without retinal degeneration) on the rotarod
- The sight-impaired C3H mice stayed on the rotarod longer than did their sighted Pde6b+ partners

JHU Pheno 2012 Lab Manual P 10
Hearing Test

Clicker

Tests That May be Affected by Hearing Loss

Cued Fear Conditioning
For learning and memory

Prepulse Inhibition
For sensorimotor gating

Other Tests

- Provoked Aggression
- Grip Strength

Aggression

Grip Strength Normal

JHU Pheno 2012 Lab Manual P 11
Grip Strength Abnormal

Grip Strength Apparatus
Images from EMPRESS

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Level 1 Behavioral Screen

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

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

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

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 for movement.

Body Tone: 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

Petting Escape. 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 an escape response; 2= easy to elicit escape response 3= Difficult to perform test because of spontaneous escape attempts

Passivity -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 off. 1= delayed or unsuccessful attempt to climb up, 2 = normal, 3=hyperactive.

Trunk Curl 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 eg hindlimb clasping ; 1=< 90° 2 = curls to 90° or more; 3= climbs up tail

Righting – Hold mouse by base of tail, Hold your other hand flat with thumb up and little finger down so as to provide a vertical surface. Bring the dorsum (back) of the mouse to the back of your hand. Once the normal mouse feels the surface of 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 - hyperactive.
**Visual Placing/Reach Touch (Y or N)**  Hold the mouse by the tail and lower it slowly and at a steady pace towards 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 the surface. 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, This test is Y or N for vision.

**Whisker response** – The whiskers are superficially stimulated using a ‘teased out’ cotton tipped applicator. This is another test that can be difficult to elicit consistently because the whiskers are often difficult to see. Touching the whiskers should elicit a response: either a cessation of “whisking” (continual movement of whiskers) or a responsive nose quiver. 0= no response; 1= difficult to elicit a response 2=normal response; 3= hyperactive response

**Ear Twitch**- 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

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

**Forelimb place** – 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

**Withdrawal**- 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 immediately return to normal position 1= slow to return; 2=rapid return 3= hyperactive response

**Biting** - Using a tight dorsal scruff, 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.

**Clicker (hearing test)** - 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)

**Grip**- 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. Normally mice can hold on upside down easily for 60 secs. Less than 60 secs is abnormal.
PHENOTYPING CORE
SPECIMEN COLLECTION

N Forbes July 2012

OUTLINE
- Survival Bleed (facial vessels)
- Blood Glucose
- Fecal Occult Blood
- Terminal Bleed
- Urinalysis

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.
  - Use dominant hand (right for most people)
  - 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.

- 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.
  - Dab/press area with alcohol pad.
  - 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,
  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 reads “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.

**FECAL OCCULT BLOOD**

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

**FECAL OCCULT BLOOD (DEMONSTRATION)**

- 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.

*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
**Fecal Occult Blood**

**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 the non-dominant side of your body.
  - 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.

- **Urine Analysis**
  - **Aim:**
    1. Collect mouse urine,
    2. Measure urine specific gravity,
    3. Test with dipstick (chemstrip).

  - **Required:**
    1. Mouse in container (opaque & small is good),
    2. Veterinary refractometer,
    3. 96 well microtiter plate, clean,
    4. Small pipette.

  - **Place plate(s) in container to cover the bottom.**
  - **Place mouse in container undisturbed 20-30 minutes.**
  - **Mouse may have urinated, or may urinate as it is lifted from the cage – be sure to hold it over the wells.**
  - **Pipette urine from well.**
  - **Specific gravity by refractometer**
    + Place 2 drops on the glass plate and close top,
    + Read Specific Gravity through the eye viewer.
  - **Specific gravity, glucose, protein by chemstrip dipstick**
    + Place 1 drop on each test pad,
    + Compare pad color with the guide on the chem. strip container.
  - **Record results**
    + Compare SG of refractometer vs dipstick.
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 you?
  - Growth curves (from body weights )
  - Bleeding
  - CBC
  - Chemistry
  - Urinalysis
  - Fecal Occult blood
  - Other:
Anatomic Pathology
In contrast to clinical pathology, which generally refers to evaluation of fluids or excreta from an animal, Anatomic Pathology refers to the pathology of the anatomical structures, organs and tissues. At least in larger animals, clinical pathology specimens often can be obtained in a clinical setting from live animals, and contribute diagnostic information to influence therapeutic interventions. Anatomic Pathology typically involves specimens obtained by biopsy or at necropsy.

a. Biopsy
It is not common practice to submit only a piece of tissue from a live mouse. However in long term studies or breeding colonies of valuable mice, such practices may become more common.

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

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

Whole animal perfusion can optimize fixation of all tissues and permit later dissection and tissue collection at the prosectors’ convenience. However perfused tissues cannot be cultured, some lesions may be difficult to identify, due to absence of blood, and similarity in color and consistency. Also fresh and perfused tissue weights should be expected to differ.

i. General Procedure

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

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

6. **Cutting board** - Inexpensive, plastic cutting boards are adequate for most purposes. They should be easy to clean and reuse, and not ruin blades too quickly. Wax and cork cannot be sanitized.

7. **Paper towels** - some tissues (e.g. skin and reproductive tract) can be ‘laid out’ on a dry paper towel and will adhere to it to facilitate examination and ensure uniform fixation.

8. **Small Metric ruler** - should usually be included in photographs. The descriptors ‘large’ or ‘small’ should be used only when accompanied by measurements of size, mass (wt), or volume (ml). Mass lesions and organs have 3 dimensions and their measurements should be recorded in 3 dimensions. E.g. a ‘spot’ may be 2x2mm, but a ‘mass’ is 2x2x2mm.

9. **Forceps** - blunt ended, serrated or toothed forceps may cause less damage. Fine pointed forceps can puncture and tear. Smooth forceps require considerable pressure to ‘grip’ a slippery tissue.

10. **Scissors** - fine, blunt ended scissors may cause less damage. Sharp tipped corneal scissors are used commonly but are likely to tear or puncture tissues.

11. **Blades** - not so useful in mouse necropsy dissections. But sharp fresh blades are critical to trimming tissues for histology. Single edge blades in hundred packs are adequate and inexpensive, but some prosectors may prefer scalpels with handles or other special blades.

12. **Syringe and needle** - 3ml syringe with a 21 gauge needle works well for infusing lung and gastrointestinal tract with fixative.

13. **Fixatives** - 10% neutral buffered formalin (10%NBF) is suitable for soft tissues for many situations. Acid alcohol fixatives and other options are being used more frequently. The primary goal (histology, immunohistochemistry, in situ hybridization) should influence the choice of fixative.

14. **Decalcifying (demineralizing) solutions** - Head and other bones must be decalcified for paraffin processing for histology. Several formic acid solutions (TBD2, Formical etc) simultaneously fix and decalcify in < 24hr. Bouin’s solution also fixes and decalcifies and maybe especially useful for mice < 2w old.

15. **Specimen containers, cassettes, labels, pencils, markers** - Label containers, cassettes, etc before starting. Pencils work best for many things. Even ‘permanent’ markers disappear with alcohols, fixatives.

**PROCEDURE**

**A External examination** - (see necropsy form below) Weigh the intact animal. Note colors of coat/skin and eyes. Note identifying marks (e.g. toe snips, ear punches), presence/absence of whiskers. External lesions (e.g. domed head, microphthalmia, skin lesions) should be recorded, and measured whenever possible (e.g. left flank, subcutaneous mass 4x4x5mm). Body condition should be assessed (e.g. thin, adequate or good body condition, obese), or scored, e.g. per Ullman & Foltz 1999.

**B Palpation** -- Gentle palpation may reveal pups or abdominal masses, or suggest the presence of abdominal fluid. When the abdomen is distended by fluid (e.g. ascites), a sterile sample can be obtained with a needle and syringe for cytology, chemical or microbiology evaluation. Mass lesions should be measured, weighed when feasible. Their consistency can be described as soft, fluctuant, firm or hard. ‘Hard’ should be reserved for boney or mineralized masses.

**C Dissection:**

1. It is helpful to always **orient animals in the same direction**, e.g. head up or head right, so that the side of the lesion can be recalled. If the animal always is examined ‘right-side-up’ (i.e. left lateral recumbency), and the prosector remembers that the ‘top’ kidney was cystic, the prosector will be certain that the right kidney was cystic.

2. **Pelt removal** facilitates assessment of subcutaneous fat (minimal, adequate, abundant), and reveals subcutaneous lesions and abdominal organs in situ. Cervical and submandibular lymphadenomegaly (enlarged lymph nodes) is evident, as is splenomegaly, or cystic kidneys, pregnancy or other lesions. The pelt can be removed easily by pinching or incising ventral abdominal skin and exerting gentle pressure.
cranially and caudally until the animal is ‘degloved’. Cut the ear canals, around the eyes, muzzle and extremities to complete pelt removal. Eyes can be left in situ and decalcified with the head.

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

3. After examining the animal with the pelt removed, **remove the ‘chain’ of salivary glands (parotid, sublingual, submandibular) and lymph nodes**, that extend from ear to ear under the chin. Place the whole chain in a cassette (#4). Further trim-in is not required unless for tumors or other lesions.

4. **Open the abdomen**, xiphoid to pubis, and examine contents in situ (in the body).

5. **Lift the sternum** by the xiphoid process and cut on both sides of it to remove it intact and expose the thoracic cavity. Place sternum flat on paper, or directly in cassette (#1), inside (marrow side) down, to fix. Examine thoracic contents, note fluid or masses, absence or enlargement of the thymus over the heart, megaesophagus.

6. **Expose the cervical trachea** by blunt dissection and use the 3ml syringe/21g needle to **infuse the lung with fixative**. The lungs should expand fully and excess fixative will reflux up the trachea. It is not necessary to clamp or tie the trachea. Generally this technique will not overfill the lungs because there is ample space around the needle for fixative to reflux. Take care after infusion to not compress the lungs during subsequent dissection.

7. **Split the mandibular symphysis** with scissors. Grasp the tongue with forceps and retract gently to **remove tongue, larynx, trachea and esophagus from the head and neck**. Continue retracting to **remove heart, thymus and lungs from the thorax**. Use blunt dissection (scissors) to free these tissues. Examine thoracic contents ex situ (out of the body). Note megaesophagus, aortic or other lesions. Separate heart, thymus, lungs. Weigh heart, thymus. Place thymus in a cassette (#1), so it is not lost. If you can not find the thymus, **note/record its absence**. Heart should fix intact. Place lungs (dorsum down), plus larynx/trachea with thyroid glands in a cassette (#2).

8. **Thyroid glands** are immediately caudal to the larynx on both sides of the trachea. They may be difficult to see without magnification, so include 2-3mm of trachea caudal to the larynx. The histology technician should understand if a cross section of trachea is desired ‘O’, or a longitudinal section. ➞ ==

9. **Split the pelvis** at the pubic symphysis to facilitate removal of ALL abdominal contents, including the urogenital tract.

10. **Grasp the diaphragm** with forceps, cut at its deepest extent and retract gently, lifting and dissecting free abdominal contents to remove them in toto from the abdominal cavity. Very little cutting should be necessary. Adrenal glands and kidneys tend to stay deep in the retroperitoneal space. They should be identified early, and blunt dissection may be necessary to remove them.
    - Nick the right kidney with a scissors or blade to facilitate subsequent identification. If you forget this, note that the right kidney normally is ‘higher’ (more cranial) than the left.
    - Splitting the pelvis facilitates removal of urogenital tract and rectum intact.

11. Examine abdominal and pelvic contents ex situ. Measure, weigh, record any abnormalities.

12. **Isolate the urogenital tract**, with rectum attached, from other viscera. Lay it flat on a dry piece of paper. Consistently orient the rectum (dorsal aspect) ‘down’ on the paper, and urinary bladder (ventral aspect) ‘up’ facing you, with left and right sides identified easily. Dissect out testes, or ovaries and uterus if these are to be weighed. These tissues also can be placed directly in fixative on the paper towel. Small ovaries or testes can be placed in a cassette (#8) at the time of dissection so that they are not lost. Testes should not be cut until they are fixed.

13. **Dissect kidneys (with adrenal glands attached)** from the other abdominal contents. Even ex situ the right kidney should be higher (more cranial, closer to liver) than the left. Remove adrenals and other attached tissue (fat, lymph nodes) from the kidneys and place them in a cassette (#3). Weigh the kidneys. Female mice usually have larger adrenals than male mice.

14. **Dissect the spleen** from other abdominal contents. Remove attached fat, mesentery and pancreas before weighing it.

15. **Dissect the liver from the GI tract**. A small lobe usually is folded into the lesser curvature of the stomach. Take care to remove it so that the entire liver is examined and weighed. Also remove remnants of diaphragm, fat and other tissue to obtain an accurate weight. When manipulating the liver,
lift it gently, or grasp parts that will not be submitted for histology, e.g., diaphragm, smaller lobes, or fibrous attachments. Unless there are lesions in other lobes or protocol requires examination of other lobes, the **median and left lateral lobes are the biggest** and yield comparable sections for histology.

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

16. **The gastrointestinal (GI) tract** should be all that remains of the abdominal contents at this point. Different methods to dissect, trim-in and section GI tract may be required for specific purposes. For a reproducible method to evaluate representative sections from different segments: Hold stomach in one hand and rectum (fecal balls) in other and stretch gently. The GI tract should stretch out but remain intact. Bluntly dissect off fatty tissues and pancreas including mesentery and lymph nodes. Place these **in toto** in a cassette (#5). Further trim-in usually is not required unless there are tumors or enlarged lymph nodes. If necessary tissues can be identified by spreading them out on dry paper.

17. With a 3 ml syringe/21g needle, infuse approximately ½-1 ml fixative gently into stomach, duodenum, ileum, cecum, colon. Unless analysis of contents of specific segments is required, tying off of these segments should not be necessary. The intact GI tract then can be submerged in fixative to facilitate identification of segments at trim-in.

D. **Decalcification/Demineralization of hard (boney) tissues:**

1. Simultaneous immersion fixation plus decalcification of mouse heads and bones can be accomplished with formic acid solutions such as TBD2 (Thermo Shandon), or Formical, Immunocal (Decal). Mouse tissues usually are sufficiently decalcified and fixed for trim-in by 24 hours, so that they can be trimmed with other fixed tissues. Advantages of this method are that it involves only a single solution; soft tissues and bones can be trimmed at the same time (sometimes <12 hours after dissection of small mice); and some immunohistochemical techniques may work. Other decalcification protocols are more complex (e.g. perfusion and/or prolonged immersion in Bouin’s solution; fixation followed by EDTA or nitric acid, but may be required for specific purposes). 50ml conical centrifuge tubes are useful for decalcifying a typical mouse head, limb and spine. The ratio of tissue to solution should be at least 1:10 (as with other fixatives) and the tissue should be submerged completely. Gentle agitation (e.g. rocker) may improve exposure to solution. Although these formic acid solutions are relatively gentle, mouse tissues may become over-decalcified within 48hrs. Over decalcified tissues are mushy and histology is uniformly 'pink', without cellular detail.

2. **Head** with skin removed can be placed directly into the decal solution. The tongue should have been removed during necropsy dissection (to facilitate removal of the pluck, and to examine the oral cavity and teeth. Skin and tongue will interfere with fixation and decalcification.

3. **Hind limb** with skin removed can be placed directly into the decal solution. Limbs can be saved in formalin for subsequent decalcification.

4. **Spine / vertebrae** (backbone) should be trimmed of extraneous tissue (e.g. ribs and body wall muscle) and placed intact into the decal solution. It is not necessary to remove paravertebral muscles as with larger animals.

5. **Sternum** usually does not require decalcification, and can be placed (marrow side down) in a cassette (#1). Cell detail of bone marrow is better when not decalcified. Most of its firm tissue is cartilage. But old, large or osteopetrotic mice may have boney sterna.

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

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

- **Cassette 1:** Thymus, sternum - **Add heart after trimming - see below.**
- **Cassette 2:** Lung, thyroid/larynx/trachea.
- **Cassette 4:** Salivary glands and associated lymph nodes.
- **Cassette 5:** Pancreas, mesentery, lymph nodes.
- **(Cassette 8: entire reproductive tract when it is small & fits nicely)**
Other tissues must be ‘trimmed’ to fit into cassettes for histologic processing. Trimming should be performed in a well ventilated area or hood.

- Used fixative should be discarded according to institutional guidelines for hazardous materials.
- After trimming, labeled cassettes should be submitted in clean formalin or alcohol depending on the processor schedule. Check with the histology lab and technician.
- Tissues to be saved should be kept in clean formalin.
- During trimming, tissues should be cut with a single clean swipe, not squished or sawed. Inexpensive single edge blades are suitable for trimming most tissues, and should be replaced as soon as they become dull so that tissue is not squished. Longer Weck-type blades facilitate trimming decalcified heads. These are sharper and have a longer cutting edge, to achieve a single clean cut.
- Decalcified tissues should cut easily (quietly). Crunchy tissues require additional decalcification. Mushy tissues are over-decalcified and the decal period should be reduced for subsequent similar specimens. Decalcified specimens should be rinsed in water (preferably distilled, deionized), and cassettes can be kept in saline ideally (or water) for histology processing within 48 hrs. Remaining, rinsed, decalcified tissue can be saved with formalin fixed tissues.
- Trimmed specimens should not 3mm thickness to fit into the cassette without creating grid marks and ‘squish artifact’.
- Systematic numbering of cassettes facilitates retrieval of archived material. The numbering system in Table 1 is an example. Lesions in an organ that require additional cassettes can be labeled with the same number followed by a, b, c, d, e.g. Liver tumors can be labeled 7a, 7b, 7c.

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

<table>
<thead>
<tr>
<th>Cassette #</th>
<th>Tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Heart (+ Thymus) (+ muscle e.g. tongue, diaphragm, soleus) (+ nondecalcified sternum if it fits)</td>
</tr>
<tr>
<td>2</td>
<td>Lung -- Entire, formalin-infused + Thyroid/larynx/trachea</td>
</tr>
<tr>
<td>3</td>
<td>Kidneys - Right/cross-section, Left /longitudinal section + Adrenals</td>
</tr>
<tr>
<td>4</td>
<td>Salivary glands with lymph nodes (ear to ear)</td>
</tr>
<tr>
<td>5</td>
<td>Pancreas + mesentery, lymph nodes (any enlarged nodes → 5a,b,c etc)</td>
</tr>
<tr>
<td>6</td>
<td>GI (Stom, Duod, Ileum, Cecum, Prox colon, Rectum)</td>
</tr>
<tr>
<td>7</td>
<td>Liver (Left Lateral + Median Lobe/G bladder sections) + Spleen (1 or 2 hemisections)</td>
</tr>
<tr>
<td>8</td>
<td>Female (Uterus + Ovaries, Vagina/bladder/rectum) Intact tract if small enough Male (Testes/Epi, Sem ves, Bladder/rectum/prostate)</td>
</tr>
<tr>
<td>9</td>
<td>Skin - dorsal neck + inguinal (mammary + clitoral/preputial gl) [+/- decal leg]</td>
</tr>
<tr>
<td>10</td>
<td>Decal Head: ears (pituitary, thalamus, hippocampus, cortex), eyes (olfactory lobes, molars), nose (olfactory + respiratory, incisors), cerebellum, medulla</td>
</tr>
</tbody>
</table>

**TRIMMING SUGGESTIONS** (trimming tissues into cassettes for paraffin processing)

1. **Cassette/slide #1:** The fixed heart can be hemisected (cut in half, longitudinally) to expose all chambers and valves, and both halves submitted. The right ventricle has a thinner wall than the left, and may ‘wrinkle’ slightly. With a little practice, the correct orientation is identified easily. For some purposes multiple cross-sections of heart are preferred. Additional tissues in cassette #1 may include thymus (inserted during necropsy dissection), sternum (non decalcified, marrow side down), representative muscle such as tongue (cross section of its thickest part), diaphragm.

2. **Cassette/slide #3:** The right kidney is transected (cross section), and the left kidney is cut longitudinally (longitudinal section) and placed in cassette (#3) with the adrenal glands. Kidney sections should include cortex and medulla. Submit 2-4 pieces (halves) of kidney if they fit easily.
3. **Cassette/slide #6:** The following GI tract segments can be included in a single cassette (#6). Swiss roll technique usually requires 2 cassettes. Lesions can be placed in separate cassettes 6a,b, c etc.
   a. Stomach - squamous (white) + glandular (pink) portions
   b. Duodenum with pancreas ~ 1 cm from pylorus, Ō-like cross section
   c. Mid-jejunum - several cm from pylorus, Ō-like cross section
   d. Ileum - from last 2 cm before cecum, Ō-like cross section, include lymphoid nodules (Peyer’s patch) if you identify them grossly
   e. Cecum - from the tip -- V- or U-like section, include pale lymphoid nodule (Peyer’s patch) if you identify it grossly
   f. Proximal colon - from 2 cm after cecum, 2 ŌO-like cross sections are nice to examine for protozoa and pinworms. Diagonal stripes discerned though the serosa are due to deep mucosal folds
   g. Distal colon/rectum - an o-like cross section from between fecal balls. Feces contain indigestible material that damages microtome knives, and are not appreciated by histology technicians

4. **Cassette/slide #7:** Cut the liver’s median lobe to include the gall bladder between the left and right parts. Cut a section of the left lateral lobe from hilus to edge. Hemisect spleen longitudinally/sagitally. The 2 liver sections and both spleen sections normally fit easily in 1 cassette.

5. **Cassette/slide #8:** female reproductive tract - when small may fit in toto in the cassette. Or trim to provide representative sections of ovaries and uterus, and 1 or 2 cross sections through rectum, vagina and urinary bladder demonstrat their anatomical relationships nicely.

6. **Cassette/slide #8:** male reproductive tract - when small may fit in toto in the cassette. Or trim to provide representative sections of testes, epididymis, seminal vesicle and coagulating gland, and a cross section through rectum and the neck of the urinary bladder to include prostate gland.

7. **Cassette/slide #10:** Decalcified head, External ear canal openings and eyes are primary landmarks. Lambdoid and coronal sutures also are useful landmarks. A right-handed prosector usually holds the nose in the left hand and cuts/sections with the right hand, starting with the most posterior/caudal section (1), and progressing anteriorly/rostrally. Use a single (1) clean stroke for each section.
   a. Cut on caudal then rostral side of ear canals for section 1 (cerebellum), and section 2 (middle and/or internal ear, pituitary, hippocampus).
   b. Cut on caudal then rostral side of eyes for section 3 (cerebrum), section 4 (eyes, Harderian glands, olfactory lobes, molar teeth), section 5 (nose, incisor roots).
   c. Long nosed (dolicocephalic) mice may require a 5th cut.
   d. Place sections 1 and 2 front/rostral down in the cassette.
   e. Place sections 3,4,5 back/caudal down in the cassette.

---

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

Adapted from Paxinos & Franklin 2001

Adapted from Popesko et al. 1992. Vol 2
Figure 2. Decalcified Mouse head - 5 transverse histology sections. Numbered in order of cut, caudal to rostral: 1. Cerebellum; 2. Ears/hippocampus/Pituitary; 3. Cerebrum; 4. Eyes, oral cavity; 5. Nose.

8. Cassette/slide #11: Decalcified hind limb - remove lateral muscles by a clean cut so that the knee and long bones can be seen on the flat (cut) surface, and minimal sectioning by the microtome will enter the bone to result in a useful histologic section. One specimen can include femur and knee joint. The other specimen can include tibia and tarsus (heel joint). Toes with claws and fur interfere with decalcification, damage microtome knives, and tend not to be appreciated by histology technicians.

9. Cassette/slide #12: Pathologists or investigators may have very specific methods for evaluation of spinal cord and vertebrae. For simple survey screening of bone, musculature and spinal cord, cervicothoracic and lumbosacral specimens can be accommodated in 2 cassettes. Cut transverse sections at each end of the cervicothoracic and lumbosacral segments, then hemisect each segment (split it longitudinally) and put the 'best” half in the cassette, along with the cross sections from the same segments. Biopsy ink or a distinctive nick in the section can be used to identify left or right sides, if desired.

Suggested References
The Virtual Mouse Necropsy -- http://www.niaid.nih.gov/dir/services/animalcare/MouseNecropsy/Necropsy.html
EULEP Mouse Necropsy http://www.eulep.org/Necropsy_of_the_Mouse/index.php
RENI Tissue trimming guide http://reni.item.fraunhofer.de/reni/trimming/index.php
### Mouse Lymph Nodes

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

<table>
<thead>
<tr>
<th>#</th>
<th>English name</th>
<th>Official name</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. ischiadic</td>
</tr>
<tr>
<td>9</td>
<td>Popliteal ln.</td>
<td>Ln. popliteus</td>
</tr>
<tr>
<td>10</td>
<td>Cranial mediastinal ln.</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. gastric</td>
</tr>
<tr>
<td>14</td>
<td>Pancreaticoduodenal ln.</td>
<td>Ln. pancreaticoduodenalis</td>
</tr>
<tr>
<td>15</td>
<td>Jejunal ln.</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>

### Mouse Mammary Glands

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

**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 5. Mouse Body Condition scoring (from Ullman & Foltz. 1999)

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, 2-...).
ANATOMIC PATHOLOGY SUBMISSION FORM

Necropsy / Biopsy Date ________________  Prosector ____________  DCM # ____________
Investigator / Dept ____________________  Contact ____________  Contact # ____________
Research ☐  Diag ☐  Teaching ☐  Charge #: ________________  Room # ____________

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

Ancillary Tests (check ‘✓’ applicable boxes) Other: ______________________________________

<table>
<thead>
<tr>
<th>CBC / Diff</th>
<th>Clin Chem</th>
<th>UA</th>
<th>Serology</th>
<th>Micro</th>
<th>Fur</th>
<th>PWP / OP</th>
<th>Urinalysis</th>
<th>Behav JW</th>
<th>Imaging</th>
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</tbody>
</table>

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

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

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

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

<table>
<thead>
<tr>
<th>Body Wt (g)</th>
<th>Liver (g)</th>
<th>Spleen (g)</th>
<th>▼ (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ID Tags / #’s (save with tissues if possible): ________________  Ear Punch: R ☐ ☐ ☐ L

Dorsum

L R

Ventrum

L R

Right

L R

Left

More information + updates at http://www.hopkinsmedicine.org/mcp/PHENOCORE/
### Cassette # | Tissues (Slides 1-10 = standard necropsy)
--- | ---
1 | □Heart + Thymus [+/- sternum, diaphragm, tongue, soleus]
2 | Lung --- entire, formalin-infused + thyroid/trachea
3 | Kidneys - right/cross, left /long + Adrenals
4 | Submandibular + parotid salivary glands + L nodes
5 | Pancreas + Lymph nodes (mesenteric chain + any enlarged \(\rightarrow\) 6a,b,c etc)
6 | GI (Stom, Duod/panc, ileum, Cecum, Prox colon, Rectum)
7 | Liver (L Lateral+ Median Lobe/G bladder) + Spleen
8 | Female (Uterus + Ovaries, Vagina/bladder/rectum)
9 | Male (Testes/Epi, Sem ves, Bladder/prostate /rectum)
10 | Skin - dorsal neck + inguinal (mammary + clitoral/preputial gl)
11 | Decal Head  ears (pituitary, thalamus, hippocampus, cortex), eyes (olfactory lobes, molars), nose (olfactory + respiratory, incisors), cerebellum, medulla
12(a,b,c...) | Spine
13 | Lesions:

---

**Mouse Lymph Nodes adapted from Dunn 1954 (+Van den Broeck, et al. 2006)**

1. Superficial cervical (or mandibular) Nodes
2. Deep cervical node
3. Mediastinal Nodes
4. Axillary Nodes
5. Branchial Nodes
   A. Thymus
   B. Spleen
6. Pancreatic (or pancreaticoduodenal) Node
7. Renal Nodes
8. Mesenteric (or jejunal) Nodes
   9. Inguinal (or iliac) Node
   10. Lumbar Nodes
   11. Sacral Nodes
   12. Sciatic Nodes
   NS Popliteal Node - Behind Knee

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Additional information (continued from front page)

---

Submitted: ___ / ___ / ___  
Completed: ___ / ___ / JHU Phenol#5012lab Manual P 29

---
PROCEDURE SUMMARY

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

I. MATERIALS

1. Prosector (recorder, photographer are nice too)
2. Relevant records & report forms
3. PPE
4. Lab coat or other protective uniform
5. Measuring tools
6. Fxative and decalifying solutions
7. Fixative
8. Decal

I. MATERIALS (CONTINUED)

9. Fixatives:
   - NBF = 10% neutral buffered Formalin
   - Bouin's solution, Zenker's solution
   - Other options
     - Bouin's, Fekete's, Telly's etc acid alcohol
     - PFA – paraformaldehyde – for ISH etc

I. MATERIALS (CONTINUED)

10. Decalifying Solutions (Decal)
   - Nitrical – Not a fixative – strong, fast
   - Decal Stat – Not a fixative, strong < 1hr!
   - Decal – Not a fixative, not as fast - overnight.
   - Immunocal – Not a fixative, gentler? overnight
   - Formalin – Not a fixative, not as fast - overnight

I. MATERIALS (CONTINUED)

11. Fixatives
   - Sodium phosphate, monobasic, monohydrate 4.0 g
   - Sodium phosphate, dibasic, anhydrous 6.5 g
   - dH2O to 1 liter

I. MATERIALS (CONTINUED)

12. Cutting Board
13. Paper Towels
14. Instruments
15. Scissors (fine-blunt & student grade)
16. Blades
17. Syringes and needles
18. Specimen containers
19. Labeled cassettes (1-10)
20. Markers

I. MATERIALS (CONTINUED)

1. External Examination
2. Check records, identification - correct animal?
3. Weight
4. EXAM - measure, quantify whenever possible
5. Palpate - measure, quantify whenever possible
6. Body condition
7. Abdominal fluid (obtain sterile sample)
8. Abdominal mass (soft, fluctuant, firm, hard)
9. Skin - Subcutaneous mass
10. Head – decalcified

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, test, urinary bladder, (+/- rectum)
9. Skin, critical prep, distal limb (+/- Decal leg)
10. Head – decalcified

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, test, urinary bladder, (+/- rectum)
9. Skin, critical prep, distal limb (+/- Decal leg)
10. Head – decalcified

Necropsy ppt P 1
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Lab 3 Forbes Brayton Necropsy PPT
IV. DISSECTION (ANIMAL ORIENTATION)

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

What color? What sex? What's your diagnosis?

- Agouti, Female, Vaginal prolapse

IV. DISSECTION (PELT REMOVAL A)

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

What color? What sex? What's your diagnosis?

- Female, Vaginal prolapse

IV. DISSECTION (PELT REMOVAL B)

- Pull anterior skin up to forelimbs
- Pull anterior skin up to ears
- Pull anterior 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

- Female's clitoral gland usually is smaller than male's preputial gland

What color? What sex? What's your diagnosis?

- Agouti, Female, Vaginal prolapse

IV. DISSECTION (PELT REMOVAL C)

- Pull posterior skin down to hind limbs
- Pull right and left hind limbs separately out of skin by holding at 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
- Record abnormalities, including location & size

- Posterior skin incised on DORSAL surface

What color? What sex? What's your diagnosis?
IV. DISSECTION: EXAMINE THE MOUSE

What do you see?

**Normal (WNL):** ample body fat, ample ingesta, fecal balls

**Abnormal:** Prolapse, Splenomegaly; Pale; probably exsanguination

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IV. DISSECTION (SALIVARY GLANDS)

Female's salivary glands are smaller than male's.

---

IV. DISSECTION (THORAX - A)

**Incise Peritoneum**
- Open to examine abdominal contents

**Remove Sternum**
- Hold xiphoid process w/ forceps
- Spread remaining ribs to open thorax and expose thoracic contents
- Lay sternum flat, internal/marrow side down
- Place in cassette #1, close, fix

**Remove Thymus**
- Place in cassette #1, close, fix
- Note/record size or absence of thymus

---

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

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**Necropsy ppt P 3**

JHU Pheno 2012 Lab Manual P 32
IV. DISSECTION (EN BLOC REMOVAL)

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

IV. DISSECTION: SPLIT MANDIBLE

IV. DISSECTION: TONGUE

IV. DISSECTION: REFLECT CAUDALLY

IV. DISSECTION: AT THE DIAPHRAGM

IV. DISSECTION: VISCERA EN-BLOC

IV. DISSECTION: SPLIT PELVIS

Necropsy ppt P 4

JHU Pheno 2012 Lab Manual P 33
IV. DISSECTION: ORGANS EN BLOC (DORSUM)

Identify:
- Lungs
- Liver
- Stomach
- Splenic (liver) left
- Kidneys
- Intestine and reproductive tract

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

Liver: Clean extraneous tissue from liver

Spleen: remove and clean off extraneous tissue

Kidneys: Remove with adrenals attached

Adrenals: still attached

Intestine and reproductive tract

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IV. DISSECTION: FEMALE REPRODUCTIVE TRACT

Ovaries

Uterine horns

Vaginas

Urinary Bladder

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IV. DISSECTION: MALE REPRODUCTIVE TRACT

Seminal vesicles

Coagulating gland

Bladder

Prostate

Vas deferens

Epididymis

Testes

Urethra

Penis

45

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

Stomach: Check for missing pieces, ovary, bladder, etc

Remove all fat etc

Spread out flat on paper towel. Fix in toto in container

Pancreas: remove and clean off extraneous tissue

Kidneys: weighing and fix (in toto in container)

Spleen: separate lobes, fix (in toto in container)

Liver: remove and clean off extraneous tissue

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IV. DISSECTION: CASSSETTE 2

Lungs

Trachea

Larynx (parotid glands)

Eosophagus

Thymus remnants

Intestine

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IV. DISSECTION: ORGANS EN BLOC (A)

Heart – WEIGH IT

Tongue

Lungs with trachea, larynx, lymph nodes, etc

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IV. DISSECTION: ORGANS EN BLOC (B)

Kidneys:
- Remove with adrenals attached
- Remove extraneous tissue from kidneys
- Weigh, fix (in toto in container)

Spleen: remove and clean off extraneous tissue

Liver: Remove all lobes

Confirm all lobes (check for small quadrates with stomach)

Remove diaphragm etc from median lobe

Weigh all lobes together

Separate lobes, fix (in toto in container)

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IV. DISSECTION: ORGANS EN BLOC (C)

Reproductive Tract:
- Separate from GI tract
- Check for missing pieces, ovary, bladder, etc
- 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/mesentary: trim from GI tract

Stomach: Check for missing pieces, ovary, bladder, etc

Remove all fat etc

Spread out flat on paper towel. Fix in toto in container

Pancreas, mesentery, etc: remove and clean off extraneous tissue

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Identify
1. Stomach
2. Cecum
3. Fecal Balls

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

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

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
   - 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/](http://reni.item.fraunhofer.de/reni/trimming/)

Cassette 1

- Thymus - already in cassette
- Sternum - Place marrow (in) side down in cassette
- Tongue - Section Longitudinally, put half in cassette
- Heart - Hemi-sect sagitally to see all chambers
  - Put both halves in cassette
- Close cassette and re-submerge

Cassette 2

- Trachea, larynx, thyroid
  - Remove extra tissue with forceps
  - Locate thyroid below larynx
  - Transect trachea below (distal to) thyroid
  - Place larynx/thyroid into cassette
  - Hoping for cross section
- Lungs - Orient dorsal side down into cassette
- Close cassette and re-submerge

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
  - Place sections in cassette Cut side down
  - If using biopsy foam, lay sections on 1 pad and cover with 2nd pad
  - Close cassette and re-submerge

Cassette 4

- Salivary glands and cervical – submandibular lymph nodes
- Should be in cassette already
  - Submandibular Glands
    - Males’ should be bigger than females’
  - Sublingual Glands
  - Parotid Glands
  - Exorbital Lacrimal Glands
VI. TRIMMING TISSUES INTO CASSETTES

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 → 0 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 → 0 on cross section
- Distal Colon (fecal balls usually)
  - 2 sections 4-5mm long → 0 on cross section
- Close and submerge cassette

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
  - Representative areas
    - Mid-sagittal inguinal – to include preputial or clitoral gland, external reproductive and rectal orifices
    - Craniofacial- dorsal neck - to include periocular, periauricular, perioral skin
    - Lesions
  - Leg – decalcified – sometimes included

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.

CASSETTE 10

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

Cassette 10 - Summary

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

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Lab 3 Forbes Brayton Necropsy PPT
Aims of perfusion:
- To use the vascular system of an animal 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
Method

1. Open Vacutainer packet and cut 3-5mm off plastic needle guard with razor.

This cuff prevents the needle from going through the heart.

2. Euthanize animal, weigh and record weight in grams.

3. Remove pelt at costal margin and reflect rostrally.

4. Cut through ribs to expose thoracic cavity.

Retention sternum if you plan to evaluate sternum marrow.

5. Fill 20ml syringe with saline-heparin.

6. Fill 20ml syringe with 10% NBF.

7. Attach blood collecting set to saline-heparin syringe and prime.

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

Method (continued)

5. Fill 20ml syringe with saline-heparin.

6. Fill 20ml syringe with 10% NBF.

7. Attach blood collecting set to saline-heparin syringe and prime.

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

9. Carefully insert needle (attached to saline heparin syringe) into left ventricle.

10. Slowly inject saline heparin (or other fixative) and watch for muscle contraction and total body stiffening.

11. Without retracting needle, remove the saline heparin syringe and attach the 10% NBF syringe.

12. Slowly, over > 1 minute, inject 10% NBF (or other fixative) and watch for muscle contraction and total body stiffening.

Incising right atrium (video)

10% NBF Perfusion (video)

Incising right atrium (video)