Mouse Necropsy

Plan for the necropsy so that it is efficient and before autolysis occurs – mouse organs die really quickly, so using the following sequence of steps will help you to do one animal in about 15-20 minutes

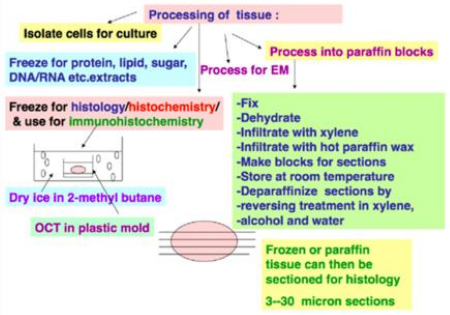
Procedure

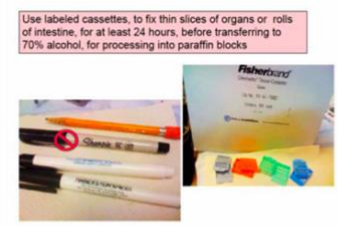
1. Label cassettes with #2 pencil so that the labeling does not disappear during processing: **mouse ID** in front, **date** on one side, and **PI name** on the other side.
2. Fill to ¾ of volume, an empty about to be discarded tissue culture bottle with 10% neutral buffered formalin (there is a bottle in the fume hood at the end of Bay MM, but if you have some that will help).
3. Remember 10 volumes of fixative (the old tissue culture bottle holds up to 20 cassettes).
4. Place blue sponges in the cassettes which will receive the spleen and pancreas, so that those organs (which tend to curl) will stay flat and thus easier for the histotechs to embed and obtain good flat sections.
5. Anesthetize and euthanize in the approved mouse chamber.
6. Spray carcass with 70% alcohol to prevent hair from flying around and causing allergies.
7. Insert scissors into the skin around the abdomen and open using the Y-shaped incision.
8. Reflect the skin up above the chin to expose the salivary glands and then dissect them out and place in a labeled cassette. Salivary glands usually have lymph nodes attached underneath. The same cassette can be used to hold the liver and ½ of each kidney.
    a. **One cassette will hold the salivary glands, liver, ½ of each kidney, and heart (added during step 10)**.
9. Hold the spleen and pull up to get the fatty looking pancreas. And place both in the cassette with the blue sponge.
    a. One cassette will hold spleen and pancreas
10. Expose the trachea and inject about 1 mL of fixative into the lungs and you will get your thrill for the day when you see the lungs expanding. Remove the lungs (ideally you should separate out the lobes so you can look at pathology in each lobe).
    a. One cassette will hold the lungs
    b. Separate the heart away and place in the cassette with the salivary glands, liver, and kidney
11. The intestines (small and large) can be cut up into short segments. Try to remove the fecal matter by squeezing the tube (fecal matter hardens during processing and can be problematic to the histotechs). If you can see the Peyer's patches—those will help with lymph node morphology.
    a. One cassette with the intestine
12. Let the organs fix overnight and change to 70% alcohol and send to histology with the Request form filled out containing the chart string information.

Optimal harvesting and processing mouse organs for histopathological examination

General points:

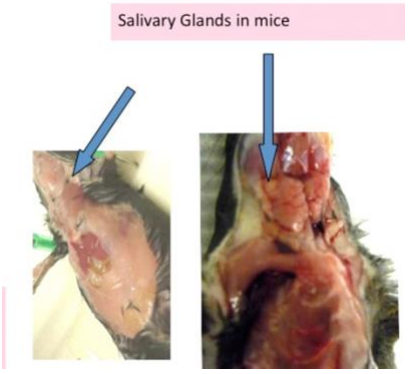
When tissues are removed from the body, during surgery, or during an autopsy, prompt onset of autolysis occurs which can inhibit efforts to isolate nucleotides or certain enzymes and proteins for various investigative efforts. The tissue thus has to be flash-frozen for extracts, or frozen in cryoprotective agents, or fixed, using different procedure-dependent fixatives, for analysis in histo-pathology as shown in Figure 1.





The THIN slices of tissue are then placed FLAT into plastic cassettes (Fisher Catalog #15-182-500E)LABELED with #2 pencil or Secureline II pens - Fisher Cat.# 1490530. DO NOT use Sharpies or lab markers, and immerse in fixative that is at least 10 times the volume of the tissue to be fixed. The cassettes will hold tissues in fixative for less than 24 hours, when they should be transferred into 70% alcohol, until they are processed, embedded in PARAFFIN WAX, and sectioned into 3-5 micron thin slices, onto COATED glass slides, using a microtome, at room temperature, for staining, and analysis of morphologic detail.

Use a #2 pencil to be sure none of the labels wash off during processing in alcohol

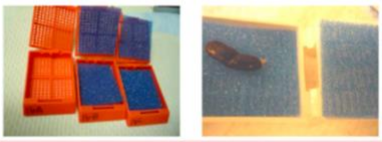


Mouse salivary glands are under the chin right after you open up the skin

Specific requirements for Skin, Spleen, Thymus, Pancreas, Adipose tissue to obtain optimal morphology.

These particular organs need to be flattened between histology sponges into cassettes, before fixing, in order to obtain the best sections for staining analyses.

This flattening before fixation allows these tissues to be oriented, such that the entire section will show large areas for analysis. The plastic cassettes that are used for fixing should be labeled with an indelible marker, Do not use a "Sharpie", which is soluble in the organic solvents which are used for processing.



Certain tissues if placed on sponges allow orientation before fixation. Label cassettes simply with an indelible pencil.

Figure 8

Flatten the spleen between sponges or it will curl up and they will not be able give you a good section to analyze

Figure 5 then shows the difference between un-inflated lungs on the left, in contrast to the image on the right, which shows inflated lungs, which are almost enclosing and surrounding the heart and pressing down on the diaphragm.

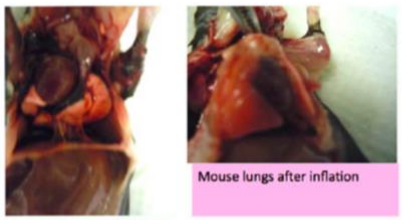


Figure 5

Inflate the lungs with fixative for processing and paraffin embedding or with 1:1 OCT: PBS for freezing

PANCREAS

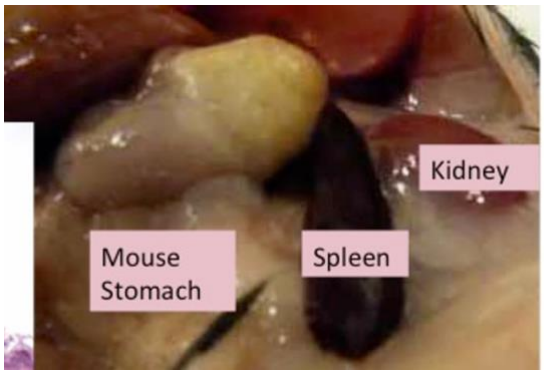
The PANCREAS is the fatty appearing structure that appears adherent to the hilus of the spleen. Remove as much of the tan colored mass, closest to the spleen (this area usually contains a lot of the islets) and place BETWEEN two sponges in an appropriately labeled embedding cassette BEFORE FIXING in buffered formalin. Good flat paraffin sections of this organ are more difficult to obtain if not fixed in this manner.

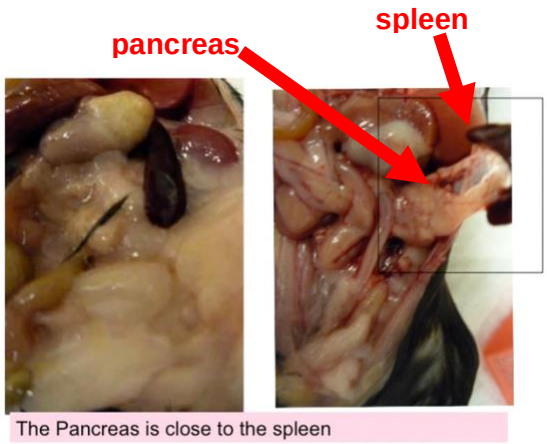
KIDNEYS Remember to carefully remove the two ADRENALS before dealing with the Kidneys. The adrenals also need special attention, because they are so small and will need to be placed in separate cassettes, perhaps with the OVARIES, which are also small. The Ovaries can be isolated at the same as time as harvesting the Adrenals and may not be easily visualized becasue they are surrounded by adipose tissue, in adjacent areas, (or by following the Uterine horns up from the pelvis).

The KIDNEYS should be cut along the middle, into two parts, to reveal cortex and medulla and arcuate arteries, so that similar areas will be comparable, between littermate controls and in mutant animals, for microscopic observation. Embed both sides flat surface down, onto the bottom of the embedding cassettes, so that the organ is fixed while flat and thus the sections will reveal better morphologic relationships

Remember to request that sections be made at less than 3 microns if possible, to ensure adequate examination of glomeruli. Additional special stains may be requested as well as immunohistochemistry

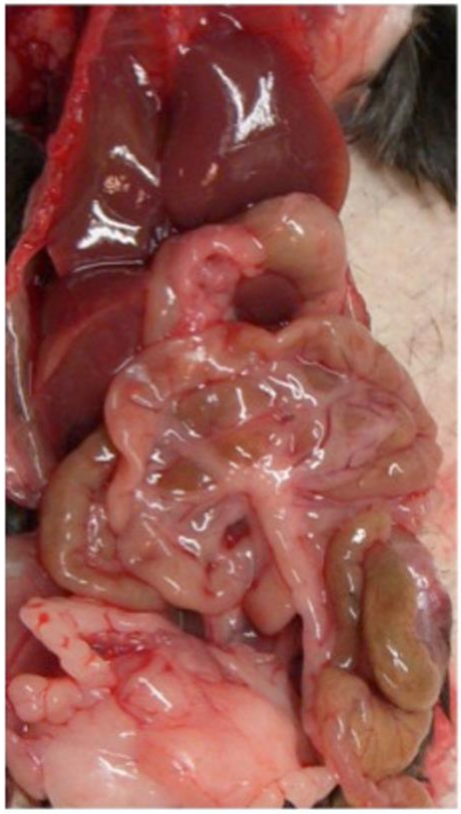
Slice each kidney in half so that the section will go through the middle to reveal any pathology





The Pancreas is close to the spleen

Pull up the spleen and the Pancreas is the fatty appearing organ attached to the spleen

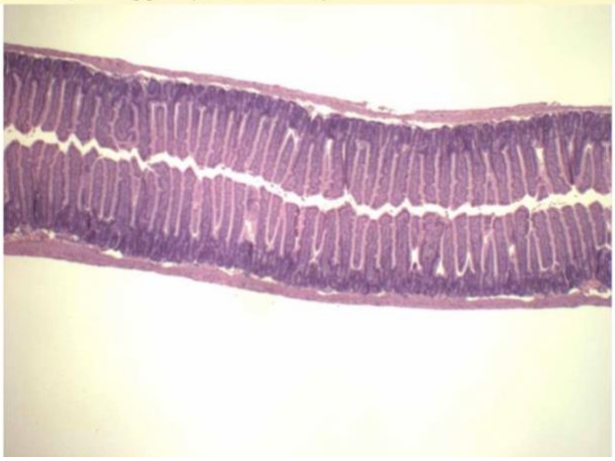


The mouse liver is under the diaphragm and the large organ which occupies the upper half of the abdomen you will see as you open the abdomen

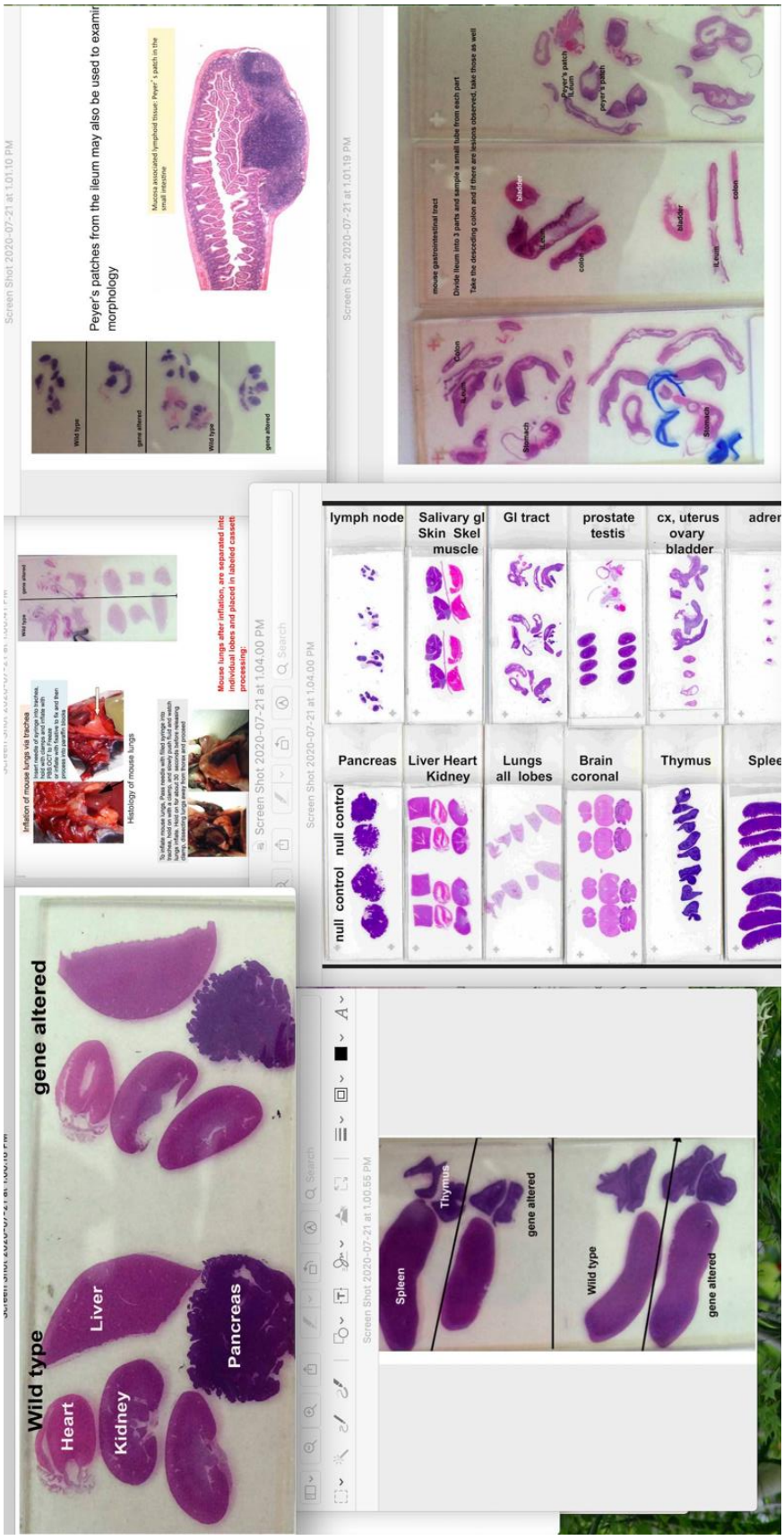
Take the large lobe on top and place in the same cassette as the two halves of the kidneys

Pancreas and spleen go between sponges in a separate cassette

Mouse small intestine samples which were not rolled, but embedded carefully as a tube, showing good preservation of tips of all villi



The intestines can be sampled as though they are short segments of TUBES—
that is sufficient for a quick survey


Peyer's patches from the ileum may also be used to examir
morphology
Wild type
gene altered
Mouse lungs after inflation, are separated into
individual lobes and placed in labeled cassett
processing:
Histology of mouse lungs
lymph node
Salivary gl
Skin Skel
muscle
GI tract
prostate
testis
cx, uterus
ovary
bladder
Pancreas
Liver Heart
Kidney
Lungs
all lobes
Brain
coronal
Thymus
null control
null control
Wild type
Heart
Kidney
Liver
Pancreas
gene altered
Spleen
Thymus
gene altered
Wild type
gene altered

References

1. Parkinson CM, O'Brien A, Albers TM, Simon MA, Clifford CB & Pritchett-Corning KR (2011). Diagnostic Necropsy and Selected Tissue and Sample Collection in Rats and Mice. J Vis Exp. (54): 2966. PMID: 21847084. PMCID: PMC3211129. DOI: 10.3791/2966.
2. Treuting PM & Pettan-Brewer C (2011). Practical pathology of aging mice. Pathobiol Aging Age Relat Dis. *1*. PMID: 22953032. PMCID: PMC3417704. DOI: 10.3402/pba.v1i0.7202.
3. UC Davis – Mouse Virtual Necropsy: http://tvmouse.ucdavis.edu/virtualNecropsy/

For additional help with mouse necropsy:

http://eulep.pdn.cam.ac.uk/Necropsy_of_the_Mouse/index.php?file=Chapter_4.html#1