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 nucleic acids or certain enzymes and proteins for various investigative efforts. This tissue thus has to be flash-frozen for extracts, or frozen in cryoprotective agents, or fixed, using different procedure-dependent features, for analysis in histopathology as shown in Figure 1.

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

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

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

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


For additional help with mouse necropsy: