Summary of Optimal Harvesting and Processing of Mouse Organs for Histopathological Examination

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

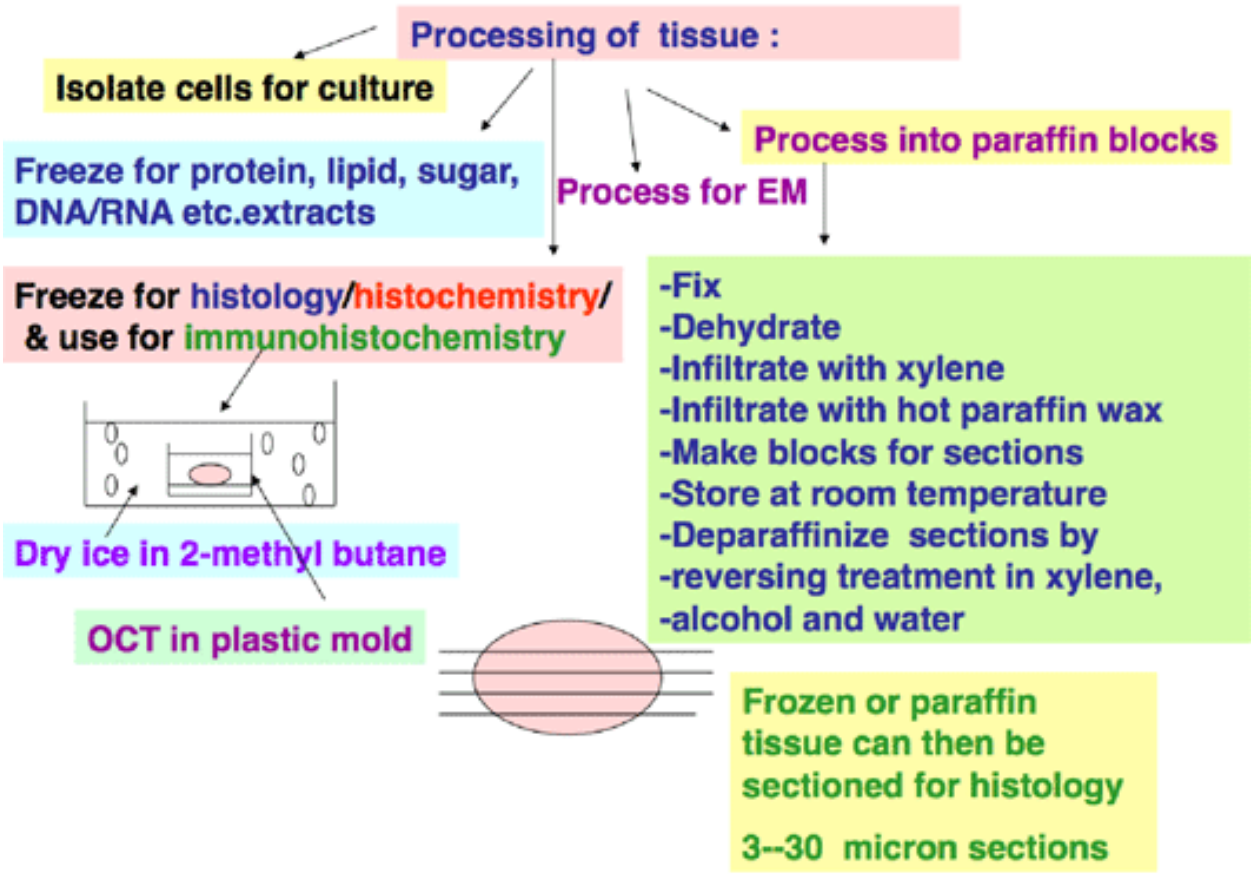

Figure 1. The first step of processing tissue for histopathological analysis is to flash-freeze for extracts, freeze in cryoprotective agents, or fix using fixatives.

Optimum Cutting Temperature Compound (OCT) is a commonly used cryoprotective agent to surround tissues prior to flash-freezing.

If tissues are placed into the freezer without using cryoprotective agents, the water molecules freeze at different rates from the freezing of the tissue.

When one then attempts to make frozen sections for use in histology or immunohistochemistry, the tissue will look “broken” and shattered with spaces where antibodies will congeal, contributing to non-specific background.

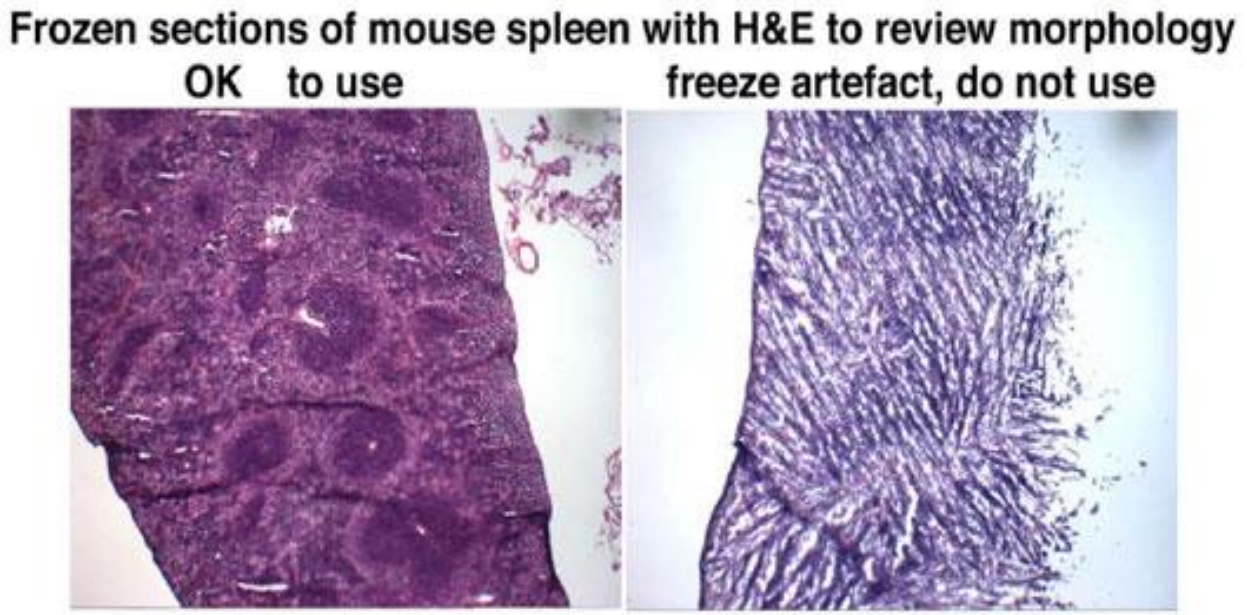

Figure 2. Use of cryoprotective agents are critical. Image demonstrates the poor morphology of a poorly frozen mouse spleen stained with hematoxylin and eosin.

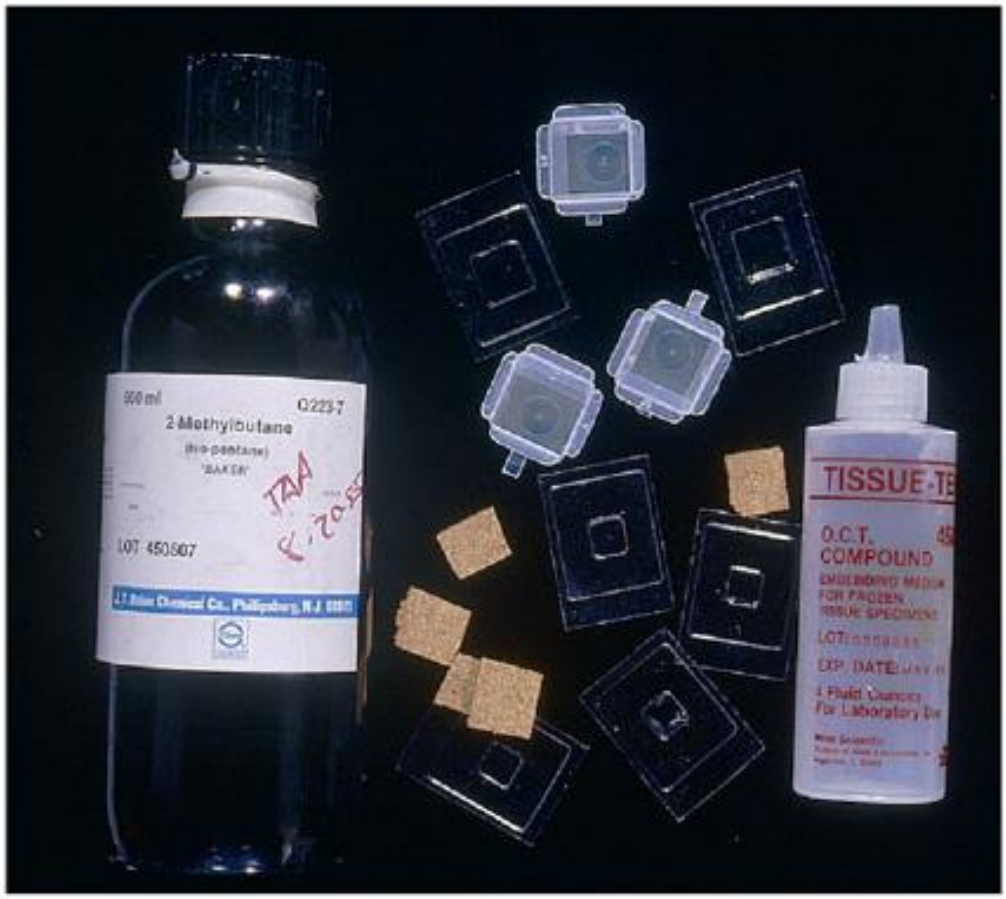

Figure 3. Materials needed to freeze fresh tissue.

Specific Requirements for Different Organs Harvested from Mice

Mouse Lungs

For the best morphological analyses, **it is best to inflate the lungs with fixative or with the freezing medium**. Although human lungs are large in comparison to mouse lungs, if they are inflated with fixative, it is possible to analyze morphologic detail optimally. Since mouse lungs collapse when the thorax is opened it is important that, after identifying the cartilage-ringed trachea, the lungs are inflated in order to obtain the best histological detail.

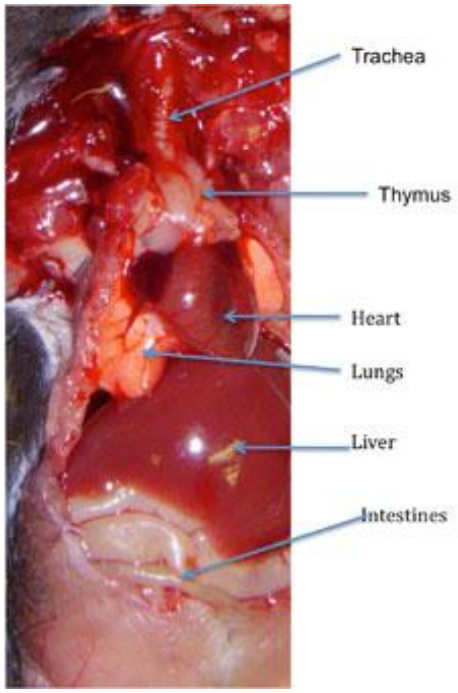

Figure 4. Open thorax of the mouse with the trachea, thymus, heart and lungs in the thorax with a partially opened abdominal cavity showing the liver and some of the intestines.

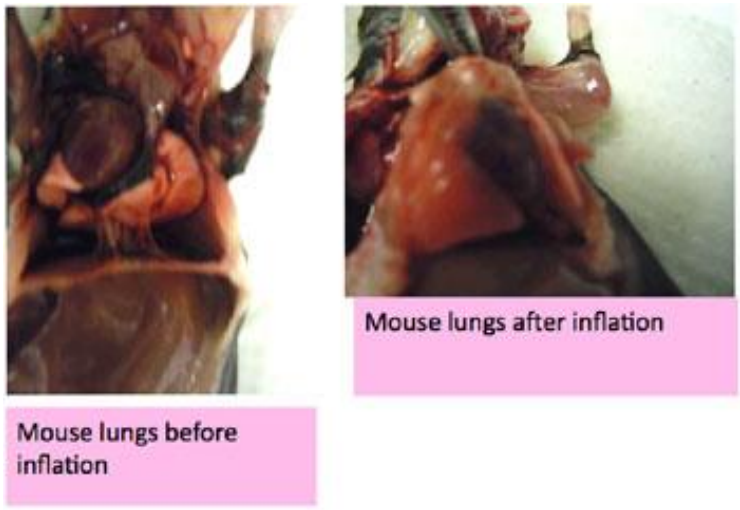

Figure 5. Lungs on the left are uninflated, in contrast to the image on the right, which shows inflated lungs that are almost enclosing and surrounding the heart and pressing down on the diaphragm.

If the mouse lungs are to be used as frozen sections it becomes even more important to inflate the lungs with a 1:1 mixture of freezing medium in buffer, before freezing for long-term storage.

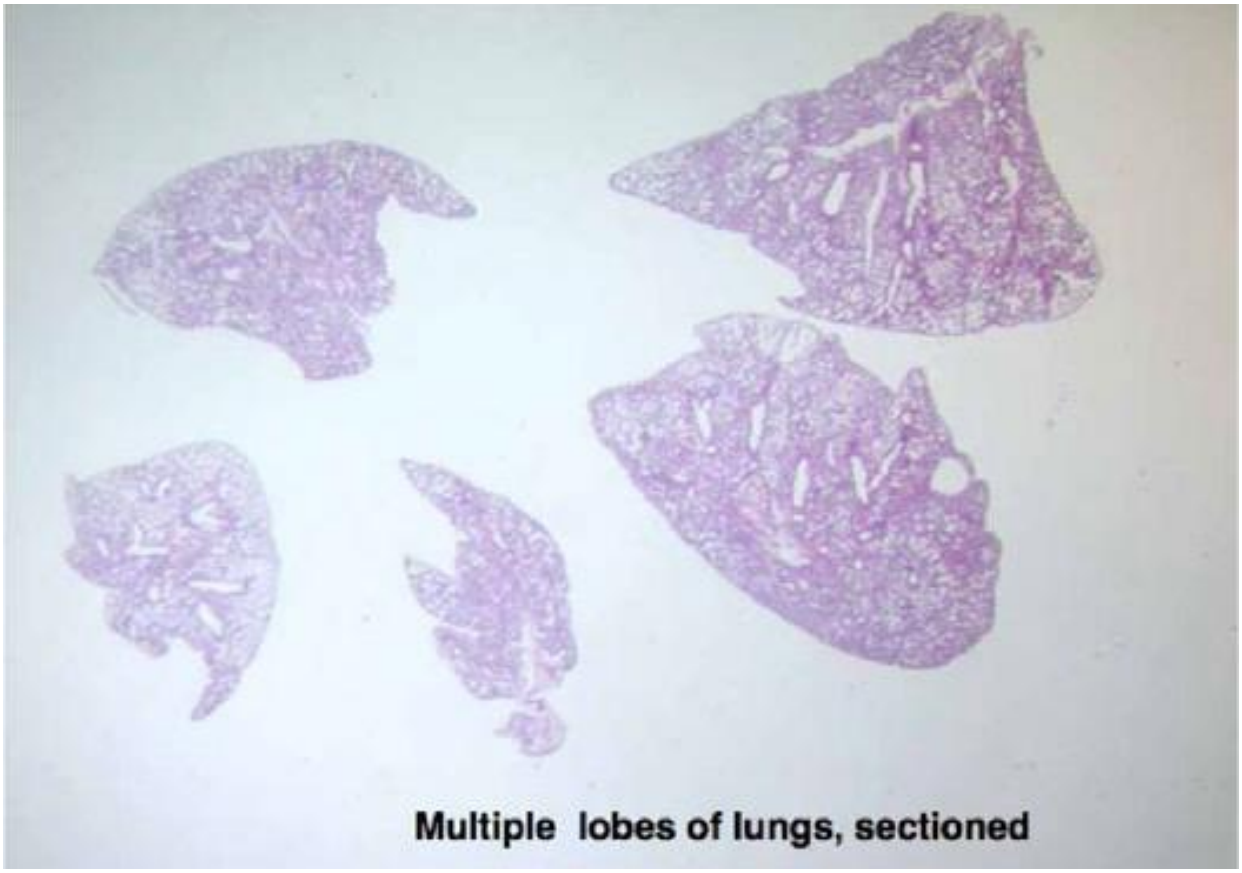

Figure 6. Stained sections of mouse lungs after each lobe has been separated and embedded before sectioning and staining.

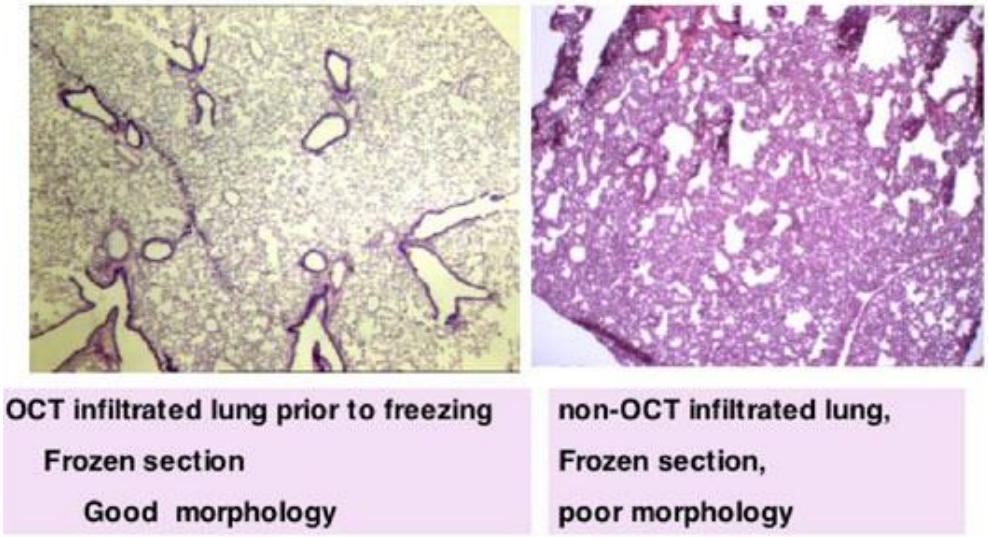

Figure 7. Left panel shows good morphology of mouse lungs that were frozen after inflation. Right panel shows poor morphology on sections from non-inflated mouse lungs.

Skin, Spleen, Thymus, Pancreas, and Adipose Tissue

In order to obtain the best sections for staining analyses, these particular organs **need to be flattened between histology sponges into cassettes *before* fixing**. 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” – the ink is soluble in the organic solvents used for processing.

Figure 8. Certain tissues, if placed on sponges, allow orientation before fixation. Label cassettes simply with an indelible marker or pencil.

Similarly, if skin is flattened before fixing, the histotechnician will correctly orient it while embedding it into paraffin wax, after processing.

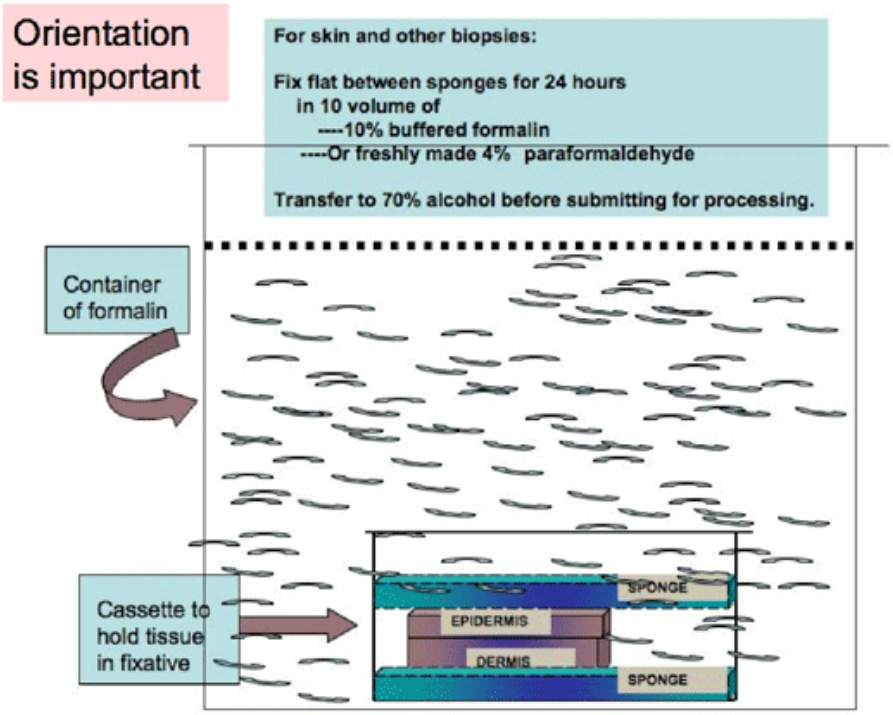

Figure 9. Orientation is important for skin specimens.

Tumors

Like most organs, tumors are three-dimensional. Therefore, before fixing, it is best to make thin slices and then flatten in correctly and simply labeled cassettes.

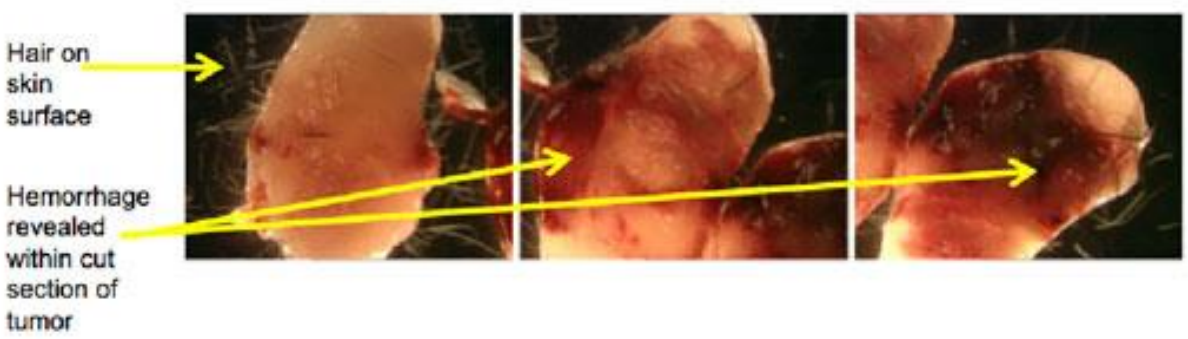

Figure 10. Tumors should be sliced thinly prior to fixing and then flattened in cassettes for fixing.

Intestine

The intestinal mucosa is extremely sensitive to prolonged periods of drying and thus must be fixed with as much haste as possible. Small segments are opened and rolled onto a stick, before immersion fixation, for processing, embedding, sectioning, and staining.

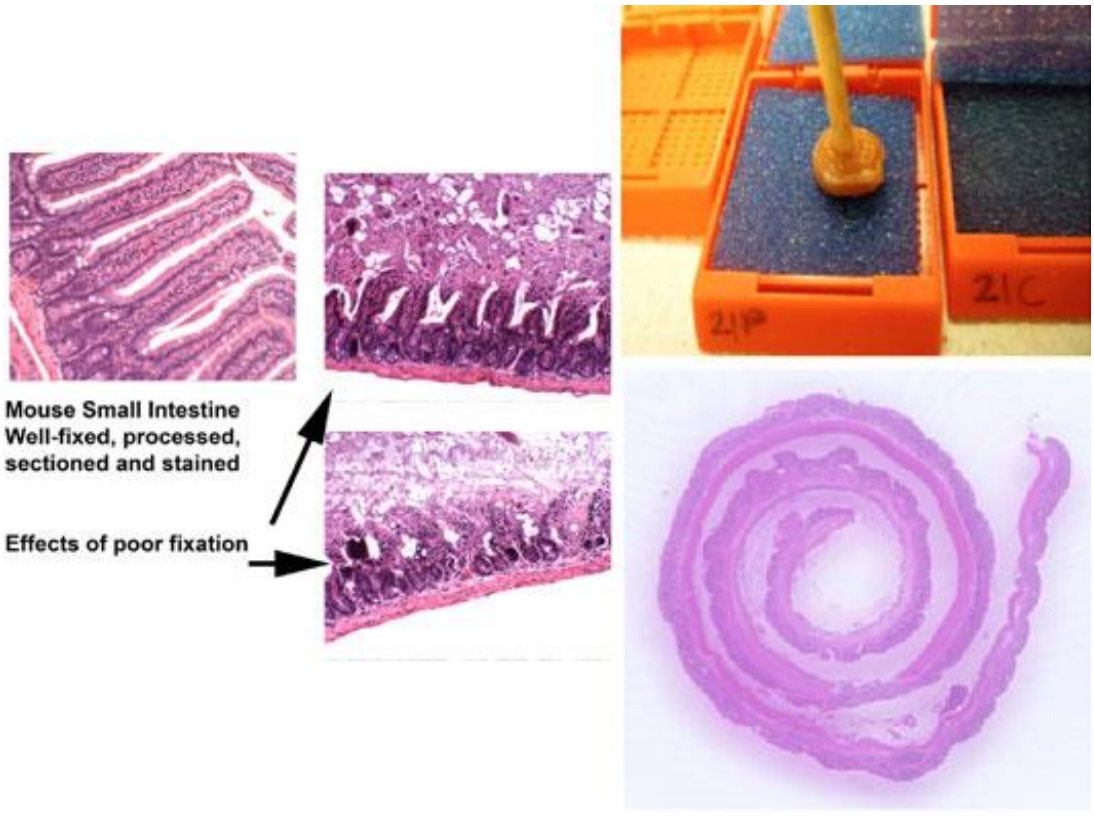

Figure 11. Preparation of intestine involves opening and rolling small sections onto a stick prior to immersion fixation.

Commonly Used Fixatives

The most commonly used fixatives are 10% neutral-buffered saline, zinc formalin, and Bouin's fixative solution.

> Fix THIN slices of tissue in 10 times its volume of fixative for AT LEAST 24 hours.
>
> Take the cassettes with the enclosed tissue in fixative to the histology lab (let them know ahead of time) so that the tissues can be processed immediately and embedded into paraffin blocks for paraffin sectioning.

Freshly made 4% paraformaldehyde is preferred for small samples and also fixes well.

> Tissues should be fixed for LESS THAN 24 hours before being processed and embedded into paraffin for sectioning and staining.

Figure 12. Commonly used fixatives. Left, 10% neutral-buffered formalin. Center, zinc formalin (requires special processing). Right, Bouin's fixative solution (quick, but hardens tissues if fixed for too long; move specimens to 70% ethanol after 6 hours).

Different fixatives are used for different purposes:

1. **10% buffered formalin** is the most commonly used fixative (Fisher Catalog#SF93-4). However, this fixative penetrates into the tissue **very slowly** (1 mm per hour), so the tissue MUST BE SLICED THINLY in order for the fixative to do its job properly.
2. **FRESHLY MADE 4% paraformaldehyde** also fixes well for perfusion fixation and renders good morphologic preparations.
3. **Bouin's fixative** fixes fast, and is especially useful when fixing embryos that have been opened a little to allow the fixative to infiltrate. However, because it fixes so well, it MUST be removed from contact with the tissue after about 6 hours of fixation. The tissue MUST be rinsed in 70% alcohol until the yellow color is not discernible.
    a. If tissues are allowed to fix in Bouin's solution for too long, they will become hard and brittle, and challenging to section, resulting in poorly prepared sections
4. **Zinc-containing fixatives** are used to enhance immunohistochemistry assays.

Figure 13. Decalcification of bones is important before processing and embedding into paraffin blocks for sectioning and staining. Using EDTA for decalcification takes longer but is advantageous in allowing the detection of antigens in immunohistochemistry assays.