**Pancreas tissue fixation and block prep**
Penn Medicine BioBank
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## I. Standard Chemical Tissue Fixation

1. Aldehyde fixatives (formalin, 10% Neutral Buffered Formalin (NBF), 4% Paraformaldehyde (PFA), gluteraldehyde) "fix" tissue (i.e., arrest biological degradation) by forming chemical "cross-links" between and within proteins.
2. The most commonly used is NBF, which is purchased in a ready-to-use form and is thus very convenient. Arguably, 4% paraformaldehyde is, in many cases, a superior fix for immunohistochemistry as it tends to yield reduced background staining but must be freshly made just before use.
3. Many researchers believe that tissue may be stored indefinitely in 10% NBF without harm. This is partly true; sections cut from such material and stained with a routine histological stain (e.g., Hematoxylin and Eosin-'H&E') will look fine.
4. Problems, however, will likely arise when such material is used for immunostaining since the cross-linking action continues and may irreversibly change the conformation (i.e., reduce the antigenicity) of antibody targets.
5. If you intend to use your tissue for immuno-staining be careful not to 'over-fix' the tissue.
6. Fixation method / time / temperature is a variable that needs to be considered when developing immunohistochemistry protocols. Significantly altering fixation times between batches of specimens can have major consequences on the quality of immunostains.

## II. Some general suggested fixation guidelines

1. For routine fixation in 10% NBF, dissect the tissue as rapidly as possible and immerse in a large volume of fix (i.e., at least 20X volume of fix to tissue). Place at 4 degrees and fix 'overnight'. After fixation, wash the tissue well in several changes of Phosphate Buffered Saline (PBS).
2. Tissue may be stored in cold PBS for short periods of time (2 or 3 days) but should be placed in 70% ethanol for extended storage. The tissue will be safe in the EtOH since there is no danger of bacterial degradation. However note that it has been reported that **proteins can be extracted** by extended storage in ethanol and that storage in PBS with sodium azide added at 4 degrees is a better choice. Safest of all is to promptly fix, wash and deliver your samples for processing and embedding.
3. Tissue may be brought to our lab for processing in either PBS or 70% EtOH.
4. The size of the specimen being fixed should be considered. A commonly quoted rate of penetration for aldehyde fixes is two-to-three millimeter per hour. This rate is, of course, variable depending on the density and other characteristics of different tissues and is simply a rule of thumb, but it follows that trying to fix very large pieces of tissue runs the risk of under-fixing the interior of the specimen resulting in biologic degradation of the constituent molecules.

5. For solid material (e.g., liver) the longest dimension should not exceed 10-15mm.
6. For very small pieces of tissue (~1-2mm range dimensions) an overnight fix may in fact be too long. One to several hours may be a better choice for this material but be aware that changing times of fixation between experiments may have serious effects on subsequent immunostains.
7. Please try to be consistent to reduce variable staining results.

## III. Some specific considerations

1. Different tissues degrade at different rates post excision. When collecting multiple tissue types it is wise to fix the enzyme-rich ones first. For example, pancreas degrades extremely rapidly and should always be taken first followed by liver and so forth.
2. Tissue hardens upon fixation and remains in whatever shape it was fixed in. This should be considered in those cases where the final orientation of the sections is important. Pancreas, for example, is very pliable when dissected and if simply dropped into fix will likely harden into some rough spheroid shape. If your intention is to look at sections with a “maximum footprint” (a term of art meaning maximum viewable area) the pancreas has to be spread out flat in a cassette lined with a biopsy sponge before fixation occurs.

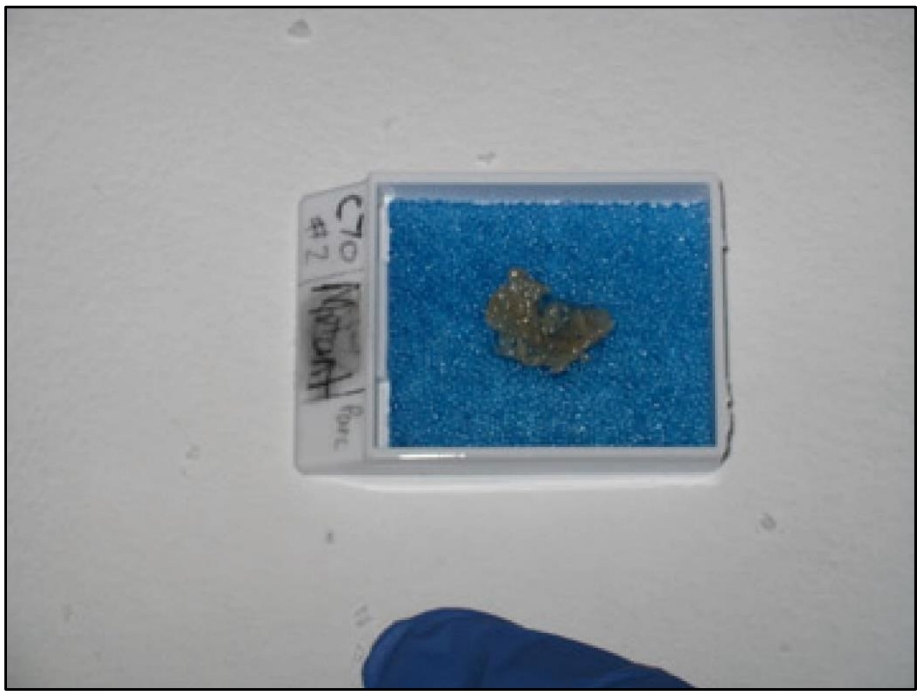


**Figure 1**

3. The quality of sections cut on a microtome suffer badly from several (avoidable) causes. During the post fixation processing step all water is extracted from the cellular spaces of the tissue and is replaced with paraffin.
4. This is carried out using a vacuum-based automatic machine in which the tissue is dehydrated via a series of graded ethanols followed by xylenes and finally liquid paraffin.
5. The processed tissue is then oriented in a mold, embedded in a paraffin block and is ready to section.

6. The density and general consistency of the tissue is more or less that of the paraffin and cutting good sections is fairly straightforward. The inclusion of materials with radically different hardness in the block, however, wreaks havoc! When such material comes in contact with the microtome blade it causes severe ripping and tearing of the entire section.
7. Things to avoid include: **fecal material** in intestine, especially in the colon where this material is very hard; **hair** is particularly bad—if you are bringing us skin to be sectioned be sure to remove the hair before fixation. This can be done using a razor blade or clippers. We can loan you our clippers if you need them. Hair can also sometimes be inadvertently included.
8. Please be careful during dissections; **sutures**-if you have done a ligation prior to dissection please be sure to not include the thread or staples with the specimen.

**Figure 2**

9. Labeling tissue cassettes. The cassettes into which the tissue is placed are subsequently exposed to ethanols and xylenes in the processor.
10. If you label your cassettes with a regular magic marker the writing will come off in the xylene. It is devastating to lose the identity of your specimens! We try to test the labeling for xylene insolubility before processing but why take the chance that we may forget to do this? Unless you have the special pens we use, **label your cassettes with pencil**!

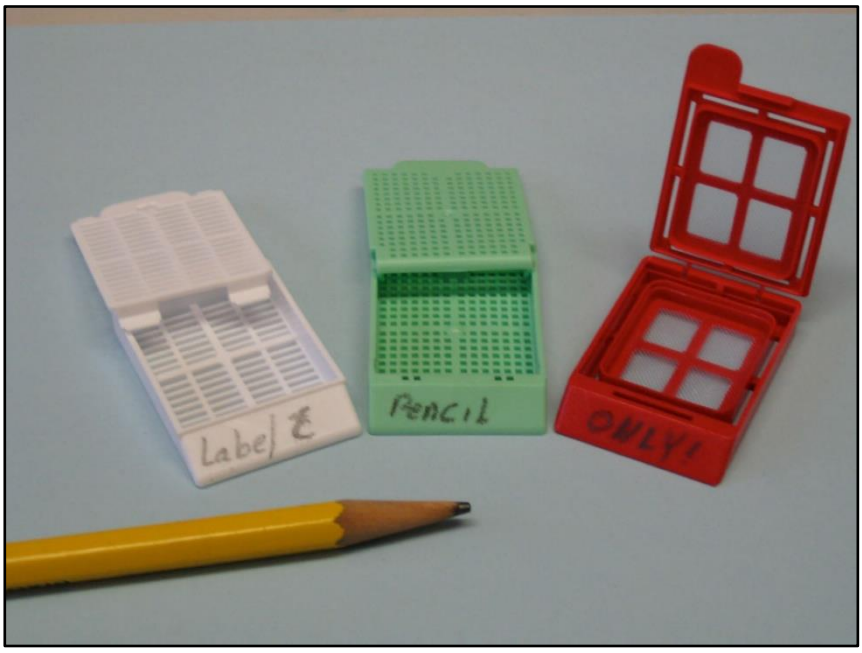


**Figure 3**