PLEASE NOTE: There is no one-size-fits-all method of tissue preparation for all experimental designs. Before harvesting tissue, you need to assess your experimental design, choose the best procedure that will meet your experiment's purpose best to properly illustrate the pathology and be compatible with the further analysis. If the unique goals of your particular research project require special handling procedures beyond this instruction, please discuss with Translational Research/Pathology Shared Resource Core Laboratory before you do anything with your precious samples.

1. Gross the samples

- **Orientation**

Before dissecting and harvest specimens, you must have clear goals in mind based on the type of specimen and the purpose of the experiment. Consider the best method to illustrate the morphology and best meet your experiment's purpose before doing anything to your samples. The following Figure-1 is a very good example of how to illustrate a lesion in a tubular sample by properly gross the tissue for best orientation during the embedding and cutting procedures. When grossing, try your best to cut the surface of interest (the part you want to see) **straight, flat and smooth** with a sharp blade or knife. You can use tissue marking dyes to mark designated terminals/margins/surfaces/points to direct our histotechnician to embed and/or cut.

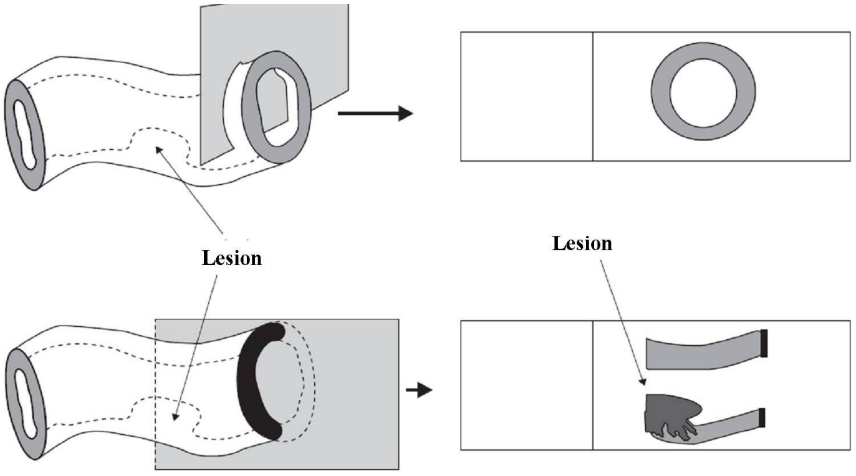

Figure-1. An example of how to properly gross the tissue to illustrate a lesion in a tubular sample. *Adapt from "Manual of Surgical Pathology (Third-Edition)"*

- **Size/Dimension**

In order to get the samples fixed and processed properly, it is very important to keep at least one dimension of a sample no more than 4mm, the maximum width × length of the sample is 20mm × 30mm. In another word, when you put the tissue in a regular tissue cassette, the tissue should never be so thick that it touches both top and bottom of the cassette; or so large that it touches both left and right sides, or both front and back sides of the cassette. If you have a special need to submit samples which are larger than described above, you should always discuss and consult with us thoroughly prior to harvesting your samples. We will customize a special processing program to properly handle these tissues, if it is really necessary.

- **Usage of Microbiopsy Cassettes, Wraps, and Foam Pads**

Microbiopsy Cassette can help to retain smaller size sample inside the cassettes, as it is usually built with smaller meshed grids. Wrap the samples in a biopsy wrap/specimen bag/lens paper (could be provided by our

Core Facility) which are small enough to be lost through the grids of the regular cassette and folded four times to make a pocket to keep the samples inside the cassettes. Never use foam pads to keep you small sample in the cassettes as it also compress the sample. Not doing so will result in a very high risk to lose your sample during the fixation, storage and processing procedures.

Although using a foam pad (could be provided by our facility) inside a cassette can keep the sample in its position, straight and/or flat during Fixation, storage and processing. The foam pad could compress your sample, slow down the penetration of the fixative and processing reagents, which may cause incomplete fixation and processing for the samples. So, it is strongly not recommended to use foam pad all the time. Use foam pads only when it is really necessary.

Also, please note that foam pads are not substitute of biopsy wrap/specimen bag/lens paper. They are used for different purpose during the sample preparation as descripted above.

- **Multiple tissue embedding**

You are allowed to put more than one tissue in a cassette, or we can combine tissues in multiple cassettes if they can be embedded in one block (extra charge may apply). Multiple tissue embedding will save your time and other resources in the future. But please do not put tissue/organs which are different a lot in size in one block; you might have to scarify the smaller/thinner ones in order to reach the best level of the bigger/thicker ones when sectioning the samples.

If you want to track your sample ID in a multiple tissue embedding block, please consult with the core laboratory. We have a series of solution according to different circumstances.

2. Sample Fixation

- **Fixatives**

There is no universal fixative that is ideal for all purposes. The following are some of the common fixatives being used:

- ❖ 10% Buffered formalin in 0.1M phosphate buffer (one of the most popular fixatives)
- ❖ 4% paraformaldehyde in 0.1M phosphate buffer(another popular fixatives)
- ❖ 2% paraformaldehyde with 0.2% picric acid in 0.1M phosphate buffer
- ❖ PLP fixative: 4% paraformaldehyde, 0.2% periodate and 1.2% lysine in 0.1M phosphate buffer

- **Sample Labeling and Fixation**

Label the cassettes (could be provided by our Core Facility) with **pencil (strongly recommended)** or **special marker that is solvent proof (resists xylene and alcohol) (**Note: cassettes must be dried for at least 3 minutes after labelling if using special marker to avoid labels washing off). Dissect, gross the tissue, put the samples in labeled cassettes, and immerse the samples in fixative as quickly as possible. Agitate the solution to ensure all tissues are completely immersed in fixative.

- **Duration of fixation**

Fixation is a physical and chemical process, and enough time must be reserved for the process to complete. Both over-fixation and under-fixation can be detrimental and may be responsible for inappropriate results for the downstream assays.

Generally, duration of fixation depends on the size and type of the samples, temperature, and the fixative being used. For the most common formaldehyde fixatives (10% buffered formalin or 4% paraformaldehyde, both penetration is approximately 0.5 mm/hour at room temperature), fixation should be completed within hours for normal size routine specimens. So, overnight (about 16 hours) fixation is fairly enough for general purpose. Larger specimens could be fixed for 24-48 hours. If the tissue or organ has a thick capsule (e.g. Kidney, Lymph Node, *etc.*) or skin (e.g. mouse embryos), the fixative will not penetrate through the capsule as rapidly as it will penetrate normal tissue. Therefore, cut the capsule or skin open on the side or use multiple punctures with a needle, to accelerate penetration of the fixative. Fixatives and dehydrants penetrate fatty tissues (such as mammary gland) much slower than other type of tissues. Fatty tissue should be cut even thinner or use prolonged procedures to achieve better fixation and processing.

- **Ratio of fixative volume**

Fixative volume: specimen size should be at least 15:1.

3. Decalcification

Bone or calcified tissues can not be cut by a microtome and must be decalcified prior to processing. Tissues that need to be decalcified can be submitted in the fixative (usually 10% buffered formalin), rather than in 70% ETOH. **This is the only case in which samples will be accepted in formalin.** Tissues for decalcification should be submitted in a separate specimen container than any other samples which do not need decalcification. **The specimen container and the paperwork must both clearly indicate which samples need decalcification prior to processing.** The decalcification step will be done by the Core Facility. If it is difficult to properly gross tissue prior to decalcification, the paperwork should clearly describe grossing instructions for grossing after decalcification.

4. Submit the Samples

Upon completion of fixation, it will be better if the samples could be rinsed with PBS (recommended) 3×10 minutes or flushed with tap water for half an hour to wash away the extra formaldehyde. Then, the samples should be transferred to a sealed container (could be provided by our Core Facility) with enough 70% ethanol to cover them. Label the request ID on the container with tape. Do not label the request ID directly on the container using a regular maker, as it can be washed off by the ethanol. Bring the sample together with the signed request form to the Core Facility for processing **as soon as possible**.

If you want to paraffin process your sample, you should never store your sample in fixative or ethanol. Tissues embedded in paraffin last for decades.

5. Common Problems Found in Submitted Samples

- **Under-fixation or over-fixation**

Neither mistakes are acceptable, but Under-fixation is the worst problem to overcome. If the sample is Under-fixed, the proteins and the nuclear acid will not be well preserved, which will lead to bad morphology and incorrect results of your further analysis. Over-fixation with formaldehyde fixatives can mask the epitopes due to the crosslink effect of the formaldehyde. Antigen retrieval techniques can help overcome this masking. But if the tissue has been fixed for a long period of time (i.e. weeks); there might be no signal could be detected even after antigen retrieval. Please follow the instruction in the grossing and fixation sections in this instruction for properly preparation of your sample to help avoid this problem.

- **Tissue too thick or too large**

Fixatives and processing solutions can not penetrate inside these tissues. The tissue will not be fixed or

processed well and it is often impossible to section such tissue. Again, tissue sections should be no thicker than 4 mm for common purpose.

- **Small tissue without tissue wrap**

Please always wrap the samples which are small enough to be lost through the holes in the cassette in a specimen bag/lens paper/biopsy wrap and folded four times to keep the samples inside the cassettes. Never use foam pads to keep you small sample in the cassettes.

- **Abuse usage of form pad**

The foam pad will compress your sample, and slow down the penetration of the ethanol, xylene and paraffin and may cause incomplete processing for the bigger samples. Therefore, use it only when it is really necessary.

- **Calcified Substances**

As a rule of thumb, any tissue submitted for processing should be easily sectioned with a scalpel blade. Bone or calcified tissues can not be cut by a microtome and must be decalcified prior to processing, or surface-decalcification must be performed after processing(less recommended).

- **Hair and hard Foreign Material**

Hair and hard foreign material can dull microtome blades. Please carefully remove hair off the sample if it is abundant on a skin specimen or dermoid cyst. Flush the gastrointestinal tracts thoroughly to remove undigested food debris and dung. Staples, sutures and clips must be removed from tissue before submission to the facility.

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