Preparation of Samples for Paraffin Processing

1. Grossing the sample

Orientation

Before dissecting and harvesting 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 that best fits the purpose of your experiment. When grossing, use a sharp blade or knife and try your best to cut the surface of interest (the part you want to see) **straight, flat and smooth**.

When tissue elements need to be oriented a specific way on a slide, embedding instructions must include the required orientation. The area of interest must be embedded in the bottom of the tissue mold. This will be sectioned first.

Size/Dimension

In order to get the samples fixed and processed properly, the maximum width × length of the sample must be less than 20mm × 30mm. It is very important to keep at least one dimension of a sample **less than 4mm**. When you place the tissue in a regular tissue cassette, the tissue should never touch all sides of the cassette. Should you require to submit a larger sample for a specific purpose discuss this need with the Histology Unit before harvesting your sample.

For sample that are smaller than 20mm × 30mm, wrap the samples in a biopsy wrap/specimen bag/lens paper (provided by our unit) so that your samples are not lost in the grid of the regular cassette. Fold the biopsy wrap four times to make a pocket to keep the samples inside the cassettes.

Foam pad can also be provided by our unit although it is strongly recommended to use it only when it is truly needed.

Hair and hard Foreign Material

Hair and hard foreign material can dull microtome blades. Please carefully remove them from 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 Histology unit.

2. Sample Fixation

Fixation is a physical and chemical process that require a certain amount of time to complete. Both over-fixation and under-fixation can be detrimental and may be responsible for inappropriate results for the downstream assays. Generally, the duration of fixation depends on the size and type of the sample,

temperature, and the fixative being used. For the most common formaldehyde fixatives (10% buffered formalin or 4% buffered paraformaldehyde), the rate is approximately 0.5 mm/hour at room temperature.

Immersion fixation

Overnight (~16 hours) fixation is sufficient for general purpose microscopy. Larger specimens may 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 to accelerate penetration of the fixative. Fixatives and dehydrates 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.

Perfusion fixation

Perfusion fixation involves dissecting an animal and flushing 4% paraformaldehyde through its circulatory system via the heart. The tissue of interest can then be extracted and fixated further with immersion in the fixative. For rodents and other small animals, transcardial perfusion is highly recommended for obtaining the best results. Following transcardial perfusion, harvested organ(s) of interest can be immersed in the fixative to ensure complete fixation.

Protocol

1. Fresh tissues should be immediately fixed in formalin 10% Neutral Buffered Formalin or in freshly prepared 4% PFA solution for 16-48 hours before submission for processing (agitation on a shaker at room temperature is recommended). Please notice that tissue for IHC staining should not be fixed longer than 24 hours.
2. Use a volume of 15-20 times fixative volume to tissue.
3. Users are welcome to trim and place the tissue samples in a suitable labeled cassette (provided by our unit) to ensure the desired orientation.
4. Bring the sample together with the signed request form to the service unit for processing upon completion of fixation. Ideally, tissues should be brought to the service unit and processed as soon as possible. If this is not possible and the tissue is designed for immunohistochemical, tissue should be transferred from formalin to 70% alcohol to stop cross-linking.

Please notice that tissue to be stained with **immunohistochemical** stains should not be fixed longer than 24 hours.

If the tissue samples are fixed with an aldehyde fixative (paraformaldehyde, glutaraldehyde etc.) for **immunofluorescence detection**, include 0.3 M glycine in the blocking buffer, before applying the primary antibody.

Sample Labeling and Fixation

If you prefer to submit the tissues after trimming we can provide you the cassettes and/ or Bio Wraps. Label the cassettes using a **pencil or a solvent proof marker (resists xylene and alcohol),** dissect and trim the tissue, place the samples in the cassettes (use bio warps if needed) and immerse the samples in a fixative as quickly as possible. Agitate the solution to ensure all tissues are completely immersed in fixative. Make sure to place the tissue sample in the desired orientation: when tissue is placed into cassettes the tissue surface that is facing down in the cassette will sectioned first. Please list any special embedding requests on submission form. Do not overcrowd the cassette. Tissue that is compressed in the cassette will not adequately fix, infiltrate, section, or stain.

3. Decalcification

Bone or calcified tissues cannot 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). **The specimen container and the paperwork must both clearly indicate which samples need decalcification prior to processing.** The paperwork should clearly describe grossing instructions for grossing after decalcification.

Please note: there is no one standard for tissue preparation for all experimental designs. Before harvesting the tissue you need to assess your experimental design, choose the best procedure that best meets your experiment's purpose in order to properly illustrate its pathology and for it to be compatible with the further analyses. If the unique goals of your particular research project require special handling procedures beyond this instruction, please discuss them with the staff of the Histology Service Unit before you begin.

References:

- "General Guide for Preparing Samples for Paraffin Processing" Translational Research/Pathology Shared Resources Core Laboratory, Sidney Kimmel Cancer Canter
- Abcam protocols
- ARUP Research Histology Lab web site