Sample preparation for tissue hacking

Fixing

Chemical fixation with formaldehyde or other chemicals

Chemical fixatives are used to preserve tissue from degradation, and to maintain the structure of the cell and of sub-cellular components such as cell organelles (e.g., nucleus, endoplasmic reticulum, mitochondria). The most common fixative for light microscopy is 10% formalin (4% formaldehyde in phosphate buffered saline). For electron microscopy, the most commonly used fixative is glutaraldehyde, usually as a 2.5% solution in phosphate buffered saline.

Frozen section fixation

is a rapid way to fix and mount histology Frozen section procedure sections using a refrigeration device called a cryostat. It is often used after surgical removal of tumors to allow rapid determination of margin (that the tumor has been completely removed).

Washing The aim of Tissue washing is to remove formalin used water.

dehydration water must first be removed in the process of dehydration. Samples are transferred through baths of progressively more concentrated ethanol (alcohol) to remove the water.

clearing This is followed by clearing agent (such as xylene) to remove the alcohol.

infiltration finally molten paraffin wax, the infiltration agent, which replaces the xylene.

Embedding

After the tissues have been dehydrated, cleared, and infiltrated with the embedding material, they are ready for external embedding. During this process the tissue samples are placed into molds along with liquid embedding material (such as agar, gelatine, or wax) which is then hardened. This is achieved by cooling in the case of paraffin wax and

heating (curing) in the case of the epoxy resins. The acrylic resins are polymerised by heat, ultraviolet light, or chemical catalysts. The hardened blocks containing the tissue samples are then ready to be sectioned.

Sectioning *Microtome*

for light microscopy, a steel knife mounted in a microtome is used to cut 4-micrometer-thick tissue sections which are mounted on a glass microscope slide. For transmission electron microscopy, a diamond knife mounted in an ultramicrotome is used to cut 50-nanometer-thick tissue.

Sections can be cut through the tissue in a number of directions. For pathological evaluation of tissues, vertical sectioning, (cut perpendicular to the surface of the tissue to produce a cross section) is the usual method. Horizontal (also known as transverse or longitudinal) sectioning, cut along the long axis of the tissue

Staining

used Haematoxylin and Eosin stains

Mounting

After staining put the Canada balsam or D.P.X(doctrine plasticizer xylene) on tissue then cover by cover slide to make permanent slide.

Cleaning & Labeling