# SRR & CVR Govt. Degree College

## Department of Biochemistry

### Workshop on Tissue Fixation and Sectioning

Date: 17/12/2020


Objective of the workshop: To make students hands on experience about the fixation and tissue slicing using microtome

Microscopic analysis of cells and tissues requires the preparation of very thin, high-quality sections (slices) mounted on glass slides and appropriately stained to demonstrate normal and abnormal structures.

Most fresh tissue is very delicate and easily distorted and damaged, and it is thus impossible to prepare thin slices from it unless it is chemically preserved or “fixed” and supported in some way whilst it is being cut.

Broadly, there are two strategies that can be employed to provide this support:

We can freeze the tissue and keep it frozen while we cut. These sections are called “frozen sections”.

Alternatively, we can infiltrate our tissue specimen with a liquid agent that can subsequently be converted into a solid that has appropriate physical properties, which will allow thin sections to be cut from it.

Paraffin wax is such an agent. This produces so-called “paraffin sections”.

Tissue processing describes the steps required to take an animal or human tissue from fixation to the state where it is completely infiltrated with a suitable histological wax and can be embedded ready for section cutting on the microtome.

**Overview of the steps in tissue processing for paraffin sections**

1. Obtaining a fresh specimen: Fresh tissue specimens will come from various sources. It should be noted that they can very easily be damaged.

Most of the water in a specimen must be removed before it can be infiltrated with wax. This process is commonly carried out by immersing specimens in a series of ethanol (alcohol) solutions of increasing concentration until pure, water-free alcohol is reached.

Ethanol is miscible with water in all proportions so that the water in the specimen is progressively replaced by the alcohol. A series of increasing concentrations is used to avoid excessive distortion of the tissue.

A typical dehydration sequence for specimens not more than 4mm thick would be:

70% ethanol 15 min

90% ethanol 15 min

100% ethanol 15 min

100% ethanol 15 min

100% ethanol 30 min

100% ethanol 45 min

At this point, all but a tiny residue of tightly bound (molecular) water should have been removed from the specimen.

sectioned at a thickness down to at least 2 µm, to form ribbons as the sections are cut on the microtome, and to retain sufficient elasticity to flatten fully during flotation on a warm water bath.

A typical infiltration sequence for specimens not more than 4mm thick would be:

wax 30 min

wax 45 min

Embedding or blocking out

Now that the specimen is thoroughly infiltrated with wax, it must be formed into a "block" which can be clamped into a microtome for section cutting. This step is carried out using an "embedding center" where a mold is filled with molten wax and the specimen placed into it. The specimen is very carefully oriented in the mold because its placement will determine the "plane of section", an important consideration in both diagnostic and research histology. A cassette is placed on top of the mold, topped up with more wax, and the whole thing is placed

on a cold plate to solidify. When this is completed, the block with its attached cassette can be removed from the mold and is ready for microtomy.

Outcome : Students well practiced the technique the wax blocks are cutter in to thin slices for staining

They understood the significance of the tissue slicing to visualize the pathological changes if any in diseased patients

2. To introduce the microtome instrument and its parts to students

The students will appreciate the significance of the technique

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