

# **BASIC TECHNIQUES**

## **Preparation of histological sections**

In order to prepare thin sections for examination by microscopy, it is necessary to preserve the tissues (fixation) and embed them in a supporting medium (such as paraffin wax or resins) prior to sectioning. Sections are usually stained in order to provide contrast.

### **1. Fixation**

In order to preserve tissues and prevent structural change or breakdown of the components of the tissues, it is necessary to stabilize or fix the tissue. The fixative needs to preserve the tissues as close as possible to the living state. The fixatives commonly stabilize or denature proteins. A widely used fixative is formaldehyde, which has the advantage of being cheap and penetrates tissues rapidly. For better fixation, it is necessary use pH buffers in the fixative.

### **2. Embedding**

The most commonly used embedding or support medium is paraffin wax, with a melting point of about 56°C. Prior to infiltration of the tissue with molten wax, it is necessary to remove all the water from the tissue (dehydration). Dehydration is achieved using an ascending series of alcohols (70%, 95%, 100%). This is followed by tissue immersion in a wax solvent such as xylene or chloroform. The tissue is then transferred to molten paraffin wax (in an embedding oven) for a couple of hours. The tissue is then placed in a square or rectangular mold, and orientated in the required position, prior to adding hot wax to form a wax block.

### **3. Microtomy**

Sections of the tissue embedded in the wax block are cut on a machine, known as a microtome, using special knives (nowadays these are disposable). Typically series or ribbons of sections are cut at a thickness of 6-8mm. The sections are transferred to the surface of a hot waterbath (where the sections flatten and lose any wrinkles). Sections are collected on glass microscope slides (standard dimensions of 3 x 1 inches). In order for the sections to adhere to the slides they are dried for up to 24 hours in a drying oven (at a temperature of about 40°C). This prevents sections falling off the slides in the later stages of preparation.

#### **4. Staining**

The most common staining technique is known as Hematoxylin and Eosin (or H&E) staining. In order to stain the sections the wax needs to be removed. This is done using a wax solvent such as xylene. The slide is then hydrated using a series of descending alcohols (100%, 95%, 70%) and then water. The slide is then immersed in Hematoxylin stain, rinsed in running water (preferably alkaline), followed by staining with Eosin, and rinsing in water.

#### **5. Permanent Mounting**

After staining the sections are again dehydrated with ascending alcohols (95%, 100%) and xylene, prior to covering with a mountant and a glass coverlip. Mountants need to have good optical properties. The slide is left for at least 24 hours for the mountant to dry. The finished (permanent) slide with its stained tissues can then be examined under the microscope.

#### **Frozen sections**

Embedding in paraffin wax is a lengthy process and during the embedding many components (such as lipids) are dissolved and lost. Enzymatic activities are also largely destroyed. A rapid alternative to wax embedding is to prepare frozen sections and in a cryostat (a microtome operated in a low temperature cabinet, usually about  $-30^{\circ}\text{C}$ ). Frozen sections can then be stained or used for enzyme histochemistry, and mounted in a suitable water-soluble mountant.

#### **Total preparations**

In some cases the tissue to be examined is a very thin membrane. In such cases the tissue does not need cutting on a microtome, but can be stained, mounted and examined directly. This is known as a total preparation. Total preparations are not as 2-dimensional as histological sections, and adjustment of focus is necessary during examination.

#### **Cell Smears**

Cell smears are a form of histological preparation that does not require sectioning. Smears can be made for example of the blood or bone marrow. Smears are also common for swabs or scrapings of epithelial cells (e.g. from the oral cavity, cervix uteri).

### **STAINING TECHNIQUES**

## 1. **Hematoxylin and Eosin (H&E)**

This is the most commonly used staining technique for histological and histopathological sections. The Hematoxylin is a basic dye that stains acidic components of cells a blue color. This characteristic is known as basophilia. Hematoxylin stains the nuclei of cells, and the RER of the cytoplasm. Eosin is an acidic dye that stains the basic components of the cells a reddish-pink color. This characteristic is known as acidophilia. Most of the cytoplasm of cells is stained by eosin. Bone matrix is also stained by eosin.

## 2. **Periodic acid-Schiff (PAS) staining**

PAS is a widely used staining technique that stains the neutral sugars of glycosaminoglycans a pink color. Common components stained positively with PAS include mucus, the basal lamina, glycogen.

## 3. **Orcein**

Orcein staining is used to stain elastic fibers a dark brown-purple color. This is used, for example, to show the elastic components in the walls of arteries, or in the matrix of elastic cartilage.

## 4. **Osmium tetroxide**

Osmium is used to stain lipids a dark black color. It is very useful for demonstrating the myelin of myelinated nerves, or lipid droplets in the liver or steroid-secreting cells.

## 5. **Oil Red O**

Oil Red O is used to stain lipids a red-orange color in unfixed frozen sections.

## 6. **Toluidine blue**

Toluidine blue is a so-called metachromatic stain. It is a blue stain that stains specific components of tissues a purple color. This change in staining color is known as metachromasia. Metachromasia is seen in the matrix of hyaline cartilage, or in the granules of mast cells.

## 7. **Impregnation**

Impregnation is a staining technique in which blocks of tissue are processed in solutions containing metals such as silver or gold, which attach to specific

components in tissues. The silver or gold are then further processed (reduced) and develop into dark metallic deposits. The stained blocks are only then sectioned. Silver impregnation is widely used in neurohistology to stain neurons and their processes. Silver impregnation techniques are also widely used to demonstrate reticular fibers.

## 8. Vital staining

Vital staining refers to the uptake of dyes (usually particulate) by cells. If we inject Trypan blue into experimental animals, the dye is rapidly engulfed by specific macrophages. We can use such vital staining to demonstrate the Kupffer cells of the liver.

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