**MLS 502**

**Laboratory posting VI**

**Assignment title: histopathology practicals**

**15/MHS06/040**

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Study the ff staining techniques and notes on procedures, control sections, indications and expected results. A. Haematoxylin and Eosin B. PAS C. G&S D. PPB E. GRAM F. ALCIAN BLUE G. Phoxine and tartrazine H. VVG I. WVG

1. Haematoxylin and eosin

Principle: Alum acts as mordant and haematoxylin containing alum stains the nucleus light blue. This turns red in presence of acid, as differentiation is achieved by treating the tissue with acid solution. Bluing step converts the initial soluble red colour within the nucleus to an insoluble blue colour. The counterstaining is done by using eosin which imparts pink colour to the cytoplasm.

Procedure:

* Deparaffinize the section and place in the xylene. Repeat the treatment.
* Hydration: Hydrate the tissue section by passing through decreasing concentration of alcohol baths and water. (100%, 90%, 80%, 70%)
* Stain in haematoxylin for 3 minutes
* Wash in running tap water until sections “blue” for 10 minutes.
* Differentiate in 1% acid alcohol (1% HCl in 70% alcohol) briefly.
* Wash in running tap water until the sections are again blue by dipping in an alkaline solution (e.g. ammonia water) followed by tap water wash.
* Stain in 1% Eosin Y for 10 minutes
* Wash in tap water for 1minute
* Dehydrate in increasing concentration of alcohols and clear in xylene
* Mount in DPX mounting media

Results:

Nuclei and other basophilic structures are blue.

 Cytoplasm and acidophilic structures are light to dark red.

Control organs: kidney, liver

1. Periodic acid-Schiff (PAS)

Principle: PAS method works by exposing the tissue to periodic acid. Periodic acid acts as oxidizing agent which oxidizes compounds having free hydroxyl group (-OH group) or amino/alkylamine group resulting in dialdehydes. These dialdehydes when exposed to Schiff’s reagent, an insoluble magenta coloured complex is formed. A suitable basic stain is used as counter stain.

Procedure:

* Bring sections to distilled water.
* Treat with periodic acid for 5 minutes.
* Rinse well in distilled water.
* Cover with Schiff’s reagent for 5-15 minutes.
* Wash in running tap water for 5-10 minutes
* Counter stain with Harris haematoxylin for approximately 15 seconds.
* Differentiate (if necessary) with acid alcohol and bluing as usual.
* Wash in tap water.
* Rinse in increasing concentration of alcohol (70, 80, 95 and 100%)
* Clear in xylene and mount as usual.

Results: Formation of insoluble magenta coloured complex denotes positive result.

Glycogen, neutral mucosubstances, basement membranes, collagen fibres, glycolipids and phospholipids will be demonstrated as pink to red to purple colour.

If diastase or -amylase is used for a negative control, the glycogen deposits are removed leaving only the plasma membrane staining pink.

The two major types of fibres are usually distinguished by different intensity of staining.

Control organs: liver, parathyroid gland

1. Gordon & Sweet's Staining

Principle: Reticulin fibres have little natural affinity for silver solutions so; they must be treated with potassium permanganate to produce sensitised sites on the fibres where silver deposition can be initiated. The silver is in a form readily able to precipitate as metallic silver (diamine silver solution). The Optimal pH for maximum uptake of silver ions is pH 9.0. A reducing agent, formalin, causes deposition of silver in the form of metal. Any excess silver in the unprecipitated state is removed by treating with hypo. Gold chloride treatment renders the preparation permanent and produces a neutral black colour of high intensity.

Procedure:

* Deparaffinise sections with xylene then take through alcohols to water.
* Oxidise in acidified potassium permanganate for 3 minutes
* Rinse in distilled water.
* Decolourise with 2% oxalic acid for 1 min
* Rinse in distilled water.
* Mordant in 4% iron alum for 10 minutes
* Rinse in distilled water.
* Impregnate in ammoniacal silver solution for 11 seconds
* Rinse quickly in distilled water.
* Immediately reduce with 10% aqueous formalin for 2 minutes
* Wash in running tap water for 2 minutes
* Tone in 0.2% gold chloride for 2 minutes
* Rinse in distilled water.
* Fix with 2% aqueous sodium thiosulphate (hypo) for 2 minutes
* Wash in water for 2 minutes
* Counterstain with neutral red for 2 minutes
* Dehydrate, clear and mount.

Result:

Reticulin fibres-Black

Nuclei-Red

Control organs: liver

1. Perls Prussian Blue

Principle: The reaction occurs with the treatment of tissue sections with acid ferrocyanide solution. Any ferric ion (Fe3+) in the tissue combines with ferrocyanide and results in the formation of a bright blue pigment called “prussian blue” or ferric ferrocyanide.

Procedure:

* Deparaffinize and bring the sections to water.
* Treat the sections with freshly prepared acid ferrocyanide solution for 10-30 minutes.
* Wash well in distilled water.
* Lightly stain the nuclei with 0.5% aqueous neutral red or 0.1% nuclear fast red.
* Wash rapidly in distilled water.
* Dehydrate, clear and mount.

Result:

Iron (hemosiderin): blue

Nuclei: red

Background: pink

Control organs: liver and spleen

1. Gram staining

Principle: The structure of the organism’s cell wall determines whether the organism is gram positive or negative. When stained with a primary stain and fixed by a mordant, some bacteria are able to retain the primary stain by resisting decolourization while others get decolourized by a decolourizer. Those bacteria which retain the primary stain are called Gram positive and those bacteria which get decolorized and then get counterstained are called Gram negative.

Procedure:

* Place the slides on the staining rods.
* Cover the smear with crystal violet stain and leave for 1 minute.
* Wash carefully under running tap water.
* Flood the smear with Lugol’s iodine solution and leave for 1 minute.
* Drain off the iodine Wash the slide for the again in a gentle stream of tap water.
* Flood the slide with the decolourizing agent then wait for 20-30 seconds. This can also be done by adding a drop by drop to the slide until the decolorizing agent running from the slides runs clear.
* Gently wash the slide under running tap water and drain completely.
* Counterstain with safranin for and wait for about 30 seconds to 1 minute.
* Wash slide in a gentile and indirect stream of tap water until no colour appears in the effluent and then blot dry with absorbent paper.

Results:

Gram Positive: Dark purple

Gram Negative: Pale to dark red

Yeast: Dark purple

Epithelial cells: Pale red

1. Alcian blue

Principle: Alcian blue is a group of polyvalent basic dyes that are water soluble. The blue color is due to the presence of copper in the molecule. The 3% acetic acid solution (pH2.5), Alcian blue stains both sulfated and carboxylated acid mucopolysaccharides and sulfated and carboxylated sialomucins (glycoproteins). It is believed to form salt linkages with the acid groups of acid mucopolysaccharides.Procedure:

* Hydrate slides to distilled water.
* 2. 3% acetic acid, 3 minutes.
* \*Alcian blue solution, microwave: Hi power, 30 seconds.
* Wash in running water for 2 minutes, rinse in distilled.
* Nuclear-fast red, 5 minutes, wash in tap water.
* Dehydrate, clear, and coverslip.

Results:

Acid mucins/mucosubstances: blue

Nuclei (using nuclear fast red): reddish pink

Control organs: Small intestine, appendix, or colon.

1. Phoxine and tartrazine
2. VERHOEFF'S VAN GIESON (VVG)

Principle: The tissue is stained with a regressive haematoxylin, consisting of ferric chloride and iodine. The differentiating is accomplished by using excess mordant (ferric chloride) to break the tissue-mordant dye complex. The dye will be attracted to the larger amount of mordant in the differentiating solution and will be removed from the tissue. The elastic tissue has the strongest affinity of the iron-haematoxylin complex and will retain the dye longer than the other tissue elements.

Procedure:

* Deparaffinize and hydrate to distilled water.
* Verhoeff's hematoxylin for 30 minutes (save solution until stain is
* completed)
* Wash in tap water.
* Differentiate in 2% ferric chloride solution, check microscopically for black fibres on a grey background.
* Rinse in water.
* Hypo for 1 minute to remove iodine.
* Wash in water.
* Counterstain in Van Gieson's for 5 minutes.
* Dehydrate, clear in xylene, and coverslip.

 Results:

Elastic fibres and nuclei- black

Collagen- red

Other tissue elements- yellow

Control organs: Artery or skin.

1. Weigert-Van Gieson

Principle: Van Gieson is used to differentiate between collagen and smooth muscle in tumours and to demonstrate the increase of collagen in diseases. When using combined solutions of picric acid and acid fuchsin, the small molecules of picric acid penetrate all of the tissues rapidly, but are only firmly retained in the close textured, red blood cells and muscle. The larger molecules of ponceau S displaces picric acid molecules from collagen fibres, which have larger pores, and allow the larger molecules to enter.

Procedure:

* Bring sections to distilled water.
* Stain nuclei with Celestin Blue 5 mins
* Rinse in distilled water
* Stain in haematoxylin 5 mins
* Wash well in running tap water 5 mins
* Flood with Curtis stain 5 mins
* Blot.
* Dehydrate rapidly in alcohols, clear and mount.

Result:

Nuclei-Blue

Collagen-Bright red

Cytoplasm, muscle, fibrin and red blood cells-Yellow

Control organs: liver