**NAME**: OGAZI, MAC-DONALD C.

**MATRIC NUMBER**: 15/MHS05/010

**COURSE CODE**: MLS 502

**COURSE TITLE**: LABORATORY POSTING VI

**LECTURER**: MR. RAJI

**QUESTIONS**

Study the following staining techniques and notes on procedures, control sections, indications and expected results.

A. Hematoxylin and Eosin

B. PAS

C. G&S

D. PPB

E. GRAM

F. ALCIAN BLUE

G. Phoxine and tartrazine

H. VVG

I. WVG

**ANSWERS**

1. **HEMATOXYLLIN AND EOSIN**

**Principle**: Hematoxylin is a basic dye which thereby stains the acidic components of the tissue (nuclei) while eosin is an acidic dye which stains the basic component of the tissue (cytoplasm). It is a routine staining technique in histopathology laboratories.

**Procedure**:

1. Take section to water
2. Stain with harris hematoxylin for 5 mins and rinse off
3. Differentiate with 1% acid alcohol briefly and rinse off
4. Blue in running tap water for 10 mins
5. Stain with eosin for 2 mins and rinse off
6. Dehydrate with ascending grades of alcohol
7. Clear in xylene
8. Mount with DPX

**Expected result**:

Nuclei – blue to blue-black

Nucleoli – blue

Cartilage – pink or light blue to dark blue depending on type and the stain used, being darkest with Ehrlich’s hematoxylin

Cement line of bone – blue with Ehrlich’s hematoxylin

Calcium and calcified bone – purplish blue

Basophil cytoplasm (plasma cells and osteoblasts) – purplish

RBCs, plasmasomes, eosinophil granules, Paneth cell granules, zymogen granules, keratin – bright orange-red

Cytoplasm – shades of pink

Muscle fibres, thyroid colloid, thick elastic fibres, decalcified bone matrix – deep pink

Collagen and osteoid tissue – light pink

**Control Organs**:

Liver, lungs, brain, stomach, spleen, kidney and every other tissue in the human body as hematoxylin and eosin stain deals with demonstration of general tissue structure. It is a routine stain.

1. **PERIODIC ACID SCHIFF (PAS) STAINING TECHNIQUE**

**Principle**: The PAS stain is a histochemical reaction in that the periodic acid oxidizes the carbon to carbon bond forming aldehydes which react to the fuchsin-sulfurous acid which form the magenta colour.

**Procedure**:

1. Deparaffinize and hydrate to distilled water.
2. Place slides into 0.5% Periodic acid for 5 minutes.
3. Rinse in distilled water.
4. Schiff's Reagent, microwave HIGH power, for 45 - 60 seconds, until deep magenta.
5. Wash in running tap water for 5 minutes.
6. Counterstain in hematoxylin for 3 minutes.
7. Wash in tap water, blue hematoxylin, rinse in distilled water.
8. Dehydrate in alcohol, clear, and coverslip.

**Expected result**: Glycogen, neutral mucosubstances, basement membranes, collagen fibers, glycolipids and phospholipids will be demonstrated as pink to red to magenta 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, kidney, parathyroid glands and skeletal and cardiac muscle

1. **GORDON AND SWEETS’ STAINING TECHNIQUE**

**Principle**: The Gordon and Sweet's silver staining method is used to demonstrate reticular (retic) fibres. This method relies on the impregnation of retic fibres with silver through oxidation and reduction. The tissue is first oxidized using potassium permanganate to enhance subsequent staining. It is then sensitized using an iron alum solution that targets and binds to the tissue element (retic fibres). The retic fibres are then impregnated by an ammoniacal silver solution that removes and replaces the sensitizer. The silver solution is reduced by 10% formalin so that a visible metallic tone highlights the retic fibres. The metallic silver is then toned and converted to metallic gold using gold chloride solution, thereby providing better chemical stability, fibre contrast, and clarity. Unreduced silver and excess gold are removed via a 5% hypo (sodium thiosulfate) solution. The tissue section may then be counter-stained with nuclear fast-red or light-green.

**Procedure**:

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

**Expected result**:

Reticulin fibres – Black

Nuclei – Red

**Control organs**: kidney, liver, spleen and lymph nodes.

1. **PEARLS’ PRUSSIAN BLUE STAINING TECHNIQUE**

**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**:

1. Deparaffinize and hydrate to distilled water.
2. Allow slides to stand in working solution for 5 minutes, in the fume hood.
3. Rinse in distilled water.
4. Immerse in Nuclear-fast red for 5 minutes.
5. Wash in tap water.
6. Dehydrate, clear, and coverslip.

**Expected result**:

Iron (hemosiderin) – blue

Nuclei – red

Background – pink

**Control organs**: Liver, bone marrow and spleen.

1. **GRAM STAINING TECHNIQUE**

**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 the decolourizer. Those bacteria that retain the primary stain are called gram positive and those bacteria which gets decolourized and then get counterstained are called gram negative.

**Procedure**:

1. Place the slides on the staining rods.
2. Cover the smear with crystal violet stain and leave for 1 minute.
3. Wash carefully under running tap water.
4. Flood the smear with Gram’s iodine solution and leave for 1 minute.
5. Drain off the iodine. Wash the slide again in a gentle stream of tap water.
6. Flood the slide with the decolorizing 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.
7. Gently wash the slide under running tap water and drain completely.
8. Counterstain with safranin for and wait for about 30 seconds to 1 minute.
9. 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.
10. Observe under microscope.

**Expected result**:

The staining results of gram stain are as follows:

Gram Positive - Dark purple

Gram Negative - Pale to dark red

Yeasts - 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 colour 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**:

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

**Expected result**:

Acid mucins/mucosubstances – blue

Nuclei - reddish pink

**Control organs**: tissue stroma, cartilage and bones

1. **PHOXINE AND TARTRAZINE**
2. **VERHOEFF’S VAN GIESON STAINING TECHNIQUE**

**Principle**: The first step in the procedure is an overstaining of the tissue section with a soluble lake of hematoxylin-ferric chloride-iodine. Ferric chloride and iodine both serve mordant functions primarily and oxidizing functions secondarily. The latter characteristic will assist in the conversion of hematoxylin dye to hematein. In addition, the iodine may serve as a dye-trapping agent, thereby retarding dye loss from selected components during the subsequent differentiation process. Differentiation, a necessary step in any overstaining process, is accomplished by use of excess mordant (a dilute solution of ferric chloride) to break the tissue-mordant-dye complex. The elastic tissues, having the strongest affinity for the insoluble complex, retain it the longest and so are coloured black in the final result. The van Gieson solution of acid fuschin and picric acid is used as the counterstain and colours the collagen bright red and other tissue elements yellow.

**Procedure**:

1. Deparaffinize and hydrate slides to distilled water.
2. Stain in Verhoeff’s solution for 1 hour. Tissue should be completely black.
3. Rinse in tap water with 2-3 changes.
4. Differentiate in 2% ferric chloride for 1-2 minutes.
5. Stop differentiation with several changes of tap water and check microscopically for black elastic fibre staining and gray background. It is better to slightly under differentiate the tissue, since the subsequent Van Gieson’s counterstain can extract the elastic stain somewhat.
6. Wash slides in tap water.
7. Treat with 5% sodium thiosulfate for 1 minute. Discard solution.
8. Wash in running tap water for 5 minutes.
9. Counterstain in Van Gieson’s solution for 3-5 minutes.
10. Dehydrate quickly through 95% alcohol, 2 changes of 100% alcohol.
11. Clear in 2 changes of xylene for 3 minutes each.
12. Coverslip with resinous mounting medium.

**Expected results**:

      Elastic fibres - blue-black to black

      Nuclei - blue to black

      Collagen - bright red

      Other tissue elements - yellow

**Control organs**: Aorta, Skin, Lungs.

1. **WEIGERT’S VAN GIESON STAINING TECHNIQUE**

**Principle**: The method is based on the affinity towards elastic fibres displayed by resorcin fuchsin, a precipitate resulting from a reaction between resorcin and basic fuchsin and ferric chloride. Since this is not a specific method, other structures such as collagen and basal membranes might be stained. Therefore, it is essential to differentiate carefully in order to obtain a selective marked staining of elastic fibres. Weigert’s “long method” differs from “quick method” in both staining mixture and procedure; it is more selective because staining mixture is less concentrated and incubation times are very long. Counterstaining with Van Gieson trichrome stain makes it possible to differentiate collagen from connective tissue showing nuclei at the same time.

**Procedure**:

1. Bring sections to distilled water.
2. Stain nuclei with Celestin Blue 5 mins
3. Rinse in distilled water
4. Stain in haematoxylin 5 mins
5. Wash well in running tap water 5 mins
6. Flood with Curtis stain 5 mins
7. Blot.
8. Dehydrate rapidly in alcohols, clear and mount.

**Expected result**:

Nuclei - Blue

Collagen - Bright red

Cytoplasm, muscle, fibrin and red blood cells - Yellow

**Control organs**: skin, tendon, bone, ligament