**Assignment Title:** Histopathology Practicals
**Course Title:** Laboratory Posting VI

**Matric no**: 15/MHS06/065
**Course Code:** MLS 502

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

ANSWER

1. HEMATOXYLIN AND EOSIN STAIN

Hematoxylin and eosin stain or haematoxylin and eosin stain (often abbreviated as: H&E stain or HE stain) is one of the principal tissue stains used in histology. It is the most widely used stain in medical diagnosisand is often the gold standard. H&E is the combination of two histological stains: Hematoxylin and eosin stain  . The hematoxylin stains cell nuclei blue, and eosin stains the extracellular matrix and cytoplasm pink, with other structures taking on different shades, hues, and combinations of these colors.The stain shows the general layout and distribution of cells and provides a general overview of a tissue sample's structure

Principle: Alum acts as mordant and hematoxylin 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 color within the nucleus to an insoluble blue color. The counterstaining is done by using eosin which imparts pink color to the cytoplasm.

Staining Procedure:

1. Place the slides with section in a metal staining rack.

2. Immerse sections in the filtered Harris Hematoxylin for 10 seconds.

 3. Remove rack to a beaker with tap water.

 4. Exchange tap water until the water is clear.

 5. Immerse sections in EOSIN stain for ~30 seconds.

 6. Remove rack to a beaker with tap water.

7. Exchange tap water until the water is clear.

8. Dehydrate in ascending alcohol solutions (50%,70%,80%,95% , 100% )

 9. Clear with xylene.

 10. Mount coverslip onto the section on glass slide .

Results:

Nuclei and other basophilic structures are blue.

 Cytoplasm and acidophilic structures are light to dark red.

Control organ : spleen and liver

1. **PERIODIC ACID–SCHIFF** (**PAS**)

**Periodic acid–Schiff** (**PAS**) is a staining method used to detect polysaccharides such as glycogen, and mucosubstances such as glycoproteins, glycolipids and mucins in tissues.

Principle: This stain is used for the demonstration of glycogen. Tissue sections are first oxidized by periodic acid. The oxidative process results in the formation of aldehyde groupings through carbon-to-carbon bond cleavage. Free hydroxyl groups should be present for oxidation to take place. Oxidation is completed when it reaches the aldehyde stage. The aldehyde groups are detected by the Schiff reagent. A colorless, unstable dialdehyde compound is formed and then transformed to the colored final product by restoration of the quinoid chromophoric grouping.

Staining 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. \* Conventional method: Schiff's Reagent, room temperature for 30 minutes.

Results:

 Glycogen, neutral mucosubstances, basement membranes, collagen fibers, glycolipids and phospholipids will be demonstrated as pink to red to purple color. 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 fibers are usually distinguished by different intensity of staining.

Control organ

skin, liver, parathyroid glands and skeletal and cardiac muscle

1. **Gordon & Sweet's Staining Protocol for Reticulin**

The Gordon and Sweet's silver staining method is used to demonstrate reticular (retic) fibers. This method relies on the impregnation of retic fibers with silver through oxidation and reduction.

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.

**Method**

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 (**Not liver cores-see technical point 4**) 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 (**optional-see technical note 5**) for 2 minutes

17   Dehydrate, clear and mount.

**Results**

* Reticulin fibres ............................Black
* Nuclei .......................................Red

Control organ : liver

Staining type: **.** Metallic Impregnation

* In this method Some metallic compounds [ammonical silver] can be reduced by tissue[melanin reticulin fiber] to the metallic state producing an opaque to the black deposition on the tissue.
1. **PEARL PRUSSIAN BLUE IRON STAIN KIT (REACTION FOR DEMONSTRATION OF IRON)**

Prussian Blue or Perls’ reaction is used to demonstrate ferric iron and ferritin. This is not a true staining technique rather, it is a histochemical reaction.

PRINCIPLE: The reaction occurs with the treatment of sections in acid solutions of ferrocyanides. Any ferric ion (+3) in the tissue combines with the 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. \*Working solution, \* microwave, 30 seconds. Allow slides to stand in solution for 5 minutes, in the fume hood.

 3. Rinse in distilled water.

4. Nuclear-fast red, 5 minutes.

5. Wash in tap water.

 6. Dehydrate, clear, and coverslip. \*Conventional method: room temperature for 30 minutes.

RESULTS:

Iron (hemosiderin) blue Nuclei red Background pink

Control organ : liver and spleen

Staining type: staining with dye

1. **GRAM STAINIG**

PURPOSE: For demonstrating gram-negative and gram-positive in tissue.

 PRINCIPLE: Both bacteria's, positive and negative, cell wall is composed of peptidoglycan,(the gram-positve has a thicker wall) and both will take up the crystal violet. The gram-negative has a a layer of lipopolysaccharide external to the peptidoglycan wall, which is disrupted in the acetone rinse, allowing the crystal violet to be differentiated out. This allows the the gram-negative bacteria to take up the basic fuchsin stain.

PROCEDURE:

1. Deparaffinize and hydrate to distilled water.

 2. Place slides on staining rack, drop crystal violet stain onto tissue section, stain for 1 minute.

 3. Wash in tap water.

4. Lugol's iodine, 1 minute.

5. Wash in tap water.

6. Blot sections dry, breath on section then quickly pour acetone over section until no color runs off.

7. Wash in tap water.

 8. Place slides on staining rack, drop Basic fuchsin on tissue sections, stain 3 minutes.

9. Wash in tap water, blot gently but not completely dry.

10. Dip quickly into acetone, 2 dips.

 11. Dip directly into picric acid-acetone mixture until a 'salmon' color.

12. Dip quickly into two changes of acetone.

13. Air dry, dip into xylene, and coverslip.

RESULTS:

Gram-positive bacteria: blue

 Gram-negative bacteria :red

Nuclei :red

Background :yellow

1. . ALCIAN BLUE

The alcian blue stain is most commonly used on tissue samples obtained from the gastrointestinal (GI) tract and is useful in diagnosing pathological processes such as Barrett's esophagus. Using alcian blue solutions of varying pH (1.0 and 2.5) also helps differentiate various types of acid mucosubstances.

PURPOSE: Alcian blue stains acid mucosubstances and acetic mucins. Excessive amounts of non-sulfated acidic mucosubstances are seen in mesotheliomas, certain amounts occur normally in blood vessel walls but increase in early lesions of atherosclerosis.

 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:

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. Nuclear-fast red, 5 minutes, wash in tap water.

6. Dehydrate, clear, and coverslip. \*Conventional Method: Alcian blue, room temperature for 30 minutes.

RESULTS:

Acid mucins/mucosubstances: blue

 Nuclei (using Nuclear fast red)|: reddish pink

1. Phoxine and tartrazine
2. Verhoeff-Van Gieson (**VVG**) **Staining**

Description: This method is used for identifying elastic fibers in tissues such as skin, aorta, etc. on formalin-fixed, paraffin-embedded sections, and may be used for frozen sections as well. The elastic fibers will be stained blue-black and background will be stained 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 fiber 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.

**Results:**

      Elastic fibers --------------------- blue-black to black

      Nuclei ----------------------------- blue to black

      Collagen -------------------------- red

      Other tissue elements ---------- yellow

**Positive Controls:**

      Aorta, Kidney, Myometrium.

G. Weigert-Van Gieson

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.

**Method**

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.

**Results**

Nuclei ....................................................Blue

Collagen .................................................Bright red

Cytoplasm, muscle, fibrin and red blood cells ......Yellow