

P.G. [Semester II] ZOOLOGY

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Topic:

Histochemistry of Carbohydrate

① Periodic Acid Schiff (PAS) staining technique for Carbohydrate.

Periodic Acid Schiff (PAS) staining is one of the most commonly performed special staining techniques in histopathology laboratory which is used to highlight molecules with high percentage of carbohydrate content such as mucin, glycogen (polysaccharides).

Principle

PAS method works by exposing the tissue to periodic acid. Periodic acid acts as oxidising agent which ~~oxidising agent~~ oxidises compounds having free hydroxyl group (-OH) 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.

Preparation of staining solution

① Periodic Acid solution

Periodic Acid: 1 gram
Distilled water 100 ml

② Schiff's reagent

Fuchsin Basic: 1 gm
Distilled water 100 ml
Sodium metabisulphite 2 gm
Conc. HCl _____ 2 ml
Charcoal activated 0.3 gm

Basic fuchsin⁽²⁾ is dissolved in boiling water. It is cooled at 50°C and filtered. Sodium metabisulphite and HCl is added to it.

This solution is stored at dark room at room temperature overnight.

Now charcoal is added and shake it for one minute and filtered.

Procedure of PAS staining

- Section of specimen is bring to distilled water.
- It is treated with periodic acid for 5 minutes.
- Rinse well in distilled water
- Now, it is cover with Schiff's reagent for 5-15 minutes.
- Wash the section in running tap water for 5 to 10 minutes.
- It is now counter stain with Heeri's hematoxylin for approximately 15 seconds.
- Differentiate (if necessary) with acid alcohol and bluing as usual.
- Now it is washed in tap water.
- Rinse in increasing concentration of alcohol (70, 80, 95 and 100%).
- It is now clear in xylene and mount as usual.

Result

Formation of insoluble magenta coloured complex denotes positive result.

uses of PAS staining

PAS stain is mainly used to highlight the molecules (structures) with high percentage of carbohydrate content such as glycogen, glycoproteins, and proteoglycans typically found in connective

tissues, glycocalyx and basal laminae. (3)

- PAS staining can be used to assist can be used to assist in the diagnosis of several medical conditions such as:

- (a) Glycogen storage disease (vs other storage disease)
- (b) Adenocarcinoma which often secretes mucin.
- (c) Paget's disease of breast.
- (d) Alveolar soft part sarcoma
- (e) staining macrophages in Whipple's disease.
- (f) Erythroleukemia, leukemia of immature RBC's.
- (g) Fungal infections (cell wall stain magenta).

Additional notes

(i) Differentiation

It is process of removing excess dyes from tissues. It is similar to decolorizing, but differs with high degree of selectivity.

Differentiation can be accomplished by solvents (ex tap water in H & E staining), pH control, mordants, oxidizers, other dyes.

(ii) Bluing

This step converts the initial soluble red colour (of haematoxylin) within the nucleus to an insoluble blue colour. Some examples of bluing solution (alkaline pH) are ammonia water, dilute lithium carbonate, Scott's tap water (potassium carbonate, magnesium sulphate and water)

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