

PERIODIC ACID SCHIFF REACTION

Purpose To demonstrate glycogen in tissue.

Principle All of modern histochemistry of the polysaccharides, mucopolysaccharides and mucoproteins is bound up with the Periodic Acid Schiff reaction (PAS) and it is necessary to consider the principles on which this reaction is based.

Periodic acid is an oxidizing agent which breaks certain carbon chain bonds in various chemical structures; thus converting them to dialdehydes. Specifically, the periodic acid Schiff reaction is based on the fact that periodic acid will cleave (oxidize) the carbon-carbon bonds of carbohydrates where these carbon atoms have adjacent hydroxyl (i.e., 1,2 - glycols) or adjacent hydroxyl and primary (-NH₂) or secondary (-NHR amino groups) (1,2 - aminohydroxy groups) to yield aldehydes that can react with the Schiff reagent.

It does not further oxidize the resulting dialdehydes and these can be localized by combination with the Schiff's reagent to give a substituted dye which is red. Carbonyl Aldehyde groups are said to be further oxidized (by reagents such as potassium permanganate, chromic acid and hydrogen peroxide) to carboxylic acid groups and thereby prevented from subsequent reaction with Schiff's reagent. It should be noted that the red dye matter formed by the union of leucofuchsin with dialdehyde is a new compound and not, as previously supposed, re-oxidized fuchsin. Diastase may be added to the tissue section prior to staining. This enzyme digests glycogen and starch so that they will not react with the PAS reagents.

Fixative 10% neutral buffered formalin.

Reagents

1% Periodic Acid

Periodic Acid ... 1.0 g

Distilled water ... 100.0 ml

Schiff's Reagent (Purchased). Discard Schiff's reagent when it turns pink or begins to crystallize.

Quality Control Kidney, liver, or known fungus control slides must be used.

Procedure

1. Deparaffinize and hydrate slides to water.
2. Digest if necessary for 20 minutes.
3. Place all slides in 1% periodic acid for 10 minutes.
4. Wash in running tap water for 10 minutes to remove all acid.
5. Place in Schiff's reagent (stored in refrigerator) at room temperature for 20 minutes.
6. Wash in running tap water for 10 minutes.
7. Stain in hematoxylin for 1-2 minutes or 15 dips.
8. Rinse lightly in water. Blue in bluing reagent. Wash in water for a few minutes.
9. Dehydrate in alcohol and clear in xylene.
10. Mount in synthetic resin.

NOTE: When using diastase place all slides, including controls, in a 0.5% amylase solution for 10 minutes. Rinse in water. Proceed with periodic acid step.

Amylase (purchased) ... 0.5 g

Distilled water ... 100 ml

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Results

PAS-Positive material ... pink to reddish

Background ... light blue

Procedure

PAS Digestion

0.5 g Amylase

50.0 ml tap water

Mix together the water and amylase. Filter. Immerse slides in this solution for 10 minutes at room temperature. Rinse well and continue with PAS stain.

References

Theory and Practice of Histotechnology, Sheehan, 1973, pp. 89-90.