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Histology Special Stain - Periodic Acid Schiff (PAS) - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of simple polysaccharides (glycogen, starch), neutral mucosubstances, some acid mucosubstances (cornea, mucin in goblet cells), basement membranes, fungus, and mucoid cells of pituitary.

II. PRINCIPLE:

The reaction is based on the oxidation of certain tissue carbohydrates to aldehydes by the use of periodic acid. Periodic acid will NOT oxidize newly formed aldehyde groups to further breakdown products (carboxylic acid), which would result in a weak Schiff reaction. The Schiff reagent is prepared by combining a pararosaniline dye (i.e. Basic Fuchsin, Fuchsin, New Fuchsin) with water, hydrochloric acid, and sodium bisulfite. In the presence of excess sulfur groups in a high acidic environment, the chromophoric groups are rearranged, and the compound becomes colorless. Charcoal is used to remove impurities (acridine compounds and excess sulfur) from the dye. During staining, one Schiff reagent will combine with two aldehyde groups. Following the Schiff reaction, washing in running water removes the sulfur groups, restoring the magenta-pink color. Hematoxylin is used to stain the nuclei.

III. SPECIMEN COLLECTION AND HANDLING:

A. Fixation

1. Any well-fixed tissue.
2. If staining for glycogen, Carnoy fixative is preferred.

3. Avoid glutaraldehyde fixation

B. Processing
   1. Standard processing.

C. Section Thickness
   1. Routine specimens- 5µ.
   2. Liver biopsies- 4µ.
   3. Kidney biopsies- 3µ.
   4. Frozen section muscle biopsies- 10µ.

D. Slide Drying
   1. 60 minutes at 60°C.

E. Type of slide
   1. Plain

IV. REAGENTS:

A. **8% Alpha Amylase**

Alpha Amylase from pig pancreas	**0.4 gm**
Distilled water	**50.0 mL**

Just before use, dissolve together.

B. **0.5% Periodic Acid**

Periodic acid	**2.5 gm**
Distilled water	**500.0 mL**

Dissolve together. Stable at room temperature for months. Discard after use.

C. **Schiff Reagent (may use vendor pre-made)**

Basic fuchsin	**5.0 gm**
Sodium metabisulfite	**5.0 gm**
Distilled water	**1000.0 mL**
1 N Hydrochloric acid	**100.0 mL**

   1. Stir together basic fuchsin and distilled water for several minutes, until dissolved.
   2. Add sodium metabisulfite, and stir for several minutes, until dissolved.
   3. Add 1 N hydrochloric acid and stir for several hours.
   4. Allow to set overnight in the dark. Solution should be amber-colored.
   5. Add 10 gm activated charcoal and shake for 1 minute.
   6. Filter. Filtrate should be water-clear.
   7. Store in refrigerator. Stable for months; may be used twice.
   8. **TEST FOR QUALITY OF SCHIFF REAGENT:**
      Place 10 mL of 37-40% formaldehyde in a beaker. Add a few drops of Schiff reagent. If the solution rapidly turns reddish purple, it is good. If the reaction is delayed, and

the resulting color is a deep-blue-purple, the solution is breaking down and should be discarded.

D. **Hematoxylin**
Use hematoxylin from routine H&E set-up

V. EQUIPMENT:

A. Balance
B. Magnetic stirrer

VI. SUPPLIES:

A. Erlenmeyer flasks
B. Graduated cylinders
C. Filter paper
D. Funnel
E. Forceps
F. Coplin jars

VII. QUALITY CONTROL

Use liver as a positive control

VIII. SPECIAL SAFETY PRECAUTIONS:

A. Alpha amylase
    1. Is an irritant.

B. Periodic acid
    1. Is a strong oxidizer.
    2. Store separately from all other chemicals.

C. Hydrochloric acid
    1. Add drop by drop to water.
    2. May cause severe skin and eye burns.

D. Sodium Metabisulfite
    1. Is an irritant.

E. Basic Fuchsin
    1. Is an irritant and a suspected carcinogen.

IX. PROCEDURE:

Step	Action	Time	Notes
1	Deparaffinize and hydrate slides through graded alcohol to distilled water.		
2	To demonstrate glycogen, a PAS digestion is performed at this time.		
	i. Place sections labeled **'with'** in 0.8% Alpha Amylase. ii. Incubate at room temperature. iii. Rinse in distilled water to remove the Alpha Amylase. iv. Place slides labeled **"without"** in distilled water during this incubation period.	20 minutes	
3	Place all slides in 0.5% periodic acid at room temperature.	5 minutes	
4	Rinse in distilled water, 2-3 changes.	5-10 seconds each	
5	Place in Schiff reagent at room temperature.	10-20 minutes	
6	.Wash in running tap water	10 minutes	
7	Counterstain in Mayer or Gill Hematoxylin.	2 minutes	
8	Rinse in running tap water.	30 seconds	
9	Dip slides in dilute ammonia water.	2-3 seconds	
10	Wash in running tap water.	5 minutes	
11	Dehydrate through graded alcohols.		
12	Clear in two changes of xylene.		
13	Coverslip.		

X. LIMITATIONS:

A. Avoid the use of Ehrlich Hematoxylin, as certain mucins will stain with it.

B. Schiff reagent must be stored in the refrigerator.

C. If Schiff reagent develops a pink tinge, it should be discarded.

XI. RESULTS:

A. Epithelial acid mucopolysaccharides (mucins) - **pink to red**
(intestinal goblet cells, bronchus mucous glands)

B. Cryptococcus neoformans - **pink to red**

C. Nuclei - **blue**

XII. REFERENCES:

A. Bancroft JD, Stevens A: Theory and Practice of Histological Techniques, 3rd ed. New York, NY, Churchill Livingstone, 1990.

B. Carson FL: Histotechnology: A Self-Instructional Text, Chicago, IL, ASCP Press, 1990.

C. Sheehan DC, Hrapchak BB: Theory and Practice of Histotechnology, 2nd edition. Columbus, Ohio, Battelle Press, 1980.

Approval Signatures

Step Description	Approver	Date
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Applicability

Royal Oak