Washington University School of Medicine
Neuromuscular Lab
CAP: 1923316
CLIA: 26D0652044
NY: PFI 3499

PERIODIC ACID SCHIFF (PAS) PROTOCOL

PRINCIPLE:

The PAS stain demonstrates glycogen and glycated compounds. 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.

QUALITY ASSURANCE:

Stain several different muscles simultaneously. PAS staining is normally present in muscle fibers and connective tissue. The degree is of pathologic interest.

SPECIMEN REQUIRED:

Snap frozen human striated muscle.

METHOD:

Fixation: None. Use snap frozen tissue.

Technique: Cut 10 - 16 micron (12 µm) sections in cryostat from snap frozen biopsy. Attach one or more sections to a No.1½, 22 mm square coverslip.

Equipment:

Ceramic staining rack - Thomas Scientific #8542-E40
Columbia staining dish - Thomas Scientific #8542-C12
Columbia staining dish(jar) - Thomas Scientific #8542-E30
Forceps Latex gloves

Reagents:

Absolute alcohol (100% ethanol) - Quantum,
FLAMMABLE Store at room temperature in a flammable cabinet
Glacial Acetic Acid -Fisher A507-500
CORROSIVE Store at room temperature
Chloroform - Baxter 049-4, **FLAMMABLE • CARCINOGEN**
Store at room temperature in a flammable cabinet
Periodic Acid - Sigma P7875, store at room temperature
Permount - Fisher SP15-100, **FLAMMABLE HEALTH HAZARD**
Reagent alcohol, ACS - histological Fisher A962-4 or HPLC A995,
FLAMMABLE, TOXIC,TERATOGENIC
Store at room temperature in flammable cabinet.
Schiff Reagent - Harleco 6073/71, Store at room temperature
Xylenes - Fisher #HC700-1GAL, **FLAMMABLE**
Store at room temperature in flammable cabinet.

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Solutions:

I.	Carnoy's Fixative (store at room temperature)	
	PREPARE IN A FUME HOOD	
	alcohol, 100 %	60 ml
	chloroform	30 ml
	glacial acetic acid	10 ml
II.	Periodic Acid Solution, 0.5 % (w/v)	
	PREPARE FRESH FOR EACH STAIN	
	periodic acid	50 mg
	dissolved in deionized water	10 ml
III.	Alcohol 50 %	
	reagent alcohol	~50 ml
	deionized water	~50 ml
IV.	Alcohol 70 %	
	reagent alcohol	~70 ml
	deionized water	~30 ml
V.	Alcohol 80 %	
	reagent alcohol	~80 ml
	deionized water	~20 ml
V!.	Alcohol 95 %	
	reagent alcohol	~95 ml
	deionized water	~ 5 ml

Staining Procedure:

1. Place the coverslip with section in a Columbia staining dish (Thomas Scientific #8542- E40).

2. Add Carnoy's fixative to dish for 10 minutes.

3. **Rinse very carefully with several exchanges of deionized water. *Sections may wash off!!***

4. Add Periodic Acid solution to staining dish for 10 minutes.

5. **Rinse very carefully with several exchanges of deionized water. *Sections may wash off!!***

6. Add Shiff Reagent for 5 minutes

7. Carefully wash with three exchanges of tap or deionized water.

8. Dehydrate in ascending alcohol solutions (50%, 70%, 80%, 95% x 2, 100% x 2) in Columbia staining dishes - Thomas Scientific #8542-E30.

9. Clear with xylene (3 - 4 x) in Columbia staining dish - Thomas Scientific #8542-E30.

10. Mount coverslip onto a labeled glass slide with Permount or some other suitable organic mounting medium

Results:

Glycogen, neutral mucosubstances, basement membranes, collagen fibers, glycolipids and phospholipids will be demonstrated as pink to red to purple color. If diastase or α-amylase is used for a negative control, the glycogen deposits are removed leaving other staining pink. Type II muscle fibers stain darker than type I. Polyglucosan bodies may stain in intramuscular nerves.

REFERENCES:

1. Thompson, Samuel W. *SELECTED HISTOCHEMICAL AND HISTOPATHOLOGICAL METHODS,* Charles C. Thomas, Springfield, IL, 1966.

2. Sheehan, D.C. and Hrapchak, B.B., *THEORY AND PRACTICE OF HISTOTECHNOLOGY,* 2nd Edition; Battelle Memorial Institute, Columbus, OH, 1987.

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