PAS - McMANNUS' PERIODIC ACID SCHIFF'S - GLYCOGEN

PURPOSE: Glycogen is present in skin, liver, parathyroid glands and skeletal and cardiac muscle. The PAS stain is used for demonstration of basement membranes, fungus secreting adenocarcinoma from undifferentiated squamous cell carcinoma, and mucosubstances secreted from the epithelia of various organs. A routine stain for liver and kidney biopsies.

PRINCIPLE: The PAS stain is a histochemical reaction in that the periodic acid oxidizes the carbon to carbon bond forming aldehydes which react to the fuchsin-sulfurous acid which form the magenta color.

CONTROL: For staining fungus; use a known positive such as those used for the GMS. Use skin, aorta or normal liver for positive PAS staining.

FIXATIVE: Any well fixed tissue.

TECHNIQUE: Cut paraffin sections 4-5 m(3m for kidney biopsies).

EQUIPMENT: Rinse glassware in DI water. Coplin jar, microwave oven.

REAGENTS:

0.5% Periodic Acid:

Periodic acid	0.5 gm
Distilled water	100.0 ml

Mix well, label with date and initial. Stable for 1 year.

Caution: Avoid contact and inhalation.

Hematoxylin, GILL-3 Purchased through Baxter.

Lillie's Cold Schiff's Reagent:

Basic fuchsin	10.0 gm
Sodium metabisulfite	18.0 gm
Distilled water	1000 0 ml
Hydrochloric acid	10.0 ml

Stir solution for 2 hours, set in a dark cool place overnight. The solution is now a clear light brown to yellow color. Add:

Activated charcoal 500.0 gm
(or two heaping spoons)

Stir. Filter through Whatman #2 filter paper into a 1000 ml graduated cylinder. Change the filter paper often. Restore volume to 1000 ml with distilled water.

Store in Refrigerator, solution is stable for 6 months.

CAUTION: Carcinogen, corrosive.

SAFETY: Wear gloves, goggles and lab coat. Avoid contact and inhalation.

Hydrochloric acid: strong irritant to skin, eyes and respiratory system. Target organ effects via inhalation on skin, respiratory, reproductive and fetal systems. Corrosive.

Schiff's Reagent: Use extreme caution, Basic fuchsin (pararosaniline) is a known carcinogen. Wear gloves, goggles, particle mask and lab coat, while preparing solution. Work under the hood, keep hot, uncapped, solutions under the hood.

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, fungus:	magenta
Nuclei	blue

NOTES:

1. To check stain, pour 10 ml of formaldehyde in cylinder, add a few drops of Schiff's, it should turn red-purple immediately.
2. Discard Schiff's if it turns pink while sitting in the refrigerator.
3. The white precipitate at the bottom of the Schiff's can be re-dissolved if desired by gently warming and stirring the solution.

REFERENCES:

Sheehan D, Hrapchak B, Theory and practice of Histotechnology, 2nd Ed, 1980, pp 164-166, Battelle Press, Ohio

Bancroft J, Stevens A, Theory and Practice of Histological Techniques, 2nd Ed, 1982, pp 188-190, Churchill Livingstone, NY

Crookham, J, Dapson, R, Hazardous Chemicals in the Histopathology Laboratory, 2nd ED,1991, Anatech

Prepared:_____By: _____

Approved:_____By:_____

PROCEDURE CARD

PAS - GLYCOGEN - FUNGUS

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RESULTS:

Glycogen, fungus:	magenta
Nuclei	blue

SAFETY/PPE: Hydrochloric acid is caustic use caution, flush with water. Avoid breathing the basic fuchsin, carcinogen. Wear gloves, goggles and lab coat.

Lillie's Cold Schiff's Reagent:

Basic fuchsin	10.0 gm
Sodium metabisulfite	18.0 gm
Distilled water	1000.0 ml
Hydrochloric Acid	10 0 ml

Stir solution for 2 hours, set in a dark cool place overnight. The solution is now a clear light brown to yellow color. Add:

Activated charcoal	500.0 gm
	(or two heaping spoons)

Stir. Filter through Whatman #2 filter paper into a 500 ml graduated cylinder. Change the filter paper often. Restore volume to 500 ml with distilled water.

Store in Refrigerator, solution is stable for 6 months.

CAUTION: Carcinogen, corrosive.

Hematoxylin, GILL-3:

Purchased thrpugh Baxter.

0.5% Periodic Acid:

Periodic acid	0.5 gm
Distilled water	100.0 ml

Mix well, label with date and initial. Stable for 1 year.

0.5% PERIODIC ACID:

Periodic acid	0.5 gm
Distilled water	100.0 ml

Mix well, label with date and initial. Stable for 1 year.

CAUTION: Avoid contact and inhalation.

DATE:_____

TECH:_____

EXPIRATION:_____

0.5% PERIODIC ACID

DATE:_____

TECH:_____

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DATE:_____

TECH:_____

EXPIRATION:_____

SCHIFF'S SOLUTION

DATE:_____

TECH:_____