PAS/D - GLYCOGEN DIGESTION - DIASTASE

PURPOSE: To determine glycogen by digesting out and staining with PAS stain.

PRINCIPLE: The diastase (or a-amylase) act on glycogen to de polymerize it into smaller sugar units, maltose and glucose, that are washed out of the section.

CONTROL: Identical sections are obtained on two separate slides. One is digested the other is not, both are stained with the PAS stain. Liver is a good control.

FIXATIVE: Any well fixed tissue.

TECHNIQUE: Cut paraffin sections at 4m to 5m

EQUIPMENT: Rinse glassware in DI water. Coplin jars, 37°C oven.

REAGENTS:

Digestion Solution:
Diastase (with a-amylase) 0.05 gm
(obtained through Ameri. Reagent)
Distilled water 50.0 ml

Make fresh, use within 15 minutes, discard.

0.5% Periodic Acid:
See PAS

Schiff's Reagent:
See PAS

Hematoxylin, Gill-3
Purchased through Baxter

SAFETY: Hydrochloric acid is caustic use caution, flush with water. Wear gloves, goggles and lab coat.

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..

Avoid contact with and inhalation of dyes.

PROCEDURE:

1. Deparaffinize and hydrate to distilled water.
2. Warm the diastase solution in the microwave for 20 seconds.
3. Place the slide labeled "PAS/D" in the warm diastase solution and into the waterbath for 15 minutes, do not over digest.
4. Rinse in running tap water, rinse in distilled.
5. Stain both slides simultaneously, following the PAS procedure.

RESULTS:

Glycogen will be stained magenta on the PAS stained slide and will be absent on the PAS/D stained slide.

Note: If slide is over digested, the tissue must be recut. Over digestion has the appearance of lace; there is no tissue left.

NOTES:

1. Do not celloidin the slides prior to digestion, it coats the tissue and digestion can not occur.
2. Saliva can be substituted, rinse out mouth, lay slide horizontally in staining dish, in the heat for 30 minutes.
3. If slide is over digested, the tissue must be recut. Over digestion has the appearance of lace - there is no tissue left.

REFERENCES:

Bancroft J, Stevens A, Theory and Practice of Histological Techniques, 2nd Ed, 1980, pp 187-188, Churchill-Livingstone, NY

Carson F, Histotechnology, A Self-Instructional Text,1st Ed, 1990, pp 122-123, ASCP, Ill

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

Prepared:_____By: _____
Approved:_____By:_____

PROCEDURE CARD

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See PAS

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