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E-312 <u>McMANUS PAS METHOD FOR</u> GLYCOGEN

FIXATION: 10% Buffered Neutral Formalin

SECTION: Paraffin, @ 6 microns

STAINING PROCEDURE: Digest using a diastase, hyaluronidase, or sialidase procedure.

- 1. Deparaffinize and hydrate to distilled water.
- 2. Oxidize in **Periodic Acid**, **0.5%**, (E-312-3), 5 minutes and rinse in distilled water.
- 3. Stain in <u>Coleman's Feulgen</u>, (E-312-1) or <u>Schiff's Reagent</u>, (E-312-1A), 15 minutes and wash in running water to develop the pink color, 10 minutes.
- 4. Counter-stain in <u>Harris Hematoxylin</u>, (E-312-2B), 6 minutes, or <u>Light Green Working Solution</u>, (E-312-2A) 5-10 seconds. Light Green is better used when delineation of fungi is required. Prepare the working solution by diluting <u>Light Green</u>, 0.2% <u>Solution</u>, (E-312-2) 1:5 with distilled water; proceed to step #7, dehydration. Tap water and ammonia decolorize Light Green.
- 5. Wash in running water and transfer to **Acid Alcohol, 1%,** (E-312-4), for 3-10 quick dips. Wash again in distilled water.
- 6. Dip in dilute **Ammonia Water, 0.3%,** (E-312-5), to blue the sections and again wash in running water for ten minutes.
- 7. Dehydrate in 95% alcohol and 100% alcohol, clear in Xylene, two changes each.
- 8. Mount.

RESULTS:

Nuclei......blue
Fungi.....red
Background, when Light Green
Is used as the counter-stain....green, pale

REFERENCES:

McManus, JFA: Stain Tech.; 23: 99 (1948).

AFIP <u>Manual of Histologic Staining Techniques</u>; L.G. Luna, 3rd ed. ed.; New York: McGraw-Hill Publications, c. 1968, p. 160.

Mowry, RW: Annals of the New York Academy of Science; <u>106</u>: 402 (1963).