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15/MHS06/064

MLS 502

1. Hematoxylin and Eosin
2. Procedure:
* Bring sections to distilled water using descending grades of alcohol.
* Stain nuclei with the alum hematoxylin for 3-5 minutes
* Rinse in running tap water
* Differentiate with 0.3% acid alcohol briefly
* Rinse in running tap water
* Rinse in Scott's tap water substitute for 10 minutes
* Rinse in tap water
* Stain with eosin for 2 mins
* Dehydrate, clear and mount.
1. Control sections: spleen, liver
2. Indications: for the demonstration of general tissue structure. It can me used to check for malignant cells as well as cancer grading.
3. Expected results:

Collagen- pale pink

 Muscle- deep pink

acidophilic cytoplasm- red

basophilic cytoplasm- purple

nuclei- blue

erythrocytes- cherry red

1. PAS
2. Procedure:
* Deparaffinize and hydrate the slide using distilled water
* Place the section in preheated diastate solution (at 37 degrees centigrade) for about an hour
* Wash the sample in running water for about 5 minutes
* Place the sections in 0.5 percent periodic acid solution for about 5 minutes
* Wash the section in distilled water
* Place the section in Schiff reagent for about 15 minutes
* Wash the section for about a minute in 0.55 percent potassium metabisulfite in order to remove excess stains
* wash in running tap water for about 10 minutes
* Counterstain using Harris's hematoxylin with acetic acid for half a minute
* Wash with running water
* Dehydrate with two changes using absolute alcohol, clear with xylene and mount to view
1. Control sections: kidney, liver
2. Indications: for the demonstration of carbohydrates in tissue samples. Can be used to monitor the basement membrane of the bowman’s capsule in kidney sections.
3. Expected results:

PAS positive substances: red or magenta

Nuclei: blue

1. Gordon & sweets
2. Procedure:
* Deparaffinize and hydrate to distilled water.
* Potassium permanganate solution, 5 minutes and Wash in water.
* 5% oxalic acid until clear then wash in distilled water.
* Iron alum solution, 10 minutes.
* Wash in running tap water, rinse in distilled, 3 changes.
* Silver solution, 7 dips, shake excess solution off slides then in Distilled water, 2 changes, 3 quick dips each.
* 10% formaldehyde solution until gray black, 30 seconds and Wash in distilled water.
* 0.5% Gold chloride, 1 minute and Rinse in distilled water.
* 5% hypo, 1 minute and Wash in tap water.
* Nuclear-fast red solution, 5 minutes and Wash in running tap water.
* Dehydrate, clear, and coverslip
1. Control tissue: liver, kidney
2. Indications: demonstrates reticulin fibers. Can be used to detect liver damage.
3. Expected results:

Reticulin fibers: black

Collagen: brown to yellow

Cytoplasm: brown to yellow

Nuclei: red

1. Perl’s Prussian blue
2. Procedure:
* Deparaffinize and bring the sections to water.
* Treat the sections with freshly prepared acid ferrocyanide solution for 10-30 minutes.
* Wash well in distilled water.
* Lightly stain the nuclei with 0.5% aqueous neutral red or 0.1% nuclear fast red.
* Wash rapidly in distilled water.
* Dehydrate, clear and mount.
1. liver, lungs
2. Indications: detection of hemosiderosis.

iv. Expected results:

* Ferric ion = blue
* Nuclei = red
* Background = pink
1. Gram F
2. Procedure:
* Deparaffinize and hydrate to distilled water.
* Place slides on staining rack, drop crystal violet stain onto tissue section, stain for 1 minute.
* Wash in tap water.
* Lugol's iodine, 1 minute.
* Wash in tap water.
* Blot sections dry, breath on section then quickly pour acetone over section until no color runs off.
* Wash in tap water.
* Place slides on staining rack, drop Basic fuchsin on tissue sections, stain 3 minutes.
* Wash in tap water, blot gently but not completely dry.
* Dip quickly into acetone, 2 dips.
* Dip directly into picric acid-acetone mixture until a 'salmon' color.
* Dip quickly into two changes of acetone.
* Air dry, dip into xylene, and coverslip.
1. Control sections: An infected appendix, or any tissue containing both negative and positive gram rods.
2. Indications: For demonstrating gram-negative and gram-positive in tissue.
3. Expected results:

Gram-positive bacteria- blue

Gram-negative bacteria- red

 Nuclei- red

Background- yellow

1. Alcian blue
2. Procedure:
* Bring sections to distilled water
* Stain in the Alcian blue solution 15 minutes
* Wash well in running tap water 5 minutes (For lower pH solutions i.e. pH 1.0/0.2, drain and blot dry, to prevent removal of stain in water).
* Rinse in distilled water
* Counterstain with neutral red stain 1 min
* Rapidly dehydrate in absolute alcohol, clear and mount.

ii. Control sections: small intestine, appendix or colon

1. Indications: it stains acid mucosubstances and acetic mucins. Excessive amounts of non-sulfated acidic mucosubstances are seen in mesotheliomas.
2. Expected results:

Acid Mucins - Blue

Nuclei - red

Erythrocytes - yellow

At pH 2.5 most acid mucins (except some of the strongly sulphated groups) - Blue

At pH 1.0 only weakly and strongly sulphated acid mucins - Blue

At pH 0.2 only strongly sulphated acid mucins - Blue

1. VVG
2. Procedure:
* Deparaffinize and hydrate slides to distilled water.
* Stain in Verhoeff’s solution for 1 hour. Tissue should be completely black.
* Rinse in tap water with 2-3 changes.
* Differentiate in 2% ferric chloride for 1-2 minutes.
* Stop differentiation with several changes of tap water and check microscopically for black elastic fiber staining and gray background. It is better to slightly under differentiate the tissue, since the subsequent Van Gieson’s counterstain can extract the elastic stain somewhat.
* Wash slides in tap water.
* Treat with 5% sodium thiosulfate for 1 minute. Discard solution.
* Wash in running tap water for 5 minutes.
* Counterstain in Van Gieson’s solution for 3-5 minutes.
* Dehydrate quickly through 95% alcohol, 2 changes of 100% alcohol.
* Clear in 2 changes of xylene for 3 minutes each.
* Coverslip with resinous mounting medium
1. Control tissue: skin, blood vessels
2. Indications: it is used to demonstrate elastic fibers.
3. Expected results:

Elastic fibers: blue-black to black

Nuclei: blue to black

Collagen: red

Other tissue structures: yellow

1. WVG
2. Procedure:
* Make a working solution of Weighert’s Iron Haematoxylin by mixing equal parts of Part A and Part B. Discard this solution after use;
* Deparaffinise sections and hydrate to distilled/deionised water;
* Stain sections with the Weigert’s Iron Haematoxylin working solution for 10 to 20 minutes. Sections should be overstained as they will be slightly decolourised by the picric acid in the Van Gieson’s stain;
* Wash in running water for 10 minutes;
* Stain sections in van Gieson’s stain for 5 minutes. Discard the solution;
* Place slides in 95% alcohol;
* Dehydrate as normal, clear with xylene and mount with a synthetic resin.
1. Control sections: aorta
2. Indications: differentiates between smooth muscles and collagen in tumors and to demonstrate the increase of collagen in diseases.
3. Expected results:

Nuclei: Blue-Black

Collagen: Brilliant Red

Muscle and Cytoplasm: Yellow