**UW Medicine - Pathology**

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Verhoeff's Elastic Stain for Demonstration of Elastic Fibers

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| Adopted Date: 06/22/05  Review Date: 09/03/10  Revision Date: 04/12/13 |

PURPOSE

To identify the method for performing the special stain of Verhoeff's Elastic Stain for demonstration of elastic fibers.

PROCEDURE

**Fixation:**

10% buffered neutral formalin.

**Sectioning:**

Paraffin sections at 5 microns.

**Solutions:**

(Use Type II de-ionized water for all solution preparation.)

**5% Verhoeff’s Hematoxylin Solution**

Hematoxylin crystals (CI #75290) 20.0 gm

Ethyl alcohol, absolute 400.0 ml

Dissolve hematoxylin in 100ml of absolute ethyl alcohol with aid of heat. Stable for four months. Discard after use.

**10% Ferric Chloride**

Ferric chloride 50.0 gm

Distilled water 500.0 ml

Stable for four months. Discard after use.

**Iodine Solution**

Iodine 5.0 gm

Potassium iodide 10.0 gm

Distilled Water 500.0 ml

Dissolve potassium iodide in 2-4 ml of distilled water add iodine, when dissolved add remaining distilled water. Stable for two months. Discard after use.

**Elastic Tissue Stain**

5% Verhoeff’s hematoxylin solution 12.5 ml

Ferric chloride, 10% 4 ml

Iodine solution 4 ml

Filter the hematoxylin solution; add the ferric chloride and iodine to the filtered hematoxylin. Prepare solution fresh daily. Discard after use.

**2% Ferric Chloride**

Ferric chloride 10 gm

Distilled water 500 ml

Discard after use.

**5% Sodium Thiosulfate (Hypo)**

Sodium thiosulfate 25.0 gm

Distilled water 500.0 ml

Stable for six months. Discard after use.

**1% Acid Fuchsin**

Acid fuchsin (CI #42685) 1.0 gm

Distilled water 100.0 ml

Stable for four months.

**Van Gieson’s Solution**

Acid fuchsin, 1% 2.5 ml

Picric acid, saturated aqueous 50 ml

Stable for one month.

Picric Acid 2.1 ml

Distilled Water 100.0 ml

Handle with care. Picric acid is explosive.

**Procedure:**

1. Deparaffinize and hydrate to distilled water.
2. Verhoeff’s elastic tissue stain for 15 minutes.
3. Rinse in running water.
4. Differentiate in 2% ferric chloride until elastic fibers are properly differentiated.
5. Rinse in running water.
6. Check slides microscopically. If elastic fibers are over differentiated, go back to step two.
7. Place in 5% sodium thiosulfate for one minute.
8. Wash in tap water for five minutes.
9. Counterstain in Van Gieson’s stain for 3 minutes.
10. Dehydrate in 95% and absolute isopropyl alcohol.
11. Clear in xylene and mount.

**Results:**

Elastic fibers blue-black to black

Nuclei blue to black

Collagen red

Other tissue elements yellow

**Comments:**

The first step in the procedure is an over staining of the tissue section with a soluble lake of hematoxylin-ferric chloride-iodine. This solution penetrates the elastic fibers easily and precipitates in them.

Differentiation, a necessary step in any over staining process, is accomplished by use of excess mordant, (a dilute solution of ferric chloride), to break the tissue-mordant-dye complex. The dye distributes itself partly as a soluble lake with the free mordant, and partly as a component of the insoluble complex. Since the amount of mordant in the complex is small in comparison with the amount in the differentiating fluid, nearly all of the dye will eventually associate itself with the differentiator. Since this is soluble, it will be removed in the next washings. The elastic tissues, having the strongest affinity for the insoluble complex, retain it the longest and so are colored black in the final result.

Sodium thiosulfate, (hypo), is used to remove the excess iodine from the solution, and tap water removes both from the tissue section. The Van Gieson’s solution of acid fuchsin and picric acid is used as the counter-stain and colors the collagen bright red and other tissue elements yellow. The period of counterstaining with the Van Gieson’s solution must not be prolonged, since the picric acid will then act to further differentiate the stain. The staining solution should be made up fresh for best results, and differentiation should be controlled by using the microscope.

REFERENCES

*Manual of Histologic and Special Staining Technics*, McGraw-Hill Book co., 1960, pg. 82. Modified by Histopathology Laboratory, Harborview Medical Center, Seattle, WA.

Written By: Director Approval:

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Histology Supervisor