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Part 9116
Revised February 2018

Elastic, Verhoeff Stain Kit - Technical Memo

KIT INCLUDES:	Part 9116A	Part 9116B
Solution A: Hematoxylin 5%, Alcoholic	125 ml	250 ml
Solution B: Ferric Chloride 10%, Aqueous	125 ml	250 ml
Solution C: Iodine, Weigert & Lugol, Aqueous	75 ml	150 ml
Solution D: Sodium Thiosulfate 5%, Aqueous	250 ml	500 ml
Solution E: Van Gieson Stain	250 ml	500 ml

COMPLIMENTARY POSITIVE CONTROL SLIDES: Enclosed with this kit are two complimentary unstained positive control slides to be used for the initial verification of staining techniques and reagents. Verification must be documented by running one Newcomer Supply complimentary positive control slide along with your current positive control slide for the first run. Retain the second complimentary control slide for further troubleshooting, if needed.

Individual stain solutions and additional control slides may be available for purchase under separate part numbers at www.newcomersupply.com.

Additionally Needed:	
Xylene, ACS	Part 1445
Alcohol, Ethyl Denatured, 100%	Part 10841
Alcohol, Ethyl Denatured, 95%	Part 10842

For storage requirements and expiration date refer to individual bottle labels.

APPLICATION:

Newcomer Supply Elastic, Verhoeff Stain Kit procedure, commonly referred to as Verhoeff-Van Gieson technique, is used to demonstrate pathologic changes in elastic fibers as well as demonstration of normal elastic tissue such as arteries and veins.

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)
Technique: Paraffin sections cut at 5 microns
Solutions: All solutions are manufactured by Newcomer Supply, Inc.

All Newcomer Supply Stain Kits are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below. Some solutions in the kit may contain extra volumes.

PRESTAINING PREPARATION:

1. Prepare fresh Verhoeff Working Solution by combining in the exact order listed, mixing well after each addition. Save for Step #4.
   a. *Solution A: Hematoxylin 5%, Alcoholic* — *20 ml*
   b. *Solution B: Ferric Chloride 10%, Aqueous* — *8 ml*
   c. *Solution C: Iodine, Weigert & Lugol, Aqueous* — *8 ml*
2. Prepare fresh Ferric Chloride 2%, Aqueous Solution for Step #6.
   a. *Solution B: Ferric Chloride 10%, Aqueous* — *10 ml*
   b. *Distilled water* — *40 ml*

STAINING PROCEDURE:

3. Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohols, 10 dips each. Wash well with distilled water.
   a. *See Procedure Notes #1 and #2.*
4. Stain in fresh Verhoeff Working Solution (Step #1) for 20 minutes.
   a. *Discard solution after successful differentiation in Step #6.*
5. Rinse in several changes of tap water.
6. Differentiate each slide individually in fresh Ferric Chloride 2%, Aqueous Solution (Step #2) with agitation; approximately 20 dips.
7. Check differentiation; rinse well in tap water and check microscopically for black elastic staining with gray background.
   a. *Repeat in Ferric Chloride 2%, Aqueous Solution if necessary until desired elastic differentiation is achieved.*
   b. *See Procedure Notes #3 and #4.*
8. Wash well in tap water.
9. Place in Solution D: Sodium Thiosulfate 5%, Aqueous for 1 minute.
10. Wash well in running tap water for 5 minutes.
11. Counterstain in Solution E: Van Gieson Stain for 3 to 5 minutes.
    a. *See Procedure Note #5.*
12. Dehydrate in two changes each of 95% and 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

RESULTS:

Elastic fibers/tissue	Blue-black to black
Nuclei	Blue to black
Collagen	Red
Other tissue elements	Yellow

PROCEDURE NOTES:

1. Drain staining rack/slides after each step to prevent solution carry over.
2. Do not allow sections to dry out at any point during staining procedure.
3. It is easy to over-differentiate in Ferric Chloride 2%, Aqueous Solution.
   a. If background is completely colorless, the section has *been over-differentiated.*
   b. *Over-differentiated sections may be re-stained in Verhoeff Working Solution (Step #4) provided sections have not been treated with an alcohol step.*
4. Slides must be individually differentiated. Timing of each slides differentiation can vary dependent upon the amount of elastic tissue present in sections.
5. Avoid prolonged exposure in Solution E: Van Gieson Stain. The picric acid element will act to further differentiate the stain.
6. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:

1. Carson, Freida L., and Christa Hladik Cappellano. *Histotechnology: A Self-instructional Text*. 4th ed. Chicago: ASCP Press, 2015. 167-169.
2. Mallory, Frank Burr, and James Homer Wright. *Pathological Technique*. 7th ed. Philadelphia, PA: W.B. Saunders Company, 1918. 118-119.
3. Modifications developed by Newcomer Supply Laboratory.
