1. Purpose

Trichrome stains are frequently used to differentiate between collagen and smooth muscle in tumors and to identify increases in collagenous tissue in diseases such as cirrhosis of the liver.

1. Principle

Trichrome procedures are so named because three dyes, which may or may not include the nuclear stain, are used. Sections are first stained with an acid dye such as Biebrich scarlet; all acidophilic tissue elements such as cytoplasm, muscle, and collagen will bind the acid dyes. The sections are then treated with phosphotungstic and/or phosphomolybidic acid. Because cytoplasm is much less permeable than collagen, phosphotungstic and phosphomolybdic acids cause Biebrich scarlet to diffuse out of the collagen but not out of the cytoplasm. Phosphotungstic and phosphomolybdic acids have numerous acidic groups that most likely act as a link between the decolorized collagen and aniline blue, the collagen dye. The pH of the phosphotungstic/phosphomolybdic acid solution may also increase selective collagen staining and aids in the diffusion or removal of Biebrich scarlet.

1. Fixative

10% neutral buffered formalin or Davidsons (Hartmann's). *Bouin’s preferred.*

1. Embedding/Sectioning

Cut paraffin sections 4 to 5 µm.

1. Quality Control

Practically every tissue has an internal control, so no other control sections are needed; however, if a control is desired, uterus, small intestine or appendix can be used.

1. Reagents

* Davidson's (Hartmann's) (commercially prepared- Poly)
* Weigert Iron Hematoxylin Stock solutions:

 Solution A (commercially prepared-Poly)

Solution B (commercially prepared-Poly)

* Biebrich Scarlet-Acid Fuchsin Solution ( commercially prepared-Poly)
* Phosphomolybdic/Phosphotungstic Acid Solution (commercially prepared-Poly)
* Aniline Blue Solution (commercially prepared-Poly)
* Acetic Acid Solution, 1% ( commercially prepared- Poly)

1. Preparation
* **Working Weigert’s Hematoxylin**: Mix equal parts (25ml each) of Weigert’s hematoxylin stock solution A and solution B in a coplin jar.
1. Staining Procedure

 1. Deparaffinize sections and hydrate to distilled water.

 2. Rinse well in distilled water.

 3. Mordant the section in Davidsons solution for 1 hour (solution is pre-heated in 56°C oven)

 4. Remove slides from oven, cool for 1 minute, and wash in running water.

 5. Rinse in distilled water.

 6. Stain sections in **Weigert’s Hematoxylin Working Solution** for 10 minutes.

 7. Wash in running water for 10 minutes.

 8. Rinse in distilled water.

 9. Stain sections in Biebrich scarlet-acid fuchsin solution for 2 minutes.

 10. Rinse in distilled water.

 11. Place the slides in phosphomolybdic/phosphotungstic acid solution for 10 minutes.

 12. Rinse in distilled.

 13. Stain sections in aniline blue solution for 4 minutes.

 14. Rinse the slides in distilled water.

 15. Place slides in 1% acetic acid solution for 4 minutes.

 16. Rinse in distilled water.

 17. Dehydrate with 95% and 100% alcohol, two changes each, and then clear with Citri-solv.

1. Results

 

 Nuclei………………………………………………………..Black

 Cytoplasm, keratin, muscle fibers…………………………...Red

 Collagen and mucus…………………………………………Blue

1. Reference

 Histotechnology, A Self Instructional Text 2nd Edition, Frieda L. Carlson 1997

Implementation Date

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| --- | --- | --- | --- |
| Author | Olga De Jesus |  | 6/11/2013 |
| Supervisor Approval | Olga De Jesus |  |  |
| Director Approval | Dr. Clyde Belgrave |  |  |

Review

 Supervisor Name Signature Date

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Revisions

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| Description: Reformatting. Modified procedure staining times. Added images. |
| Author: Olga De Jesus 6/11/2013 |
| Supervisor approval:  |
| Director Approval (major revisions only): |
| New Procedure Number |
| Description: |
| Author: |
| Supervisor approval: |
| Director approval: |
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