

MASSON'S TRICHROME STAIN

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.

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 with the acid dyes. The sections are then treated with phosphotungstic and/or phosphomolybdic acid. Because cytoplasm is much less permeable than collagen the phosphotungstic and phosphomolybdic acids cause the 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. Probably the pH of the phosphotungstic/phosphomolybdic acid solution also increases selective collagen staining and aids in the diffusion or removal of Biebrich scarlet.

Fixative Bouin's solution is preferred but 10% neutral buffered formalin may be used.

Reagents

Bouin's Solution

Picric Acid, saturated aqueous solution ... 75.0 ml
Formaldehyde, 37-40% ... 25.0 ml
Glacial acetic acid ... 5.0 ml

Weigert's Iron Hematoxylin

Solution A

Hematoxylin ... 10.0 g
Alcohol, 95% ... 1,000.0 ml

Solution B

Ferric chloride, 29% aqueous solution ... 20.0 ml
Distilled water ... 475.0 ml
Glacial acetic acid ... 5.0 ml

Working Solution

Mix equal parts of solutions A and B.

Biebrich Scarlet-Acid Fuchsin Solution

Biebrich scarlet, 1% aqueous solution ... 360.0 ml
Acid fuchsin, 1% aqueous solution ... 40.0 ml
Glacial acetic acid ... 4.0 ml

Phosphomolybdic-Phosphotungstic Acid Solution

Phosphomolybdic acid ... 25.0 g
Phosphotungstic acid ... 25.0 g
Distilled water ... 2,000.0 ml

Aniline Blue Solution

Aniline Blue ... 25.0 g
Glacial acetic acid ... 20.0 ml

1% Acetic Acid Solution

Glacial acetic acid ... 1.0 ml
Distilled water ... 99.0 ml

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, appendix or fallopian tube will provide good material.

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Procedure

1. Deparaffinize sections and hydrate to distilled water.
2. Rinse well in distilled water.
3. Mordant the section in Bouin's solution for 1 hour at 56°C.
4. Remove slides from oven, allow to cool, and wash in running water until the yellow color disappears.
5. Rinse in distilled water.
6. Stain sections in Weigert's hematoxylin for 10 minutes.
7. Wash in running water for 10 minutes.
8. Rinse in distilled water.
9. Stain sections in Biebrich scarlet-acid fuchsin for 2 minutes. If desired, the solution may be saved for *one more run only*.
10. Rinse in distilled water.
11. Place the slides in phosphomolybdic-phosphotungstic acid solution for 10-15 minutes. Discard this solution.
12. Stain sections in aniline blue solution for 5 minutes. If desired, this solution may be saved for *one more run only*.
13. Rinse the slide in distilled water.
14. Place the slides in 1% acetic acid solution for 3-5 minutes. Discard this solution.
15. Dehydrate with 95% and absolute alcohol, two changes each.
16. Clear with two or three changes of xylene and mount with synthetic resin.

Results

Nuclei black

Cytoplasm, keratin, muscle fibers red

Collagen and mucus blue

Plate 8-4, p. 267 Histology: A Self-Instructional Text, Fried Carson, 1990.

Procedure Notes

If desired, collagen may be counterstained with light green instead of aniline blue. Changes are described on page 144 of Histology: A Self-Instructional Text.

References

Histology, A Self-Instructional Text, Freida L. Carson, 1990, pp. 142-144, and Plate 8-4, p. 267.