medilines

MSB Trichrome

for Fibrin

DESIGN TO DEMONSTRATE

Fibrin (insoluble fibrillar protein formed by polymerisation of fibrinogen, which is one of the plasma proteins and is seen in tissues where there has been tissue damage.)

REAGENTS & PREPARATIONS

MSB Solution A	
MSB Solution B	
MSB Solution 1:	Mix A and B in equal volume (Solution will remain stable for one weak. Preferably prepare fresh)
MSB Decolourizer	Ready to use
MSB Solution 2:	Ready to use
MSB Solution 3:	Ready to use
MSB Solution 4:	Ready to use
MSB Solution 5:	Ready to use

TISSUE SAMPLE

4-5µ paraffin sections of 10% neutral buffered formalin.
Trichrome stains often benefit from initial Bouin, formal sublimate or B5 fixation. Zenker and Helly fixation is usually satisfactory.

Note: Formalin variants are generally sufficient, but staining can be intensified by secondary fixation (Mordant) in Bouin's Fluid

Place section after the 1st step of following staining procedure in Bouin's fluid for an hour at 56-60°C (or microwave 1 minute, allow to stand for 15 minutes} and wash in running tap water for 5 minutes to remove Picric Acid.

STAINING PROCEDURES

1. Remove Paraffin through xylene, rehydrate through graded ethanol and bring sections to distilled water just prior to staining.
2. Place into MSB **Solution 1** for 5-7 minutes
3. Place in **Decolourizer** for 5-10 seconds. Wash in running tap water for 30 seconds and rinse in distilled water.
4. Rinse in 95% Ethanol and Shake excess alcohol from the slides.

5. Place into **MSB Solution 2** for 3-5 minutes.
6. Wash with distilled water. Just enough to remove MSB solution 2.
7. Place into **Masson Solution 3** for 5-10 minutes.
8. Rinse in distilled water.
9. Place into **Masson Solution 4** for 5-10 minutes until no red remains in the collagen
10. Rinse in distilled water
11. Place into **Masson Solution 5** for 2-4 minutes(control microscopically)
12. Rinse in distilled water
13. Dehydrate up through graded ethanol to absolute ethanol.
14. Clear in xylene and mount with a resinous medium.

Expected results

- Fibrin – Clear Red
- Muscle – Pale Red
- Red Blood Cells – Yellow/Orange
- Collagen – Blue
- Nuclei – Blue/Black

Positive Controls

Inflammatory appendix due to peritonitis (fibrinous appendix), Control: skeletal or cardiac muscle, soft cartilage.

Notes

1. Overnight formalin fixation is usually satisfactory, but avoid rapid fixation with formalin and short processing, as this produces tissues that stain poorly even with the secondary fixation specified
2. Once a satisfactory stain has been achieved, the time should remain constant. Any change in fixation, processing or section thickness warrants reviewing the staining time.
3. Avoid microbial contamination of reagents; otherwise increase in nonspecific staining may occur.
4. The user must validate any changes made to the factory-released procedure.
5. Store reagent packs in an upright position when not in use.
6. Keep reagent packs out of direct sunlight and away from heat-generating sources.
7. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.

Reference

Drury, R.A.B. and Wallington, E.A., (1980)
*Carleton's histological technique Ed. 5*Oxford University Press, Oxford, UK.
Culling C.F.A., (1974)
Handbook of histopathological and histochemical techniques Ed. 3 Butterworth, London, UK.
Bancroft, J.D. and Stevens A. (1982)
Theory and practice of histological techniques Ed. 2 Churchill Livingstone, Edinburgh & London, UK.