RUSSELL-MOVAT PENTACHROME STAIN KIT PROCEDURE

**KIT COMPONENTS**
- 100 ml 10% ALCOHOLIC HEMATOXYLIN
- 100 ml 10% FERRIC CHLORIDE
- 100 ml UNIVERSAL IODINE SOLUTION
- 100 ml 2% FERRIC CHLORIDE
- 100 ml 5% SODIUM THIOSULFATE
- 100 ml 3% GLACIAL ACETIC ACID
- 100 ml 1% ALCIAN BLUE SOLUTION
- 100 ml CROCEIN SCARLET-ACID FUCHSIN
- 200 ml 1% GLACIAL ACETIC ACID
- 200 ml 5% PHOSPHOTUNGSTIC ACID
- 100 ml ALCOHOLIC Saffron SOLUTION

**INCLUDED:**
- 100 ml 5% SODIUM THIOSULFATE
- 100 ml 3% GLACIAL ACETIC ACID
- 200 ml 1% GLACIAL ACETIC ACID
- 200 ml 5% PHOSPHOTUNGSTIC ACID

**PRINCIPLE:**
This kit demonstrates collagen, elastic fibers, fibrinoid, mucin, muscle, and nuclei.

**SPECIMEN:**
Any well fixed paraffin embedded tissue cut at 4 to 5 microns.

**QUALITY CONTROL:**
American MasterTech Scientific Recommended Control Slide:
Elastic Fibers, CSEA; Muscle (striated), CSMUS25

**VERHoeff's Elastic Stain:** Mix these components in the order shown below just prior to use!

1. 10% ALCOHOLIC HEMATOXYLIN...........................20 ml
2. REAGENT ALCOHOL...........................................20 ml
3. 10% FERRIC CHLORIDE .......................................20 ml
4. UNIVERSAL IODINE SOLUTION..........................20 ml

**PROCEDURE:**
1. Deparaffinize slide using Xylene or a Xylene Substitute and hydrate through alcohols.
2. Rinse slide in Distilled water.
3. Place slide in Verhoeff's Elastic Stain for 15 to 20 minutes.
4. Rinse slide in lukewarm running Tap water for 5 minutes, followed by Distilled water.
5. Differentiate section in 2% FERRIC CHLORIDE until elastic fibers are sharply defined. *(Check using microscope!)*
6. Rinse slide in Distilled water and place in 5% SODIUM THIOSULFATE for 1 minute.
7. Rinse slide in running Tap water for 5 minutes.
8. Place slide in 3% GLACIAL ACETIC ACID for 3 minutes.
9. Place slide directly in 1% ALCIAN BLUE SOLUTION for 15 to 30 minutes or until mucins are blue.
10. Rinse slide thoroughly in warm running Tap water for 1 minute, then rinse in Distilled water.
11. Place slide in CROCEIN SCARLET-ACID FUCHSIN for 2 minutes.
12. Rinse slide through 3 changes of Distilled water.
13. Dip slide 5 times in 1% GLACIAL ACETIC ACID.
14. Place slide in 2 changes of 5% PHOSPHOTUNGSTIC ACID for 2 to 5 minutes each. Check section under microscope; stop differentiation when connective tissue is clear and before the elastic fibers are de-stained.
15. Dip slide 5 times in 1% GLACIAL ACETIC ACID.
16. Dehydrate slide through 3 changes of fresh Absolute Alcohol.
17. Place slide in ALCOHOLIC Saffron Solution for 15 minutes.
18. Dehydrate slide through 3 changes of fresh Absolute Alcohol.
19. Clear slide through 3 changes of fresh Xylene or Xylene Substitute.
20. Coverslip using a permanent mounting media.

**RESULTS:**
- Elastic fibers, nuclei: **BLACK**
- Collagen: **YELLOW**
- Mucins: **BLUE TO GREEN**
- Muscle: **RED**
- Fibrinoid: **INTENSE RED**

**REFERENCES:**
Movat HZ: Demonstration of all connective tissue elements in a single section; Arch Path 1955, 60: 289.