**Modified Russell-Movat Pentachrome**

Samples should be fixed in 4% PFA for 24-48 hours, paraffin embedded and sectioned at 5 um sections

**Solutions:**

1. **10% Alcoholic Hematoxylin (we use 5%)**

 25 grams of hematoxylin in 500mL of 100% ethanol

1. **10% Ferric Chloride**

 50 grams Ferric Chloride in 500mL distilled water

1. **Verhoeff’s Iodine Solution**

 Measure 2.0 grams iodine and mix together with 4.0 grams potassium iodine

 Slowly add 100mL distilled water (stirring often)

1. **Verhoeff’s Elastic Stain Working Solution – Make Fresh Every Time**

 Add IN THIS ORDER

 100mL of 5% Alcoholic hematoxyln

 50mL 10% Ferric Chloride

 Mix well

 50mL Verhoeff’s Iodine Solution

1. **2% Ferric Chloride Differentiating Solution**

 100mL Ferric Chloride

 400mL distilled water

1. **5% Sodium Thiosulfate (Hypo) Solution**

 25 grams into 500mL distilled water

1. **3% Glacial Acetic Acid**

 15mL galacial acetic acid into 485mL distilled water

1. **1% Alcian Blue**

 5 grams of Alcian blue 8GS

 485mL distilled water

 15mL glacial acetic acid

1. **Crocein Scarlet-Acid Fuchsion Stock**

 Solution A

 0.5 grams of Crocein Scarlett

497.5mL distilled water

2.5mL glacial acetic acid

 Solution B

 0.1 grams acid fuchsin

99.5mL distilled water

0.5mL glacial acetic acid

 Crocein Scarlet-Acid Fuchsin Working Solution

 5mL glacial acetic acid in 495mL distilled water

1. **5% Phosphotungstic Acid Solution**

 25 grams in 500mL distilled water

1. **Alcoholic Saffron Solution – Make at least 24 hours before staining and allow to stir**

 15 grams Saffron du Gatinais

 250mL 100% ethanol

**Procedure**

1. Deparaffinize and hydrate slides to water;

 5 min each xylene, xylene, 100% ethanol, 100% ethanol,

 95% ethanol, 70% ethanol, water 2 changes.

1. Stain in Verhoeff’s elastic stain working solution for 30 minutes. Alternative protocol:Stain in Verhoff’s elastic stain for 15-30 mins;

 12 mins for rat balloon injuries.

1. Place in lukewarm running water for 6-20 mins;

 rat balloon injuries were in running water for 5 minutes.

1. Place slides in distilled water for 5 minutes.
2. Differentiate in 2% ferric chloride solution by dipping slides for 10 seconds. Quickly rinse in distilled water. Check slides under a microscope and determine whether or not to move on. 25 seconds for rat, 35 seconds for embryos. Check for differentiation.
3. Place in 5% sodium thiosulfate solution for 1 minute.
4. Wash in tap water for 5 minutes. Rinse with distilled water.
5. Place in 3% glacial acetic acid solution for 3 minutes.
6. Place directly in 1% alcian blue solution for 15-30 minutes.
7. Rinse thoroughy in running warm tap water for 10 minutes. Rinse with distilled water.
8. Stain in Crocein scarlet-acid fuchsin solution for 2 minutes.
9. Rinse in two changes of distilled water (5 dips each).
10. Rinse in 1% acetic acid solution (5 dips).
11. Place in 5% phophotungstic acid for 1 minute.
12. Rinse in 1% acetic acid solution (5 dips).
13. Rinse in distilled water (5 dips).
14. Rinse in 100% ethanol (5 dips).
15. Dehydrate slides in 100% ethanol (2 changes 3 minutes each).
16. Stain in alcoholic saffron solution for 15 minutes.
17. Rinse slides in 100% ethanol (3 changes 3 minutes each).
18. Rinse in xylenes (2 changes 3 minutes each).
19. Mount with resinous medium (paramount, cytoseal).

**Results**

Nuclei……………………………………………black

Elastic fibers………………………………….black

Collagen………………………………………..yellow

Ground substance and mucins………blue to green

Muscle………………………………………….red

Fibrinoid……………………………………….intense red

**Avoid exposure of saffron solution to water.**