

APPENDIX C: STAINING PROTOCOL

a) PROTOCOL OF HEMATOXYLIN AND EOSIN (H&E) STAIN:

Before the staining, the slides were undergoing deparaffination and dehydration until distilled water.

STAINING PROTOCOL:

- | | |
|-----------------------------|-------------------------------|
| 1. Harris' alum Hematoxylin | -20-25 seconds |
| 2. Running tap water | -Remove the excess stains |
| 3. 0.2% HCl | -3 seconds |
| 4. Running tap water | -3 seconds |
| 5. 0.2 % NaHCO ₃ | -3 seconds |
| 6. Running tap water | -Until the sections turn blue |
| 7. Eosin Solution | -1 minutes |

DEHYDRATION:

- | | |
|------------------------|------------------|
| 1. 95% Alcohol I | -Rinsed |
| 2. 95% Alcohol II | -Rinsed |
| 3. Absolute Alcohol I | -3 minutes |
| 4. Absolute Alcohol II | -3 minutes |
| 5. Xylene I | -3 minutes |
| 6. Xylene II | -3 minutes /More |

The slides were then mounted with Canada balsam.

b) PROTOCOL OF ACCUSTAIN TRICHROME STAIN (MASSON) HT-15
[SIGMA]:

The slides where undergo deparaffination and rehydration before staining until distilled water.

STAINING PROTOCOL:

- | | |
|---|--|
| 1. Modant in Bouin's Soution. | -56 C for 15 minutes
/Room Temperature for overnight. |
| 2. Running tap water | -Until yellow color removed |
| 3. Distilled water | -Rinsed briefly. |
| 4. Working Weigert's Hematoxylin | -5 minutes |
| 5. Running tap water | -5 minutes |
| 6. Distilled water | -Rinsed |
| 7. Biebrich Scarlet-Acid Fuchsin | -5 minutes |
| 8. Distilled water | -Rinsed |
| 9. Phosphomolybdic/Phosphotungstic Acid | -5-10minutes |
| 10. Aniline Blue | -5 minutes |
| 11. Distilled water | -Rinsed |
| 12. 1% acetic acid | -3-5 minutes |

DEHYDRATION:

- | | |
|-------------------|------------|
| 1. 95% Alcohol I | -3 minutes |
| 2. 95% Alcohol II | -3 minutes |
| 3. Xylene I | -3 minutes |
| 4. Xylene II | -3 minutes |
| 5. Xylene III | -3 minutes |

COVER SLIPPING:

With DPX (xylene based) mount and dry overnight at room temperature.

c) PROTOCOL OF ACCUSTAIN ELASTIC STAINS [SIGMA]:

The slides were undergo deparaffination and rehydration before staining until distilled water.

STAINING PROTOCOL:

- | | |
|---|-------------|
| 1. Working Elastic Stain | -10 minutes |
| 2. Distilled water | -Rinsed |
| 3. Working Ferric Chloride Solution | -1-2minutes |
| 4. Running Tap Water | -Rinse |
| 5. (If over differentiated, return to step 1) | |
| 6. 95% Alcohol | -Rinsed |
| 7. Distilled water | -Rinsed |
| 8. Van Gieson Solution | -1-3minutes |

DEHYDRATION:

- | | |
|-------------------------|-----------------|
| 9. 95% Alcohol II | -Rinsed |
| 10. 95% Alcohol II | -Rinsed |
| 11. Absolute Alcohol I | -3 minutes |
| 12. Absolute Alcohol II | -3 minutes |
| 13. Xylene I | -3 minutes |
| 14. Xylene II | -3 minutes |
| 15. Xylene III | -3 minutes/more |

COVER SLIPPING:

With DPX (xylene based) mount and dry overnight at room temperature.