

PARAFFIN H/E STAINING PROTOCOL
For use on Leica AutostainerXL (Last updated 3/24/2023)

OBJECTIVE: To identify nuclei verses cytoplasm and other tissue elements

RECOMMENDED CONTROL TISSUE: Appendix, small bowel or any well-fixed tissue routinely stained in lab.

ID of Control Used: _____ **Type of Tissue:** _____

Reagent	Vendor	Catalog #	Was this a new Bottle (Y/N)? Yes, check lot log.
Harris Hematoxylin	Epredia	72711	
Glacial Acetic Acid	Fisher	A35-500	
Ammonium Hydroxide	Fisher	A669-500	
Eosin Phloxine 515	Surgipath	3801606	

SPECIAL INSTRUCTIONS: Hematoxylin – Filter Daily (if fresh or when reuse). Milli-Q H2O, Bluing and Acid Alcohol – change daily. Hematoxylin and Eosin – Change Biweekly or after 400 slides. Dump all other reagents after 200 slides or as needed. Rotate/change as needed.

REAGENT PREPARATION:

- 1) Acid Alcohol* – Add 3 ml of Glacial Acetic Acid to 3L 70% Ethanol.
- 2) Ammonia water** – Add 1ml Ammonia Hydroxide to 3.8L DI H2O.

PROTOCOL:

1. Prepare 4µm Paraffin sections on glass slides
2. Run Program 1 on Leica Autostainer XL. Place rack into Load drawer and press ‘Load’. Program is as follows:

<ol style="list-style-type: none"> 1) Oven 64°C 15 mins. 2) Station 1 Xylene 5 mins. 3) Station 2 Xylene 5 mins. 4) Station 3 100% ETOH 45 secs. 5) Station 4 100% ETOH 45 secs. 6) Station 5 95% ETOH 1 min. 7) Station 6 Milli Q H2O 1 minute 8) Station 8 Harris Hematoxylin 7 min 9) DI WASH 5 3 mins. 10) Station 9 0.1% Acid Alcohol*15 sec 11) WASH 4 1 min. 	<ol style="list-style-type: none"> 12) Station 10 Ammonia Water** 1min. 13) WASH 3 30 secs. 14) Station 11 70% ETOH 1 min. 15) Station 12 Eosin 1:15 min. 16) Station 13 95% ETOH 30 secs. 17) Station 14 100% ETOH 1 min. 18) Station 15 100% ETOH 1 min. 19) Station 16 Xylene 1 min. 20) Station 17 Xylene 1 min. 21) Station 18 Xylene 1 min. 22) EXIT Xylene 1 min.
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3. Machine beeps when cycle is complete; Press Exit and remove rack.
4. Coverslip

EXPECTED RESULTS: Nuclei – blue, Cytoplasm and other tissue – various shades of pink, Erythrocytes and eosinophilic granules – bright pink to red.

CASE IDs/SLIDE NUMBERS: