Pathology Core Protocol

Protocol Section:	Staining Procedures-Manual	**Policy No:**	P-CMHD2-HE-01
Protocol Subject:	Haematoxylin and Eosin Stain	**Effective Date:**	
		Date Reviewed:	6 January 2003
		Date Revised:	September 22, 2003

Solutions:

Iodine Solution(for Mercury pigment removal): add saturated alcoholic iodine solution to 70% alcohol until it is dark brown in colour.

Sodium thiosulphate: 3% aqueous

Harris' Haematoxylin:

Haematoxylin	20 gm
95% alcohol	120 ml
Aluminum ammonium sulphate	240 gm
Distilled water	2400 ml
Sodium iodate	1.2 gm
Glacial acetic acid	96 ml

Dissolve the haematoxylin in the alcohol. Mix the alum and water and bring to a boil. Remove from heat and add the alcohol and haematoxylin solution. Bring to a rapid boil again. Remove from heat, let it settle a minute then add the sodium iodate, very slowly at first. Bring to a boil again, then cool quickly in cold water. When cool, add glacial acetic acid. Filter before use.

Acid Alcohol: 1% hydrochloric acid in 70% alcohol.

Scott's Tapwater Substitute:

Magnesium sulphate	80 gm
Sodium bicarbonate	14 gm
Distilled water	4000 ml

Dissolve salts separately, mix and add a crystal of thymol.

Eosin Staining Solution:

1% aqueous Eosin Y	400 ml
0.1% aqueous Phloxine B	400 ml
95% alcohol	3200 ml
Glacial Acetic acid	16 ml

Procedure:

1. Deparaffinize in xylene; two changes, total of 3 minutes.
2. Absolute alcohol; three changes, 10 dips in each.
3. 95% alcohol; 10 dips.
4. If sections are B5Fixed, treat with iodine for 5 minutes, and wash. Then treat with sodium thiosulphate for 1 minute.
5. Wash well in running tapwater.
6. Stain in Harris haematoxylin for 5 minutes.
7. Wash in water, differentiate in acid alcohol 3 to 6 dips.
8. Wash well in water, blue in Scott's Tapwater substitute 1 minute.
9. Wash well in running water.
10. Rinse in 95% alcohol.
11. Stain 1 minute in eosin, making sure stain covers slides completely.
12. Wash well in running water.
13. Dehydrate in 95% alcohol and 3 changes of absolute alcohol, 10 dips in each.
14. Clear in 3 changes of xylene, 10 dips each, and mount.

Results:

Nuclei: blue

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Cytoplasm and inter-cellular substances: shades of pink and red
Cells with much RNA or acid mucopolysaccharide: purplish

Note:

Mayer's haematoxylin can be substituted for Harris'. The solution is much weaker, requires longer staining time (15 minutes), no differentiation, and must be changed every week.

Mayer's Haematoxylin

Haematoxylin	1 gm
Aluminum ammonium sulphate	50 gm
Distilled water	1000 ml
Sodium iodate (NaIO3)	0.2 gm
Citric acid	1 gm
Chloral hydrate	50 gm

Dissolve the haematoxylin first using gentle heat, then add each ingredient in order of method and dissolve each one completely before adding the next. Stain keeps well for months.

Reference: Bancroft, J.D., Stevens, Alan: Theory and Practice of Histological Techniques

Issued by Lab Manager: _____ **Date:** _____

Approved by Facility Management: _____ **Date:** _____