Title	Haematoxylin and Eosin Staining of Tissue Sections
SOP Code	SOP118_01
Effective Date	01-Sep-2012

Site Approvals

Name and Title (typed or printed)	Signature	Date dd/Mon/yyyy

1.0 PURPOSE

This Standard Operating Procedure (SOP) outlines standardized procedures and process of staining tumour tissue sections, using haematoxylin and eosin (H&E). The SOP does not cover detailed safety procedures for handling Human Biological Materials (HBMs) or hazardous chemicals.

2.0 SCOPE

The policy applies to all repository personnel responsible for sectioning and staining tissue preserved in paraffin or Optimal Cutting Temperature (OCT) blocks.

3.0 RESPONSIBILITIES

Staining is performed by the laboratory or histology technician or trained personnel designated by the biorepository.

4.0 DEFINITIONS

See Glossary of Terms.

5.0 PROCEDURE

Note: Times specified for the steps in the protocol may be modified to suit lab specific reagents, which may vary slightly in strengths and composition.

5.1 Staining of Formalin Fixed Paraffin Embedded Tissue Sections

5.1.1 Have materials and equipment ready. Have reagents and equipment ready.

5.1.2 Take FFPE sections that have been cut or slides from storage.

5.1.3 Dewaxing

REAGENT	**TIME**
Xylene/Toluene	4 minutes with occasional agitation
Xylene/Toluene	4 minutes with occasional agitation

5.1.4 Rehydration

REAGENT	**TIME**
100% Ethanol	10 dips
100% Ethanol	10 dips
85% Ethanol	10 dips
70% Ethanol	10 dips
Water wash	4 minutes

5.1.5 Staining

REAGENT	**TIME**
Harris Haematoxylin	4 minutes
Water wash	5 minutes
Acid- Alcohol (destain)	1-7 dips (as needed to destain to required degree)
Water wash	8 minutes
Ammonia water	2 minutes
Water Wash	5 minutes

Eosin	2 minutes
Water Wash	Quick Rinse

5.1.6 Dehydration

REAGENT	**TIME**
70% Ethanol	10 dips
85% Ethanol	10 dips
100% Ethanol	10 dips
Clear in Xylene/Toluene	10 dips
Clear in Xylene/Toluene	10 dips

5.1.7 Coverslip slides with mounting medium.

5.18 Staining Results: Nuclei (Deep Blue); Cytoplasm and connective tissue (shades of pink).

5.2 Staining of OCT Embedded Tissue Sections

5.2.1 Note: OCT Embedded tissue tend to lift off and slide more easily during staining. The use of adhesive slides may alleviate this problem. Also, Eosin staining is depressed while staining of nuclei is enhanced.

5.2.2 Staining

REAGENT	**TIME**
10% Formalin	1-2 minutes
Water Wash	10-20 dips
Harris Haematoxylin	1 minutes
Water wash	10-20 dips
Acid- Alcohol (destain)	1 –10 dips (or as needed to destain to required degree)
Ammonia water	10 –20 dips
Water	10 –20 dips
Eosin (1%)	30 seconds
Water Wash	Quick Rinse

5.2.3 Dehydration

REAGENT	TIME
70% Ethanol	10 dips
85% Ethanol	10 dips
100% Ethanol	10 dips
Clear in Xylene/Toluene	10 dips
Clear in Xylene/Toluene	10 dips

5.2.4 Coverslip slides with mounting medium such as Permount.

5.2.5 Staining Results: Nuclei (Deep Blue); Cytoplasm and connective tissue (shades of pink).

6.0 REFERENCES

Health Canada, Food and Drug Regulations, Part C, Division 5, Drugs for Clinical Trials Involving Human Subjects, (Schedule 1024), June 20, 2001.

Health Canada, Guidance for Industry, Good Clinical Practice: Consolidated Guideline, ICH Topic E6, 1997.

2011 NCI Best Practices for Specimen Resources. Office of Biorepositories and Biospecimen Research, National Cancer Institute, Bethesda, MD. http://biospecimens.cancer.gov/bestpractices/2011-NCIBestPractices.pdf

ISBER Best Practices for repositories: Collection, storage, retrieval and distribution of biological materials for research. Cell Preservation Technology 6(1), 3-58, 2008 http://www.isber.org/Pubs/BestPractices2008.pdf

CTRNET Standard Operating Procedures, Canadian Tumour Repository Network, http://www.ctrnet.ca/operating-procedures

7.0 REVISION HISTORY

SOP Code	Effective Date	Summary of Changes
SOP118_01	01-Sep-2012	Original version