CTRNet Standard Operating Procedure Haematoxylin and Eosin Staining of Tissue Sections			
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Subject:	Haematoxylin and Eosin Staining of Tissue Sections	Category	Material Handling and Documentation

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REVISION HISTORY

SOP Number	Date Issued	Author (Initials)	Summary of Revisions
8.3.007	Jan 2008	JdSH	Initial version

1.0 PURPOSE

Tissue samples are collected from patients that have been through the informed consent process and agreed to participate in the tumour repository program. Tumour tissues are preserved and valuable for specific research studies. Formaldehyde fixed and paraffin embedded (FFPE) tissue and frozen (OCT) embedded tissue can be sectioned for studies needing preservation of histo-morphology of the specimen. Staining of the section with Haematoxylin and Eosin (H&E) is employed universally for microscopic examination of tissue. It facilitates interpretation of pathology, identification of tissue, study of tissue composition and accurate tumour grading. If many sections are cut from a tissue block, H&E sections may have to be done at intervals to ensure representation of the tumour.

2.0 SCOPE

The Standard Operating Procedure (SOP) describes how sections of tissues should be stained. The SOP does not cover detailed safety procedures for handling Human Biological Materials (HBMs) or hazardous chemicals and it is recommended that personnel follow institutional safety guidelines.

3.0 REFERENCE TO OTHER POLICIES AND SOPS

1. CTRNet Policy: POL 005.001 Records and Documentation
2. CTRNet Policy: POL 002.001 Ethics
3. CTRNet Policy: POL 004.001 Privacy and Security
4. CTRNet Policy: POL 007.001 Material and Information Handling Policy
5. CTRNet Generic Procedure: FS 002.001 CTRNet Generic SOP for Collection and Processing of Tumour Tissue
6. CTRNet SOP: 8.3.005 Preservation of Tissue: Paraffin embedding
7. CTRNet SOP:8.3.006 Sectioning of Paraffin and OCT Embedded Tissue
8. CTRNet SOP: 8.1.002 Biohazardous Material Waste Management

4.0 ROLES AND RESPONSIBILITY

The policy applies to all personnel from CTRNet member repositories who are responsible for sectioning and staining tissue preserved in paraffin or OCT blocks.

Tumour Bank Personnel	**Responsibility/Role**	**Site Specific Personnel and Contact Information**
Lab Technician	May be responsible for staining tissue sections on slides	
Histology Lab Technician	May be specifically responsible for processing FFPE tissues, sectioning paraffin and frozen (OCT) samples and staining tissue sections	

5.0 MATERIALS, REAGENTS EQUIPMENT AND FORMS

The materials, equipment and forms listed in the following list are recommendations only and may be substituted by alternative/equivalent products more suitable for the site-specific task or procedure.

Materials and Equipment	**Materials and Equipment (Site- Specific)**
Markers, ink and pens	
Eosin	
Harris Haematoxylin (filtered)	
Xylene/Toluene	
Tap Water	
Ethanols	
HCl	
Coplin Jars for staining	
Slide racks for staining and drying slides	
Forceps	

Mounting Medium such as Permount	
Coverslips	

See Appendix A for details on preparing reagents used in this staining procedure

6.0 DEFINITIONS

Formalin Fixed Paraffin-embedded (FFPE) Tissue: Tissue that has been fixed Formalin and set in paraffin.

Haematoxylin: Haematoxylin is a yellowish brown natural dye which is extracted from the heartwood of the tree *Haematoxylum campechianum* (Formula:$C_{16}H_{14}0_6$). It is used as a biological stain.

Eosin: The reddish biological stain Eosin ($C_{20}H_6O_5Br_4Na_2$) is the most common counterstain to haematoxylin in the H&E method.

OCT: "Optimal Cutting Temperature" compound is the name used for polyethylene glycol/sucrose-based freezing medium. OCT preserves ultrastructure and prevents tissue from desiccation, degradation, acts as an insulator from thermal variation and minimizes crystal formation. It is especially useful for preserving fresh frozen tissue that may need to be sectioned.

Preservation: Use of chemical agents, alterations in environmental conditions or other means during processing to prevent or retard biological or physical deterioration of a specimen.

Staining: A procedure in which a dye or a combination of dyes and reagents are used to colour the constituents of cells and tissues

7.0 PROCEDURES

This procedure is intended to ensure that tissue sections are stained in a consistent manner. As mentioned earlier stained sections are valuable for studying tissue morphology and structure. Microscopic examination of stained sections facilitate identification of tissue and components. Consistency in procedure is important for obtaining comparable and reliable test results. Times specified for the steps in the protocol may be modified to suit lab specific reagents which may vary slightly in strengths and composition.

7.1 Staining of Formalin Fixed Paraffin Embedded Tissue Sections

1. Treat all tissue as potentially infectious.
2. Staining is performed by the laboratory or histology technician or trained personnel designated by the tumour repository.

3. Have materials and equipment ready. Have reagents and equipment ready.
4. Take sections FFPE sections that have cut or slides from storage
5. **Dewaxing**

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

6. **Rehydration**

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

7. **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
Eosin	2 minutes
Water Wash	Quick Rinse

8. **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

9. Coverslip slides with mounting medium such as Permount.
10. Staining Results:

    Nuclei (Deep Blue)

    Cytoplasm and connective tissue (shades of pink)

7.2 Staining of OCT Embedded Tissue Sections

1. The main difference from the protocol described for FFPE sections above is that the 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.

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

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

4. Coverslip slides with mounting medium such as Permount.
5. Staining Results:

    Nuclei (Deep Blue)

    Cytoplasm and connective tissue (shades of pink).

8.0 APPLICABLE REFERENCES, REGULATONS AND GUIDELINES

1. Declaration of Helsinki. http://ohsr.od.nih.gov/helsinki.php3 http://www.wma.net/e/policy/b3.htm
2. Tri-Council Policy Statement; Ethical Conduct for Research Involving Humans; Medical Research Council of Canada; Natural Sciences and Engineering Council of Canada; Social Sciences and Humanities Research Council of Canada, August 1998. http://www.pre.ethics.gc.ca/english/policystatement/policystatement.cfm
3. Human Tissue and Biological Samples for use in Research. Operational and Ethical Guidelines. Medical Research Council Ethics Series. http://www.mrc.ac.uk/pdf-tissue_guide_fin.pdf
4. Best Practices for Repositories I. Collection, Storage and Retrieval of Human Biological Materials for Research. International Society for Biological and Environmental Repositories (ISBER). http://www.isber.org
5. US National Biospecimen Network Blueprint http://www.ndoc.org/about_ndc/reports/NBN_comment.asp
6. Jewell, S. et al. 2002, Analysis of the Molecular Quality of Human Tissues, an experience from the Cooperative Human Tissue Network. Am. J. Clin. Pathol. 118:733-741.
7. Guideline – Fresh Tissue Working Group of BIG and NCI breast cancer Cooperative Groups http://ctep.cancer.gov/forms/guidelines_fresh_tissue.pdf
8. SOP No.3 (Draft 1). November 15, 2005. Standard Tissue Sectioning. NCIC CTG. Ontario.
9. Snell L. and P. H. Watson. 2006, Breast Tissue Banking: Collection, Handling, Storage and Release of Tissue for Breast Cancer Research. Methods Mol Med. 120:3-24.
10. Recommendations of FFPE Working Group of BIG and North American breast Cancer Groups. http://ctep.cancer.gov/forms/draft_ffpe_tissue.pdf

Appendix A. Concentrations

Ammonia water

Tap Water	1000 mls
Concentrated Ammonium Hydroxide	2-3 mls

Acid-Alcohol

1% HCl (concentrated) in 70% Ethanol