CTRNet Standard Operating Procedure Haematoxylin & Eosin Staining of Tissue Sections			
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Approved By:	CTRNet Management Group (CMG)	01-May-2012
	Per: Brent Schacter	26-June-2012

1.0 PURPOSE

Tissue samples are collected from patients that have been through the informed consent process and agreed to participate in the tumour biobank program. Tumour tissues are preserved and are valuable for specific research studies. Formalin fixed and paraffin embedded (FFPE) tissue and tissue frozen in Optimal cutting temperature (OCT) compound can be sectioned for studies needing preservation of histomorphology of the specimen. Staining of the sections 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

This standard operating procedure (SOP) describes how sections of tissues should be stained.

3.0 REFERENCE TO OTHER CTRNET SOPS OR POLICIES

Note: When adopting this SOP for local use please reference CTRNet.

3.1 *CTRNet Policy: POL 5 Records and Documentation*
3.2 *CTRNet Policy: POL 2 Ethics*
3.3 *CTRNet Policy: POL 4 Privacy and Security*
3.4 *CTRNet Policy: POL 7 Material and Information Handling*
3.5 *CTRNet Standard Operating Procedure: SOP 08.03.005 Preservation of Tissue: Paraffin Embedding*
3.6 *CTRNet Standard Operating Procedure: SOP 08.03.006 Sectioning of Tissue - Paraffin and OCT Embedded Tissue*
3.7 *CTRNet Standard Operating Procedure: SOP: 08.01.002 Biohazardous Material Waste Management*

4.0 ROLES AND RESPONSIBILITIES

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

Tumour Biobank Personnel	**Responsibility/Role**
Histology Laboratory Technician/Technologist	May be specifically responsible for processing FFPE tissues, sectioning paraffin and frozen (OCT) samples and staining tissue sections.

5.0 MATERIALS, 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)
Solvent resistant markers, ink, pencils, 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 and droppers	
Coverslips	
Eosin	

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

6.0 DEFINITIONS

See the CTRNet Program Glossary: http://www.ctrnet.ca/glossary

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 facilitates 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 laboratory specific reagents, which may vary slightly in strengths and composition.

The following steps are based on procedures utilized at the Manitoba Breast Tumour Bank.

7.1 Staining of Formalin Fixed Paraffin Embedded Tissue Sections

7.1.1 Treat all tissue as potentially infectious.

7.1.2 Staining is performed by the laboratory or histology technician/technologist or trained personnel designated by the tumour biobank.

7.1.3 Have materials and equipment ready. Have reagents and equipment ready.

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

7.1.5 Dewaxing

REAGENT	TIME	EQUIPMENT
Xylene/Toluene	2-4 minutes with occasional agitation	Slide holder Staining dish with lids In fume hood
Xylene/Toluene	2-4 minutes with occasional agitation	Slide holder Staining dish with lids In fume hood

7.1.6 Rehydration

REAGENT	TIME	EQUIPMENT
100% Ethanol	2 minutes	Slide holder Staining dish
100% Ethanol	2 minutes	Slide holder Staining dish
85% Ethanol	2 minutes	Slide holder Staining dish
70% Ethanol	2 minutes	Slide holder Staining dish
Slowly running water wash	5 minutes	Slide holder

7.1.7 Staining

REAGENT	TIME
Harris Haematoxylin	4 minutes
Slowly running water wash	5 minutes
Acid- Alcohol (1% HC1 in 95% ethanol) (destain)	1-5 dips (as needed to destain to required degree)
Slowly running water wash	8 minutes
Ammonia water	2 minutes
Slowly running water wash	5 minutes
Eosin	2 minutes

7.1.8 Dehydration

REAGENT	TIME
70% Ethanol	5-10 dips
85-95% Ethanol	10 dips
100% Ethanol	2 minutes
Clear in Xylene/Toluene	2 minutes
Clear in Xylene/Toluene	2 minutes

7.1.9 Coverslip slides with mounting medium such as Permount.

7.1.10 Staining Results:

- Nuclei (Deep Blue)
- Cytoplasm and connective tissue (shades of pink)

7.2 Staining of OCT Embedded Tissue Sections

7.2.1 The main differences from the protocol described for FFPE sections above are due to the tendency for OCT embedded tissue to lift off and slide more easily during staining procedures. The use of adhesive slides may alleviate this problem. Also, Eosin staining is depressed while staining of nuclei is enhanced.

7.2.2 Staining

REAGENT	TIME
If using 95% Ethanol	1-2 minutes
70% Ethanol	1-2 minutes
Slowly running water wash	1-2 minutes
Harris Haematoxylin	30 seconds
Slowly running water wash	1-2 minutes
Acid-Alcohol (destain)	1-2 dips (or as needed to destain to required degree)*
Slowly running water wash	1-2 minutes
Ammonia water	60 seconds
Slowly running water wash	1-2 minutes
70% Ethanol	1 minute
95% Ethanol	1 minute
Eosin (1%)	30 seconds

- **When dipping, do so very slowly to minimize section loss.**

7.2.3 Dehydration

REAGENT	TIME
70% Ethanol	5 dips*
85% Ethanol	10 dips*
100% Ethanol	1 min
100% Ethanol	1 min
Clear in Xylene/Toluene	2 mins
Clear in Xylene/Toluene	2 mins

*** When dipping, do so very slowly to minimize section loss.**

7.2.4 Coverslip slides with mounting medium such as Permount.

7.2.5 Staining Results:

- Nuclei (Deep Blue)
- Cytoplasm and connective tissue (shades of pink)

8.0 APPLICABLE REFERENCES, REGULATIONS AND GUIDELINES

8.1 Declaration of Helsinki
http://www.wma.net/en/30publications/10policies/b3/index.html

8.2 Tri-Council Policy Statement 2; 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, December 2010.
http://www.pre.ethics.gc.ca/eng/policy-politique/initiatives/tcps2-eptc2/Default/

8.3 Human Tissue and Biological Samples for use in Research. Operational and Ethical Guidelines. Medical Research Council Ethics
http://www.mrc.ac.uk/Utilities/Documentrecord/index.htm?d=MRC002420

8.4 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/Search/search.asp?zoom_query=best+practices+for+repositories

8.5 US National Biospecimen Network Blueprint
http://biospecimens.cancer.gov/resources/publications/reports/nbn.asp

8.6 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.

8.7 Guideline – Fresh Tissue Working Group of BIG and NCI breast cancer Cooperative Groups

8.8 SOP No.3 (Draft 1). November 15, 2005. Standard Tissue Sectioning. NCIC CTG. Ontario.

8.9 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.

8.10 Recommendations of FFPE Working Group of BIG and North American breast Cancer Groups.

9.0 APPENDICES

9.1 Appendix A - Concentrations

10.0 REVISION HISTORY

SOP Number	Date revised	Author	Summary of Revisions
8.3.007	Jan 2008	JdSH	Initial version
8.3.007 e1.0	June 2012	CMG	• Grammatical and formatting throughout • Definitions removed • Revision History moved to bottom • Reference links updates • Updated SOP references • Section 2.0: Deleted “The SOP does…” • Section 7.0: Added: “The following steps…” • Section 7.1.5 & 7.1.6 Tables revised to include Equipment column. • Section 7.1.7 – Procedure outlined. • Section 7.2.2 - Table revised. • Section 5: added Eosin to the table • Deleted first half of section 7.2.2 (redundant)

CONCENTRATIONS

Ammonia water

Tap Water	1000 mls
Concentrated Ammonium Hydroxide	2-3 mls

Acid-Alcohol
1% HCl (concentrated) in 70% Ethanol