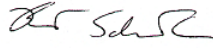


Tissue Micro Arrays from Paraffin Embedded Blocks

CTRNet Standard Operating Procedure Tissue Micro Arrays from Paraffin Embedded Blocks			
SOP Number:	08.03.010	Version:	e2.0
Supersedes:	8.3.010 e1.0	Category:	Material Handling and Documentation – Solid Tissue
Approved By:	CTRNet Management Group (CMG)		01-June-2012
	Per: Brent Schacter		28-June-2012

1.0 PURPOSE

Formaldehyde fixed and paraffin embedded (FFPE) tissue can be sectioned for studies needing preservation of histo-morphology. Conservation of the tissue resource is important to maximize the number of studies that can be conducted. Tissue Micro Arrays (TMAs) provide a cost-effective and efficient method of conserving tissue samples. TMAs have been used for molecular and immunohistochemical studies and are a valuable tool for evaluation of patient material. The purpose of this document is to outline standardized procedures for CTRNet biobanks to follow when creating TMAs from paraffin embedded tissue blocks.

2.0 SCOPE

This standard operating procedure (SOP) describes how TMAs should be constructed from FFPE tissue blocks. 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 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 08.01.002 Biohazardous Material Waste Management
- 3.7 CTRNet Standard Operating Procedure: SOP 09.004 Material Request and Release

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4.0 ROLES AND RESPONSIBILITIES

The SOP applies to all personnel from CTRNet member biobanks who are responsible for creating TMAs from FFPE tissue blocks.

Tumour Biobank Personnel	Responsibility/Role
Laboratory technician/technologist or Histology Laboratory Technician/Technologist	Responsible for organizing blocks, creating a template and constructing the TMA.
Pathologist	Reads slides and chooses sections of blocks to be cored

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)
Markers, ink and pens	
Microtome	
Hot water bath (set at 40-45°C)	
Microtome blades	
Manual tissue arrayer	
Punches with stylets (0.6- 2 mm in diameter)	
Recipient block holder	
Donor block bridge	
Tray to hold slides	
Beecher tool set for adjusting the arrayer	
Oven (set at 50-52°C)	
Appropriate labels for slides	
Labeled electrostatically charged slides (such as Superfrost+)	
Tray to hold blocks to be cored	
Tray to hold blocks that have been cored	
Slide storage boxes and/or slide shippers	

6.0 DEFINITIONS

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

7.0 PROCEDURES

This procedure is intended to ensure that tissue samples are preserved for multiple research studies and are created and sectioned in a safe, consistent and efficient manner while eliminating the risks of contamination and loss of molecular and structural integrity. The use of TMAs provides the special advantage of potentially allowing improved standardization of testing.

Consistency in procedure is important for obtaining comparable and reliable test results.

7.1 Generation of a TMA – Collecting Blocks and Information

- 7.1.1 Treat all tissue as potentially infectious.
- 7.1.2 To eliminate wastage of a tissue resource, TMA generation is performed only by experienced laboratory or histology technicians/technologists or trained personnel designated by the tumour biobank.
- 7.1.3 Have materials and equipment ready.
- 7.1.4 Gather H&E (Hematoxylin and Eosin) slides for all cases for the pathologist to read.
- 7.1.5 Determine for every block if the depth of the tissue in the block is still sufficient for use in a TMA recipient block.
- 7.1.6 Collect information about the case and diagnosis from the archiving database as needed for the study.

7.2 Generation of a TMA – Reviewing Blocks

- 7.2.1 The pathologist examines the slides/ tissue blocks and marks areas that are suitable to represent the tumour as per the basis of the research study the block is being designed for. A fine felt-tipped waterproof marker is used for marking the slides.
- 7.2.2 The marked areas are matched to the corresponding paraffin blocks.
- 7.2.3 These same areas are then marked on the paraffin block using a medium tipped marker, taking care not to damage the surface of the block by applying excessive pressure. This marks the area where the core should be removed from the donor block.

7.3 Generation of a TMA – Creating the Template

- 7.3.1 Use spreadsheet software such as Microsoft Excel to map out the template of the TMA. Design map to best accommodate the variety of cases, number of samples, matching normal tissue, purpose for array etc. A standard layout for a 0.6 mm core array would be to use 10 x 6 core grid which can be repeated several times (sectors) to fit the available space in the recipient block.
- 7.3.2 All cases on the array should be positioned randomly to avoid bias from immunohistochemical staining artifacts and biases introduced due to prior knowledge of case parameters.
- 7.3.3 It is good practice to insert recognizable cores at indicator positions. For example, use Mercurochrome-stained liver tissue cores at both the beginning (1 core) and end (3 cores) of the experimental cores to secure orientation and ensure correct case identification.
- 7.3.4 Print the spreadsheet. This is the array map.

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7.4 Generation of a TMA – Recipient Block

- 7.4.1 Make a large blank paraffin block (25 mm x 37 mm) using a cassette mould of 15 mm in depth or more.
- 7.4.2 Check the newly made block for air bubbles and ensure that the block is firmly attached to the cassette.
- 7.4.3 Gather all blocks to be cored and place them in ordered rows in a tray. The order of the blocks in the tray should represent the order of the cores in the TMA.
- 7.4.4 Using a tissue arrayer such as Beecher manual tissue arrayer, measure out and mark gently on the surface of the recipient block the four corners of the array to ensure a good fit. The edges of the array should fit at least 4mm from the edge of the recipient block
- 7.4.5 Create the TMA using the Beecher manual tissue arrayer following the manufacturers procedures.
- 7.4.6 As each core is placed into the recipient block the block identification number should be noted on the array map. The number must be taken directly from the FFPE block to ensure that the map is an accurate representation of the actual block and not any pre-planned array map. After an FFPE block is used, return the blocks to a box in the same order as used to generate the recipient block. This system will avoid confusion as the number of the block and the order of the block in the storage box can be used to verify position in the TMA.

7.5 Generation of a TMA - Sectioning

- 7.5.1 Section with a new microtome knife.
- 7.5.2 Cut sections at 5 μ or less (2-3 μ).
- 7.5.3 Float the sections in a distilled water bath. Set the temperature of the water bath to no more than 5° C below the melting temperature of the paraffin used in the construction of the array. To avoid inversion of the sections on the microscope slide ensure that the sections are floated “face-up”.
- 7.5.4 Remove sections after 5-20 seconds in the water bath and mount on electrostatically charged slides (e.g. Superfrost +). Pay careful attention to orientation of the array at this step.
- 7.5.5 Dry the slides overnight at room temperature and then bake the slides for 20 minutes at 50° C before moving to storage.

7.6 Storage of TMAs

- 7.6.1 Some antigens require more stringent protection from oxidation and may require the use of freshly cut TMA slides.
- 7.6.2 Keep a beaker of melted paraffin in a 60° C incubator.
- 7.6.3 Quickly dip the air-dried slide in the paraffin once.
- 7.6.4 Place the slide on a flat surface and allow to cool.
- 7.6.5 The slides can be stored in slide storage boxes at room temperature for extended periods of time. Limit exposure to temperature variations and moisture.
- 7.6.6 Non-paraffin dipped/protected slides can be kept at 4° C for up to 2 months in a standard microslide box. This is sufficient for most antigens.
- 7.6.7 Record storage location.

7.7 Release of TMAs

- 7.7.1 Note that TMAs contain human biological material and release of TMAs for research studies must be according to procedures outlined in *CTRNet SOP 09.004 Material Request and Release*.

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 Milanes-Yearsley, M. et al. Tissue Micro-Array: A cost and Time-Effective Method for Correlative Studies by Regional and National Cancer Study Groups. *Mod Pathol* 2002;15(12):1366-1373.
- 8.7 Guideline – Fresh Tissue Working Group of BIG and NCI breast cancer Cooperative Groups
- 8.8 SOP No.1 (Draft 1). June 13, 2005. Tissue Microarray Construction. NCIC CTG. Ontario.
- 8.9 TMA Generation SOP. Manitoba Breast Tumour Bank.
- 8.10 Recommendations of FFPE Working Group of BIG and North American breast Cancer Groups.
- 8.11 Yale Tissue Microarray Construction Protocols: Version 1.0, 1/2001.
http://www.yalepath.org/dept/research/YCCTMA/YTMA_protocol.pdf

9.0 APPENDICES

None

10.0 REVISION HISTORY

SOP Number	Date revised	Author	Summary of Revisions
FS 002.001	2005	JdSH	CTRNet Generic SOP for Collection and Processing of Tumour Tissue
8.3.010	2008	JM JdSH	Revised to deal specifically with creating TMAs from paraffin embedded tissue blocks
8.3.010 e1.0	June 2012	CMG	<ul style="list-style-type: none"> • Grammatical and formatting throughout • Definitions removed • Revision History moved to bottom • Reference links updates • Updated SOP references • Section 1.0-Revised “arrayer” • Section 7.5.1-Revisions to sections 7.5.3 and 7.5.5.