



**SOP Reference: BCNTB/SOP/003**

Standard Operating Procedure for

Snap freezing fresh tissue

Version number: 2


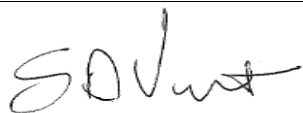

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BCNTB Advisory Council	Professor Wayne Phillips	Chair		May 2017

Document review history			
Version Number	Revision History	Name of Reviewer/Author	Review date
1.0	1 <sup>st</sup> Issue	Uma Ekbote	January 2012
2.0	Admin edits, changes to participating centres, changes to sample processing, updated HTA codes of Practice and Standards	Rosie Robertson Angie Berwick, Fabricio Barros and Sameena Iqbal	April 2016, December 2016, April 2017

## **1.0 PURPOSE AND SCOPE**

- 1.1 Rapid freezing of tissues provides the best preservation of DNA and RNA and will give the greatest yields and quality of nucleotides from subsequent extractions.
- 1.2 Samples are frozen in the quickest time possible to prevent degradation of nucleotide components. Rapid freezing also helps preserve morphology by reducing the size of ice crystals which form in the cell and cause the membranes to rupture.

## **2.0 DEFINITIONS**

- 2.1 The Breast Cancer Now Tissue Bank shall be referred to as the Tissue Bank.
- 2.2 Material refers to any Tissue with the Tissue Bank.
- 2.3 Tissue refers to any tissue or fluid taken from the human body.
- 2.4 The Institutions are the University of Leeds, University of Nottingham and Barts Cancer Institute, Queen Mary University of London.

## **3.0 REFERENCES**

- 3.1 Human Tissue Act 2004
- 3.2 Human Tissue Authority, Codes of Practice and Standards, April 2017;
  - 3.2.1 Code A: Guiding principles and the fundamental principle of consent
  - 3.2.2 Code E: Research
- 3.3 BCNTB/SOP/001: Specimen Collection
- 3.4 BCNTB/SOP/024: Data Collection
- 3.5 Control of Substances Hazardous of Health (COSHH) and Risk Assessments will be site specific adhering to national guidelines.

## **4.0 REQUIREMENTS**

- 4.1 Procedure should be carried out by trained member of Tissue Bank staff.
- 4.2 Equipment
  - 4.2.1 Sterile scalpel
  - 4.2.2 1L Dewar filled two thirds filled with liquid Nitrogen.
  - 4.2.3 Appropriate safety equipment.
  - 4.2.4 Container of dry ice for transportation of frozen samples from laboratory to the -80°C freezer.
  - 4.2.5 Disinfectant.
  - 4.2.6 Chopping board.

4.2.7 Aluminium foil.

## **5.0 HAZARDS AND PRECAUTIONS**

*All Members of staff **must be signed up to the relevant University/NHS Control of Substances Hazardous to Health (COSHH) risk assessment protocol before carrying out this procedure.***

- 5.1 Staff should also have Hepatitis B vaccination under the guidance of Occupational Health Service.
- 5.2 **Liquid Nitrogen:** Liquid Nitrogen poses serious burns and asphyxiation risk.
- 5.3 **Fresh Tissue:** Unfixed tissue poses increased infection risk, and gloves should be worn and standard laboratory practices followed.
- 5.4 **Scalpel:** Caution should be taken when handling sharp implements. Scalpels should be disposed of in designated Sharps bin.

## **6.0 PROCEDURE**

- 6.1 Prepare 1L Dewar of Liquid Nitrogen (Liq. N<sub>2</sub>) prior to collection of samples.
- 6.2 Check signed consent and sample datasheet according to local guidelines (**See appendices A, B, and C**).
- 6.3 Vials where applicable or possible should be pre-labelled. Where handwritten, labelling should be legible and in permanent ink.
- 6.4 Using a sharp, sterile, fresh scalpel for each patient/sample, divide the sample into individual aliquots to be stored in 1.8ml screw-top cryovials. The number of aliquots will depend on the size of the tissue available to bank.
- 6.5 Each sample aliquot should be 0.5 to 1.0 cm<sup>3</sup> to ensure it can be placed in the cryovials tube and also removed easily.
- 6.6 Place tissue pieces in aluminium foil for snap freezing in Liq. N<sub>2</sub>, leave for a minimum of 1 minute while sample freezes..
- 6.7 Place these frozen sample into individual labelled cryovials.
- 6.8 Record the time and number of samples in site-specific Tissue Bank worksheets (**See appendices A, B, and C**).
- 6.9 Repeat the process until all samples are frozen.
- 6.10 Samples must be entered into site-specific Tissue Bank worksheets (**See appendices A, B and C**).
- 6.11 These details are then recorded in the local database and then exported to Tissue Bank central database as per (BCNTB/SOP/024).
- 6.12 Place samples into -80°C Freezer.

## **7.0 Appendix A Leeds:**

## Tissue Sample Workflow

Patient ID:



LS16-.....

### COLLECTION

Histology No.: \_\_\_\_\_ Consent Checked by (initial): \_\_\_\_\_  
Date of surgery: \_\_\_\_\_ Date at cut-up: \_\_\_\_\_ Time at cut-up: \_\_\_\_\_  
Pathologist: \_\_\_\_\_ Side: Left / Right / Bilateral

### PROCESSING

#### Normal samples

- Frozen Aliquot IDs: \_\_\_\_\_  
Time Frozen: \_\_\_\_\_
  - FFPE Aliquot IDs: \_\_\_\_\_ Time into Formalin: \_\_\_\_\_
- Tissue Type: Normal adjacent (1-2cm from lesion) / Normal surround (2-10cm from lesion) /  
Contralateral / Risk Reducing / Reduction Mammoplasty

#### Tumour samples

- Frozen aliquot IDs: \_\_\_\_\_  
Time Frozen: \_\_\_\_\_
  - FFPE aliquot IDs: \_\_\_\_\_ Time into Formalin: \_\_\_\_\_
- Tissue Type: Primary tumour / Lymph node / DCIS / Tumour bed / Fibroadenoma / Sarcoma /  
Phyllodes / Other (specify)

Time in Freezer: \_\_\_\_\_ Tissue taken for culture?: No / Yes (specify) \_\_\_\_\_

### STORAGE

#### Frozen Storage Locations

LTB \_\_\_\_\_ : \_\_\_\_\_  
BEX \_\_\_\_\_ : \_\_\_\_\_  
BCC \_\_\_\_\_ : \_\_\_\_\_

#### FFPE Storage Locations

LRTB (Drawer 9): \_\_\_\_\_  
BCCTB (Drawer 10): \_\_\_\_\_  
Date of FFPE Processing:     /     /

### Notes

Sheet filled by:

Signature:

Sample Workflow Sheet \_\_version 2

## 8.0 Appendix B: Nottingham

### Nottingham University Hospital Biobank Worksheet

#### Tissue

Sheet number ..... of .....

*Consent ID number		*Biobank specimen number		*Histology number	
*Date and time specimen removed			*Tissue type		
Level of consent  (details if not full)					
Additional notes (include delay details)					
Aliquots taken by (name, date and time)		Aliquots frozen by (name, date and time)		Aliquots fixed by (name, date and time)	

- \*= Required field at specimen reception

Aliquot ID	Sample type (Tumour Peritumour Normal)	Frozen or fixed  (Process date and program if fixed – duration of fixation details required)	Initial storage location

## **9.0 Appendix C: Barts Cancer Institute Breast Tissue Bank**

Information recorded on samples from surgery lists

- Collection time
- Freezing time
- Number of vial for each type of tissue such as tumour, surround, adjacent where applicable.