TISSUE BANK AT THE IU SIMON CANCER CENTER

Standard Operating Procedure (SOP) 005V8.0

Processing and Storage of Breast Tissue
SPREC TIS-BPS-N-B-SNP-A-C [1]
SPREC TIS-BPS-N-B-PXT-G-P [1]

Effective Date: January 6, 2023
Author: Rana German, M.S., Biospecimen Manager

Approved by:

Michele Cote, PhD., KTB Executive Director

Materials:
Cryostorage tubes: Corning 2.0ml Cryogenic Vials. (Fisher cat. #0337421)
Polypropylene Specimen Containers: (Fisher cat. #14 955 103)
Liquid Nitrogen: (ICTSI-SSF R3 C156)
Disposable Forceps: (Avantor cat. #12576-934)
Embedding (Tissue) Cassettes: (Fisher cat. #15-182-708B)
10% Neutral Buffered Formalin: (Thermo Scientific #5701)
Ethanol- Histology Grade Absolute (Fisher Scientific cat. # BP28184)
CryoPod™ Carrier (Brooks Life Sciences)
Containers 40ml for shipping specimens in Ethanol (Avantor cat. # 89213-158)
Appropriate PPE: Gloves, Lab Coat, Eye protection
Histology Bucket 5L (Fisher cat. # 23-111-251)

Normal breast tissue is procured following SOP 001V8.0 (Acquisition of Normal Breast Tissue and Blood at a Tissue Collection Event) or SOP 012V6.0 (Acquisition of Normal Breast Tissue and Blood from an Operating Room) from donors meeting all eligibility criteria as listed here: https://komentissuebank.iu.edu/researchers/sop.php

Tissue Processing:

FIXED TISSUE:

-FIXATION:
Within 10 minutes (or less) of procurement tissue cores are received from the biopsy room on an absorbing pad and separated into individual cores using a sterile disposable forceps. Two breast tissue cores are placed separately into two prelabeled embedding cassettes. (Tissue cassettes are labeled in #2 pencil lead or by bar code label prior to tissue acquisition). The cassettes are closed and put immediately into a bucket with 10% NBF (2.5L is sufficient to submerge the cassettes in the 5L histology bucket) at room temperature where the tissue is fixed for 16-24 hours. The time of beginning of fixation (when cassette is put into 10% NBF) is written on a preprinted spreadsheet with sample IDs. At the end of the collection event, the buckets with tissue cassettes are closed and sealed with their lid and transported in the fume hood to the KTB lab and allowed to remain at room temperature for up to 24 hours.

-STABILIZATON:
After 16-24 hours, the cassettes are removed from the 10% NBF bucket under a chemical fume hood by lifting the cassettes' holder and removing it from the bucket and placing the holder with the cassettes into a bucket filled with 70% Ethanol (2.5L is sufficient to submerge the cassettes in the 5L histology bucket). Then, the cassettes are transferred to shipping containers.

-SHIPMENT:
Four (4) cassettes are added to a 40mL shipping container filled with 70% Ethanol, closed tightly with the container's lid and the tissue container is sealed in a plastic bag and allowed to remain at room temperature for up to 7-10 days. If the cups have not been sent to processing within 72 hours, they can be stored at 2-8°C for up to 4 weeks. Tissue containers are shipped to a processing facility where the processing protocol for paraffin embedding is strictly adhered to. A 5μm section of the resulting paraffin block is obtained for hematoxylin and eosin staining. A digital image of the H&E is entered into the database.

Universal Precautions are MANDATORY. Eye protection is mandatory every time liquid nitrogen is handled to protect against injury due to splashing. Standard laboratory personal protective equipment (e.g., closed toe shoes, full cover of legs and feet, and goggles) will be worn when handling coolants.

FRESH CORES:
If requested by investigator, **within 10 minutes (or less) of procurement** one or two breast tissue cores are placed in investigator supplied media in a labeled 50 ml conical tubes and transported immediately at RT or on wet ice to the on-site processing lab.

FRESH FROZEN:
Flash freezing in liquid nitrogen provides excellent specimen integrity and a wide array of options for tissue analysis [3]. **Within 10 minutes (or less) of procurement,** enough liquid

nitrogen (LN_2) to cover the specimen is transferred to a new polypropylene container. Using clean metal forceps, or disposable plastic forceps, the remaining cores (after taking the 2 cores for fixation process) are immediately placed into LN_2 and snap frozen for 30-60 seconds. The frozen cores are placed in labeled, chilled cryovials. (All cryovials pre-labeled with bar-coded labels prior to tissue acquisition). The cryovials are scanned with a barcode reader, logged into cryoboxes and held in a charged CryoPod™ Carrier or charged LN_2 dry shipper for transport to the storage facility.

If using metal forceps, used forceps are placed in a bleach solution (1/10 bleach/water) in an emesis basin. When time allows or a clean forceps is needed, the forceps in the bleach solution are gently scrubbed with a brush, rinsed in water and allowed to air dry.

Temperature of specimens following acquisition and Snap Freezing but prior to storage: Cryovials containing the specimens are to be held at ≤ -150°C in an LN_2 CryoPod™ Carrier or LN_2 dry shipper until transfer to final storage location.

Storage of Tissue: Once all the samples are procured, the cryovials are transported in CryoPod™ Carriers or LN_2 dry shipper to the SSF LN_2 tank. Tissue samples are stored in liquid nitrogen vapor, (-166.2°C to -195.1°C from top to bottom of tower).

Temperature for collection and processing: All tissue procurement and processing events are done at room temperature.

Standardization: All variables including the time between excision and snap-freezing and time stored in LN2 vapor phase prior to shipment and/or utilization will be entered into the database.

Oversight: All adverse and unexpected events will be recorded in the database and will be addressed by the Internal Advisory Committee. This includes all phases of the process: donation, processing, storage and retrieval, and utilization.

References:

1. Sabine Lehmann et.al. International Society for Biological and Environmental Repositories (ISBER) Working Group on Biospecimen Science. Standard Preanalytical Coding for Biospecimens: Review and Implementation of the Sample PREanalytical Code (SPREC).Biopreservation and Biobanking Vol. 10 No.4, 2012

2. https://biospecimens.cancer.gov/global/pdfs/NCI_BEBP_Snap-freezing_of_Post-surgical_Tissue_Biospecimens.pdf

Bibliography

- Karlsson JO, Toner M. *Long-term storage of tissues by cryopreservation: critical issues.* Biomaterials. 1996; **17**(3):243-56
- Morente MM, Mager R, Alonso S, Pezzella F, Spatz A, Knox K, Kerr D, Dinjens WN, Oosterhuis JW, Lam KH, Oomen MH, van Damme B, van de Vijver M, van Boven H, Kerjaschki D, Pammer J, Lopez-Guerrero JA, Llombart Bosch A, Carbone A, Gloghini A, Teodorovic I, Isabelle M, Passioukov A, Lejeune S, Therasse P, van Veen EB, Ratcliffe C, Riegman PH. *TuBaFrost 2: Standardising tissue collection and quality control procedures for a European virtual frozen tissue bank network.* Eur J Cancer. 2006; **42**(16):2684-91.
- Qualman SJ, France M, Grizzle WE, LiVolsi VA, Moskaluk CA, Ramirez NC, Washington MK. *Establishing a tumour bank: banking, informatics and ethics.* Br J Cancer. 2004; **90**(6):1115-9
- Villalba R, Eisman M, Fornes G, Mesa A, De Torres P, Ariza MJ Gutierrez Guzman A, Gomez Villagran JL. *Implementation of a Quality Plan (ISO 9002) In a Regional Tissue Bank.* Cell Tissue Bank. 2001; **2**(1):45-49.
- Leyland-Jones, Brian R. et al., *Recommendations for Collection and Handling of Specimens from Group Breast Cancer Clinical trials, from Onsite Collection through Shipping to the Central Bank.* Journal of Clinical Oncology 2008 26:34, 5638-5644
- Campbell LD, Astrin JJ, DeSouza Y, Giri, J, Patel AA, Rawley-Payne M, Rush A and Sieffert N. *The 2018 Revision of the ISBER Best Practices: Summary of Changes and the Editorial Team's Development Process.* Biopreservation and Biobanking 16(1): 3-6. https://doi.org/10.1089/bio.2018.0001

Electronic Resources

- ISBER Best Practices: Recommendations for Repositories https://www.isber.org/page/BPR
- NCI Best Practices for Biospecimen Resources:
- https://biospecimens.cancer.gov/bestpractices/2016-NCIBestPractices.pdf
- Snap-Freezing of Post-Surgical tissue Biospecimens: https://biospecimens.cancer.gov/global/pdfs/NCI_BEBP_Snap-freezing_of_Post-surgical_Tissue_Biospecimens.pdf