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Tissue Procurement: Fixation with 10% NBF

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ABSTRACT

Excess tissue material, not necessary for patient diagnosis or pathological assessment, is collected under a waiver of consent or from patients who have consented to a research study. The standard procedure for tissue harvesting includes fixation in 10% neutral buffered formalin (NBF). Fixation helps to preserve tissues from decay and autolysis by halting biochemical processes, such as the digestion of proteins by proteolytic enzymes, and protects the sample from bacterial contamination. Fixation also increases the mechanical strength and stability of the treated tissues, which helps maintain cell morphology and provides a hard surface for sectioning. For these reasons, 10% NBF is routinely used to fix tissues for a variety of research applications.

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GUIDELINES

All collections of remnant tissue for research are under the direction of the Certified Pathology Assistant (CPA), resident or pathologist. The procurement technician will work closely with the Surgical Pathology Suite to ensure the sample remains as sterile as possible and samples are collected quickly. Procurement technician are responsible for logging the time points to provide the investigator with an accurate “post excision time” which is recorded in the Donor IT repository application.

MATERIALS TEXT

Materials and Equipment

1. Container with wet ice
2. Appropriate container for resected tissue (sterile Urine cups)
3. Markers
4. Sterile Blades (scalpels and razor blades) and Forceps
5. FFPE cassettes (QAQC) and 10% formalin
6. Gloves, PPE (lab coat, eye and face shield)
7. Labels (appropriate for collection and storage parameters)
8. Donor IT Repository System and Investigator Request database
9. Sharps disposal
10. Biohazardous waste

Reference to other CHTN SOPs or Policies

- Consent documentation
- Technical Requirements and Training
- Collection of Clinical and Pathologic Data
- Biosafety Guidelines
- Anatomic site-specific SOPs
- Shipping Biological Specimens

SAFETY WARNINGS

- Collections are to occur using standard biosafety guidelines. This SOP does not cover detailed safety procedures for handling biological materials.
- This SOP does not cover ethical regulations pertaining to consent. It is meant to provide guidance on the harvesting of remnant human tissue for research.

BEFORE STARTING

- AT LEAST 20x the volume of 10% NBF must be used to ensure proper fixation. Fixation occurs at a rate of 1 mm per hour. Over-fixation causes tight cross-linking and under-fixation can compromise the tissue (putrefaction) and hamper some assays.
- Fresh shipments utilizing 10% NBF must be properly labeled and secured to prevent leakage during transit.

Procurement

1 Research samples are to remain on wet ice until dissection occurs. Tissues should never be allowed to desiccate or become contaminated by surrounding tissue or other donors. Therefore, working quickly and safely is critical.

2 Working with one anatomic site and tissue type at a time, remove the tissue and place on clean wax paper or disposable cutting board to assess the tissue.

3 The procurement plan should be visible on the Donor IT Repository application and the appropriate receptacles for procurement should be within reach.

4 Working quickly, obtain a “clean procurement kit” that has been autoclaved and sterilized. Remove contents (scalpel, razor, forceps) and remove any grossly necrotic tissue and fatty tissue from the specimen.

5 Remove any cauterized areas and surgical wire/strings/staples that may be present.

6 Identify normal or normal adjacent tissue and procure according to Investigator Tissue Request.

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7 Segment the normal tissue into the required number of aliquots and place in pre-labeled cassette (surgical pathology #, bar code, age/sex/race, weight and anatomic site).

8 Place in 20x volume of 10% NBF and make a notation of the time the sample went into formalin.

9 Based on the size of the tissue, fix the required length of time (4 to 24 hours).

10 After the fixation period, remove the cassette and place in 70% ETOH until scheduled processing.

Collection of Tumor Tissue

11 Collection of tumor tissue:
- Assess the tumor grossly to determine the number of possible segments that can be made without compromising the integrity of the sample.
- Remove a representative segment of tissue that grossly reflects the tumor tissue to submit for QA/QC and place in 10% formalin.
- Section the remaining tissue per investigator preparation request.
- Avoid obtaining samples that contain marker dye.
- Weigh the specimen and note the size in cm and enter into the Donor IT application.

Collection of Normal Tissue

12 Collection of normal tissue:
- Remove as much fat, inflamed tissue, and necrotic tissue as possible.
- Remove as much of the marker dye as possible.
- Ensure sample contains abundant epithelial or mucosa layer.
- Normal tissue removed is annotated by providing the distance in cm from the disease tissue or tumor.

Collection of Disease Tissue

13 Collection of disease tissue:
- Remove as much fat, marker dye, and necrotic tissue as possible.
- Grossly describe the tissue if suspicious.

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