Breast Tissue Collection and Processing 3

3.1 Overview

A major emphasis in the Kenya Breast Health Study will be on classifying tumors by hormone receptor and growth factor receptor status. This study will allow investigators, in the Kenyan population, to assess associations with treatment and survival by tumour molecular subtypes. In order to get an accurate picture of the prevalence of molecular subtypes of tumors, we will attempt to collect tissue samples at biopsy from all Cases enrolled in the study. If a Case had a breast biopsy prior to her enrollment in the study, study staff should attempt to obtain tissue from that biopsy. If the original diagnostic core biopsy cannot be obtained, a tissue sample should be obtained from the excisional surgery if the Case undergoes breast surgery while in the study. Breast tissue specimens in the Kenya Breast Health Study are collected from all participants, at a minimum biopsy samples that are preserved in formalin-fixed and paraffin-embedded (FFPE) are obtained for pathologic diagnosis and subsequent molecular assays including immunohistochemistry and future genetic assays. This collection requires close coordination with the surgical theatre staff and pathology department staff. Further, a subset of patients will also have fresh frozen (FF) and adjacent normal tissues collected alongside FFPE from surgery.

3.2 Breast Biopsy Procedures

Many women will have core biopsies to determine pathologic diagnosis of cancer. Typically, when a needle core biopsy is used for diagnosis, the surgeon collects 4-6 tissue cores for women with a suspicious breast lump for pathologic assessment and diagnosis. Buffered formalin containers for immediate fixation will be used. This will enable the samples to be immediately fixed, preventing problems in assessing tumor tissue markers.

The protocol for collection of breast biopsies is designed to optimize histopathologic preservation and maintain biomolecular integrity in a setting where tissue processing cannot occur within the normal 24 (+/-8hr) hour window on a vacuum-enabled tissue processor. The protocol has been validated in the National Cancer Institute, Tissue Array Research Program (TARP) Lab, and is currently employed by the Genotype-Tissue Expression study.

3.2.1 Breast Tissue Core FFPE Procedure

The pathologist will use the following breast tissue core fixation procedure for the study.

1. Using a 16-gauge biopsy needle, the surgeon will obtain 4-6 breast tissue cores.
2. One to two cores will placed in each labeled cassette.
3. Up to three cassettes with 2- tissue cores will be placed in one prefilled 60ml container with 30ml 10% neutral buffered formalin.

4. Fixation of the tissue cores should proceed at room temperature (not to exceed 40° C) for 16-32 hours.

5. The tissue cassette should be tapped to remove excess fixative, and wiped with a tissue.

6. After 16-32 hours in formalin, the core biopsy specimens will be processed by hospital pathologists to make a diagnosis using their current tissue processing and embedding procedures.

NOTES:

- Fixative should be maintained at room temperature (not to exceed 40° C) prior to use. Fixative with precipitate or past the expiration date should not be used. Do not store fixative containers in direct light.

- No residual fixative is present during shipping, and does not need to be noted on shipping documents as a chemical hazard. Specimens should be shipped as "diagnostic specimens/tissue" rather than biologic specimens, as they are chemically preserved.

- If excisional biopsies are performed, the same procedures noted above should be followed. The pathologist should core the tissue from the excisional biopsy and then follow steps 1-6.

3.2.2 FFPE Tissue Processing Protocol at AKU

The AKU pathologist and technical staff will use the following tissue processing procedure for the study.

1. First, the water from the tissues must be removed by dehydration. This is usually done by immersing tissues in a series of alcohols (e.g., 70% to 95% to 100%).

2. The next step is called "clearing" and consists of removal of the dehydrant with a substance that will be miscible with the embedding medium (paraffin). The most common clearing agent is xylene.

3. Finally, the tissue is infiltrated with paraffin.

    a. Paraffins can be purchased that differ in melting point and in various consistencies. A product called paraplast contains added plasticizers that make the paraffin blocks easier for some technicians to cut.

A vacuum can be applied inside the tissue processor to assist penetration of the embedding agent. The above processes are almost always automated for the large volumes of routine tissues processed. Automation consists of an instrument that moves the tissues through the various agents on a preset time scale. Newer processors are controlled by computers rather than cam wheels and have sealed reagent wells to which a vacuum and/or heat can be applied.

Table 3-1. TARP Standard Processing Times Using Tissue Tek VIP 5

Solution	Conc (%)	Time (hr:min)	Temp (Celsius)	P/V	Mix
Formalin	10	—	—	ON	SLOW
Formalin	10	—	—	ON	SLOW
Alcohol	70	30 min	—	ON	SLOW
Alcohol	95	40 min	—	ON	SLOW
Alcohol	95	40 min	—	ON	SLOW
Alcohol	100	40 min	—	ON	SLOW
Alcohol	100	40 min	—	ON	SLOW
Alcohol	100	40 min	—	ON	SLOW
Xylene	—	45 min	—	ON	SLOW
Xylene	—	45 min	—	ON	SLOW
Paraffin	—	30 min	60	ON	SLOW
Paraffin	—	30 min	60	ON	SLOW
Paraffin	—	30 min	60	ON	SLOW

Paraffin	—	30 min	60	ON	SLOW

Processing times can be altered according to preference and programs can be modified for scheduled finish time and any alterations to processing.

Tissues that come off the tissue processor are still in the cassettes and must be manually put into the blocks by a technician who must pick the tissues out of the cassette and pour molten paraffin over them. This "embedding" process is very important, because the tissues must be aligned, or oriented, properly in the block of paraffin.

(See Embedding Protocol for materials and procedure)

3.2.3 Maintenance

The following maintenance procedures must be followed for the study.

1. After each run, the processor must operate a cleaning cycle to remove excess paraffin in the chamber with washes of xylene and 100% alcohol.
2. Reagents should be changed every five cycles or as needed (depending on the volume of specimens processed).

3.3 Fresh Frozen tumour tissue collection

Breast tissues obtained from breast mastectomy or lumpectomy procedures will be processed with the aim of understanding the genetic changes that can lead to development of breast cancer in the Kenyan population. Documentation of tissues should be note on breast tissue form see below.

3.3.1 Materials/Reagents

Equipment	Materials	Reagents
Forceps	Blades	Neutral buffered Formalin
Blade handle	Slides	Liquid nitrogen
-80° Freezer	Sterile Microtube 2ml	
Knife Handle	Grossing Knife	

3.3.2 Tissue collection and processing

- Once the tissue is delivered to histology department the pathologist immediately takes tissue samples of the tumor periphery, tumor center, near normal and distant normal respectively. A max of 3 sections are collected for each category and put in a 2ml microtube.
- The samples are then labelled with the Study ID number and the tissue section number, e.g AKU001, FT11(tumor periphery sample 1)
  - FT11-13 series- Tumor periphery
  - FT21-23 series- Tumor centre
  - FN11-13 series- Near normal
  - FN21-23 series- Distant normal

- After labelling, immediately snap freeze the tissues by dipping in liquid nitrogen (dip into ladle and then into the liquid nitrogen). Secure the top of the dipping ladle with gauze before dipping it back into the tank to avoid the tubes popping out in to the tank.
- Document on the tissue form the required details (e.g. date, time, surgeon, pathologist) as indicated on the form and file it in the participant's folder.
- Fix the remaining breast tissue in neutral buffered formalin.
- Transfer the tissues to -80C Freezer for long storage
- For tissue samples from other study sites, confirm that they are well labelled with the study number and series number and have accompanying forms duly filled.
- Transfer the tissues to -80C Freezer for long storage.

APPENDIX 3.1

Tissue Collection Form

STUDY ID……..

Patient Name……

KH No …….

Histology Number…….

Facility…….

Doctor's Name……...

Surgery Type……

Date and Time Specimen Removed……...

Date and Time Received in Lab……

Date and Time Put in Formalin……..……

Date and Time of specimen processing……

Tumor Size……

Frozen Tissue	Tumor	Near Normal (within 2 cm of tumor)	Distant Normal (>2 cm from tumor)
Study ID KAIC	FT11	FN11	FN21
KAIC	FT12	FN12	FN22
KAIC	FT13	FN13	FN23

FFPT Tissue	Tumor	Near Normal (within 2 cm of tumor)	Distant Normal (>2 cm from tumor)
Study ID KAIC	T11	N11	N21
KAIC	T12	N12	N22

Technologist (Name)……..

Pathologist (Name)…….