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Optimization of Breast Biopsy and Mastectomy Sample Collection Procedures for Biobanking, Personalized Medicine, and Research Applications

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Abstract

Breast cancer is the most prevalent cancer affecting women, particularly in Asia, with the highest incidence in the Philippines. Despite ongoing efforts, the understanding of breast cancer biology remains inadequate, compounded by the lack of effective screening programs and timely treatments. Addressing these issues, biobanks and other repositories for biological specimens have gained global momentum. The University of the Philippines- Philippine General Hospital (UP-PGH) has established a cancer biobank, demonstrating the viability of a government-supported initiative for cancer research in the Philippines. The study involves a prospective observation of 100 Filipino breast cancer patients at various disease stages, leveraging the expertise of UP-PGH, Philippine Genome Center (PGC), and University of the Philippines Diliman - Institute of Biology (UPD-IB). It integrates the collection, processing, and storage of biospecimens alongside comprehensive patient data. Procedures include RNA extraction, library preparation, and sequencing. Additionally, a subset of patients participates in an *in vitro* three-dimensional (3D) cell culture experiment for chemotherapy pre-screening. The aggregated data aims to uncover therapeutic strategies that can lead to personalized treatments where standard clinical approaches are inadequate. Key techniques include ultrasound-guided core needle biopsy, meticulous normal breast tissue collection, and precise tumor percentage determination to ensure high-quality RNA extraction, sequencing, and 3D cell culture. The study also addresses challenges encountered in prior biobanking endeavors. Ultimately, this research aims to contribute significantly to breast cancer research,

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positioning itself as a model for collaborative research, biobank establishment, and the advancement of precision medicine in developing countries, such as the Philippines.

Introduction

Breast cancer stands as the most prevalent form of cancer among Filipino women. In Asia, the Philippines exhibits one of the highest breast cancer mortality rates and lowest mortality-to-incidence ratio[1]. The rates may have been greatly affected by the absence of screening programs and timely and effective treatment for breast cancer. In the country, breast cancer is typically diagnosed in later stages since most cancer patients only seek consultation at advanced cancer stages. In the Philippines, where a high incidence rate of breast cancer is observed, 53% of breast cancer cases were diagnosed in Stages III and IV, and only 2-3% were diagnosed in Stage I[1,2,3]. Aside from promoting screening uptake and patient education on breast cancer, breast cancer-related research and development should be explored further to decrease breast cancer incidence in the country.

The establishment of biobanks across the globe allowed the acceleration of medical advances. Biobanks are used for the storage and preservation of scientifically valuable biological specimens for future research and development[4]. Since then, biobanks have been used for translational research[5] and improving personalized medical approaches[6]. In Asia, biobanks are mostly concentrated in East Asia, especially in China, Japan, and Korea[4]. In low- to middle-income countries in Asia, such as the Philippines, the concept of biobanking is still emerging. Biobanks in the Philippines are mostly owned by private healthcare institutions, where the health and exposure status of the broader Philippine demographics may not be well-represented.

The establishment of the cancer biobank at the University of the Philippines Manila (UPM) Philippine General Hospital (PGH) has proven the feasibility of establishing a government-supported cancer biobank that can advance cancer-related studies in the Philippines[7]. Building upon this foundation through a collaborative effort that bridges medical expertise, research innovation, and healthcare policy, we endeavor to create a positive shift in the landscape of breast cancer care and research in the Philippines. With that, there is an imperative need to have a cancer phenome biobanking workflow and an expanded approach that streamlines the collection of extensive and stratified patient-specific data on a national scale. This study describes the protocol for breast biopsy and mastectomy sample collection procedures, focusing on their integration into the biobanking framework. Anticipating not only advancing our understanding of breast cancer pathogenesis within the context of the Filipino population but also facilitating the development of precision treatments that align with the unique genetic, environmental, and clinical attributes of affected individuals.

Protocol

This is a prospective, observational study with a collection of de-identified biospecimens (tumor and normal breast tissue) and clinicopathologic, treatment, and outcome data from 100 Filipino breast cancer patients of various stages. This is a collaborative work between the Philippine General Hospital (PGH), the Philippine Genome Center (PGC), and the Institute of Biology (IB) of the University of the Philippines Diliman, each with designated tasks.

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The protocol was registered in the Research Grants Administration Office (RGAO-2020-0933), and reviewed and approved by the University of the Philippines Manila Research Ethics Board (UPMREB) for implementation under ethics clearance number UPMREB 2020-822-01. All laboratories used adhere to the biosafety guidelines of each institution. Recruiting patients from PGH ensured the variance and representation in terms of clinicopathologic factors, ethnicity, and environmental exposures. A standard operating procedure (SOP) developed and validated in previous projects was utilized as the framework for the acquisition, collection, transportation, and other logistical arrangements related to the samples.

NOTE: See **Figure 1 - Diagrammatic workflow of the protocol** for the summary of the protocol workflow and the roles of each institution involved in the study.

1. Pre-breast sample collection procedure

1. Identify and screen the eligible patients for study inclusion during a clinic consult or preoperative conference.
    1. Inclusion criteria for cancer patients
        1. Include Filipino, female, aged 19-75 years old diagnosed with Stage 1, 2, and 3 breast cancer, and Filipino females aged 19-70 years old diagnosed with Stage 3B and 4 breast cancer.
        2. Include clinically suspected or pathologically diagnosed cases of invasive breast cancer of any stage.
        3. Include patients who will undergo surgical treatment (modified radical mastectomy or radical mastectomy) either upfront (Clinical Stage I or II) or post-neoadjuvant chemotherapy (Stage IIIB, IIIC). Stage IIIA patients may undergo either upfront surgery or neoadjuvant therapy as per the physician's recommendations. For Stage IV patients, those willing to undergo core needle biopsy of the breast tumor and adjacent normal tissue. Stage IV breast cancer patients will not undergo surgical treatment.
        4. Include Stage III breast cancer patients who have undergone neoadjuvant therapy prior to recruitment, and there is a complete medical and clinical treatment response record.
        5. Include patients who can understand and sign informed consent.
    2. Exclusion criteria for cancer patients
        1. Exclude patients who have prior or concurrent malignancy, including prior diagnosis of breast cancer.
        2. Exclude patients with bilateral breast cancer.
        3. Exclude patients who underwent prior neoadjuvant radiotherapy or hormonal therapy.
        4. Exclude patients who underwent prior core or incisional biopsy where there is no consent to repeat biopsy procedure for those patients who will undergo neoadjuvant chemotherapy (since no pre-chemotherapy fresh tumor specimen can be obtained)
        5. Exclude patients who underwent prior excisional biopsy with no residual tumor.

6. Exclude patients with comorbidities precluding surgical management or neoadjuvant chemotherapy.

3. Withdrawal criteria

1. Inform participants that they can withdraw anytime for safety or other concerns without affecting their medical care. This non-interventional study will not impact the choice of treatment for participants.

2. Secure consent from the patient and fill out the data collection form.

NOTE: The assigned fellow-in-charge (FIC) or the consultant, together with the university research associate (URA), secures the consent.

3. Obtain and verify the patient's schedule for the core needle biopsy procedure after confirming their participation in the study.

4. Conduct procedures in compliance with the guidelines set and approved by the Ethics Board Committee and Biosafety Committee of the institution.

2. Breast biopsy sample collection procedure

NOTE: See **Figure 2** for the detailed workflow of the core needlebreast biopsy procedure for stage III and IV patients, showing the distribution of samples for each procedure.

1. Pre-label tubes before specimen collection as GB, meaning Genomics - Breast (the study the specimen is under), xxx which is the participant number and can range from 001 to 100, or more, and the letters A, C and D. The following are the letter codes to be used in labeling the tubes:

A - means core needle biopsy, tumor tissue, for sending to PGC

C - means core needle biopsy, tumor tissue, for biobanking

D - means core needle biopsy, normal tissue

1. Assign the samples submitted for sequencing with the laboratory's label: "BC" and two two-digit sample numbers (e.g., BC01, BC21). Each sample has a corresponding "GB" code which correlates to the code assigned during specimen collection.

2. Obtain up to nine tissue cores using a 14 G core needle biopsy gun under ultrasound guidance, in an outpatient clinic, operating room, or other aseptic environment.

1. For each core, obtain a breast sample approximately 2 cm in length at the center of the tumor and 1 cm away from the tumor for adjacent normal tissue. Collect two types of specimens from each participant: tumor tissue and normal tissue.

2. Hand each sample to the URA for placement into a petri dish containing phosphate-buffered saline (PBS) solution.

NOTE: In this study, core needle biopsies were performed only on patients with Stage III and IV breast cancer. For Stage III patients, core needle biopsies provided chemotherapy-naïve tumor samples. For Stage IV patients, core needle biopsies served as the primary tumor sample, as mastectomy was not performed. For patients with Stage I and II breast cancer, no core needle biopsies were performed. Instead, tumor and adjacent normal tissues were obtained from mastectomy specimens.

3. Cut the biopsy specimens in half to distribute evenly between the study and histopathological analysis.

1. For tumor tissue, obtain up to 9 cores per study patient using a 14-G needle.
2. Separate the tumor cores obtained into 2 groups in the Petri dish: samples for RNA analysis (6 cores) and samples for biobanking (3 cores).
3. Cut each core into 2 lengthwise, but in cases where it is difficult to do this, cut the cores into 2 crosswise. Use a sterile blade to cut the cores. Send half of the tissue core for RNA analysis or store for biobanking. Send the other half of the tissue core to Pathology for routine histopathological analysis.
4. For normal tissue, obtain up to 3 cores per study patient using a 14 G needle. Use a different needle from the one used to obtain tumor tissues to avoid contamination. In instances where this is not possible, obtain normal breast tissues first before getting tumor tissues.
5. Place each normal tissue obtained in a Petri dish similar to what was done with the tumor tissues, and cut the cores in half. Distribute the samples for the study and to the Pathology Department for the histopathological analysis.

4. After sample collection, distribute the samples among the tubes, following a manner and order of priority.
    1. Place half of the cores in the designated 2 mL cryovial pre-filled with 1.25 mL of RNA stabilization solution (all cores for RNA analysis placed together in 1 vial and all cores for biobanking in a separate vial, maintain samples in cold temperature at all times) and place the corresponding halves of these cores in ordinary specimen vial/tube and fixed with 10% neutral buffered formalin (10% NBF), for histopathological analysis. Ensure that the two corresponding half-groups, the one going to the designated center (e.g., samples sent for RNA analysis), and the corresponding Pathology specimen have the same specimen code.
5. Complete the surgical pathology forms. Together with the forms, submit the Pathology specimens fixed with formalin to Pathology for routine histopathology.
6. Seal with parafilm the core needle biopsy samples soaked in tubes containing RNA stabilization solution (ideally 5:1 ratio of solution to tissue, so if 0.25 cm tissue, 1.25 mL of RNA stabilization solution).
7. Store the samples at 4 °C for 24 h, and then eventually transfer it in the ultra-low freezer, with a temperature of -80 °C.

    NOTE: A guide on storage will be discussed in Section 4 (Storage, packing and transport of specimens).

3. Breast mastectomy sample collection procedure

1. Follow the pre-breast sample collection procedure in Section 1.

    NOTE: Stage I, II, and III patients enrolled in the study will undergo mastectomy at the Operating Room (OR). For Stages I and II patients, mastectomy will be done upfront, while for Stage III patients, mastectomy will only be done upon completion of 8 cycles of neoadjuvant therapy. For stage 4, no mastectomy samples are collected. See **Figure 3** for the detailed breast mastectomy sample collection protocol showing the distribution of samples per procedure.
2. Coordinate the schedule with the collaborators and the Pathology department. Do this at least 2 days prior to the target date of the procedure. In case of changes to the

set schedule, inform the concerned personnel right away to avoid any conflicts in the schedule.

3. Pre-label the tubes before specimen collection as GB, meaning Genomics Breast (the study the specimen is under), xxx which is the participant number and can range from 001 to 100, or more, and the letters E and F. The following are the letter codes to be used in labeling the tubes:

   E - means mastectomy, tumor tissue, for sending to PGC, IB, and for biobanking

   F - means mastectomy, normal tissue

   1. Assign the samples submitted for sequencing with the laboratory's label: "BC" and two digit sample number (e.g., BC01, BC21). Each sample has a corresponding "GB" code which correlates to the code assigned during specimen collection.

4. Fill out the surgical pathology forms during the surgery, while waiting for the specimen. Send updates to the pathologist-in-charge for them to make any necessary preparations for the specimen collection.

5. Place the specimen in a clean plastic/specimen bag right after removal and documentation of the breast specimen. Ensure the mastectomy specimen is immediately brought to the Pathology department for grossing to minimize the cold ischemic time. Do not place the specimen in formalin upon submission.

   NOTE: Sample action photos during mastectomy are shown in **Figure 4** (Representative images of sample collection during mastectomy procedure).

6. Obtain two types of samples from each specimen upon grossing: tumor tissue and normal tissue.

   1. Tumor tissue

      1. Obtain a minimum of one block (preferably more) measuring 1 cm × 1 cm × 1 cm of tumor tissue per specimen.

      2. Cut the obtained tissue block further into two parts, with each half block measuring 1 cm × 0.5 cm × 0.5 cm. Hand over half of the tissue block to the URA for biobanking and analysis, and then fix the other half in formalin for routine histopathology.

      3. Evenly trisect the half block of fresh tissue. This will be intended for RNA sequencing, biobanking, and organoid culture.

      4. For RNA sequencing: Immediately place in pre-chilled, pre-labeled 5 mL cryovials, pre-filled with 2.5 mL RNA stabilization solution (not exceeding 30 min, must be kept cold at all times).

      5. For biobanking: Immediately place in pre-chilled, pre-labeled, and pre-filled 5 mL cryovials with 2.5 mL RNA stabilization solution (not exceeding 30 min, must be kept cold at all times).

      6. For organoid culture: Immediately place in pre-chilled, pre-labeled, and pre-filled 15 mL conical vials with 5 mL of tissue storage solution and 0.01 mL Primocin (not exceeding 30 min, must be kept cold at all times). Pool together in one conical tube one-third of all the tumor blocks obtained from different sections of the specimen.

   2. Normal tissue

1. Obtain a minimum of one block (preferably more) measuring 1 cm × 1 cm × 1 cm of normal breast tissue per specimen.
2. Cut the obtained tissue block further into two parts, with each half block measuring 1 cm × 1 cm × 0.5 cm. Hand over half of the tissue block to the URA for biobanking and analysis, and then fix the other half in formalin for routine histopathology.
3. Cut the tissue block equally into two for the study. These will be intended for RNA sequencing and biobanking.
4. For RNA sequencing: Immediately place in pre-chilled, pre-labeled 5 mL cryovials, pre-filled with 2.5 mL cryovials with RNA stabilization solution (not exceeding 30 min, maintain at cold temperature at all times).
5. For biobanking, immediately place in pre-chilled, pre-labeled, and pre-filled 2.5 mL cryovials with RNA stabilization solution (not exceeding 30 min, maintain at cold temperature at all times).

   NOTE: Tumor samples labeled "E" are typically obtained from three or more different sections of the specimen. To avoid confusion, tumor samples intended for RNA analysis and biobanking should be placed into separate tubes labeled as E1, E2, E3, etc., while pooled tumor samples intended for cell culture should be placed into tubes labeled simply as E. For samples labeled E1, E2, and E3, the relative position/location of each tissue sample with respect to the tumor (e.g., center, 10 o'clock position) should be recorded.
7. Seal with parafilm the cryovials containing the samples obtained from the mastectomy.
8. Store the cryovials in 4 °C for 24 h, and then eventually transfer them to the ultra-low freezer, with a temperature of -80 °C for long-term storage.
9. Pack the conical tube containing the pooled tumor blocks and prepare it for transport. These tumor blocks will be used for cell culture into organoids.

4. Storage, packing, and transport of specimens

1. Immediately seal with parafilm all cryovials containing the tumor and normal tissues and store these at 4 °C for 24 h. After 24 h, transfer the tubes to -20 °C for around 2-4 h and then eventually transfer them to -80 °C indefinitely, for long-term storage until transport.
2. Update the specimen tracker and database prior to transport of samples for RNA analysis, including the results of routine histopathology and tumor percentages, to ensure the identity of the sample.
3. Accomplish a transportation manifest for each sample, prior to transport. Ensure that the packing process is quick and the tubes containing the samples are not left at room temperature (RT) for a prolonged period.
   1. Seal all cryovial caps with parafilm to prevent leakage.
   2. Place all the tubes in a test tube rack.
   3. Set the temperature logger and place it over the rack alongside the tubes for transport.
   4. Wrap the tubes with absorbent material, then with bubble wrap, and place the items inside the

resealable bag. Ensure that the temperature logger is recording temperature and that the tubes are intact and positioned upright before sealing the items inside the bag.

5. Place the gel packs on the bottom of the polystyrene shipping box, then set the resealable bag containing the tubes on top of the gel packs. Put another layer of gel packs on top of the bag. Make sure the tubes are sandwiched between gel packs to ensure the specimens will be kept cold during transport.
6. Close the polystyrene box and put it inside a cardboard shipping box. If there is no large cardboard box available, use a larger polystyrene box. Place the completed transportation manifest inside a ziplock bag and put it inside the box. Placement of the items inside the box is shown in **Figure 5**.
7. Seal the cardboard box and place the appropriate shipping label outside the box.

4. Inform the recipient of the specimen collection schedule and when the target sending of samples will be.
    1. For organoid culture, only tumor tissues derived from mastectomies are sent and utilized. Ensure these are delivered fresh, on wet ice, and within 24 h from specimen collection. Once the samples are packed and the box is sealed, quickly book a courier to transport them. Ensure that the boxes will not be left in high-temperature areas for prolonged periods, as a severe temperature drop or rise can compromise the viability of the samples.
    2. Prior to handing off to the courier, take note of the number of vials/tubes shipped and the time the courier left the Biobank. Upon arrival of the package, ensure that the recipient takes note of the following: Time of arrival and receipt at the designated center, temperature at arrival, number of vials/tubes received, and any compromised specimen
    3. For RNA analysis, check if the samples to be delivered have a corresponding histopathology result and tumor percentages from the Surgical Pathology Division, prior to sending samples for RNA analysis. Identify and select the tissue block with the highest tumor content for each patient to be submitted for RNA extraction. Transport samples only those with tumor percentages.
    4. Send the samples in groups of no more than 40 to facilitate batch processing. Choose any combination of tumor tissue from core needle biopsy or mastectomy and normal tissue from mastectomy. Review the following conditions if normal tissue from core needle biopsy is to be transported:
    -Enrolled as Stage 3, but histopathology result shows Stage 4
    -Enrolled as Stage 3, but histopathology result shows loco-regional progression of disease.
    5. Ensure that all samples are transported on wet ice. Similar to the protocol being followed when sending samples for 3D cell culture, once the specimens are packed and the box is sealed, promptly book a courier for transport. Avoid leaving the boxes in high-temperature areas for prolonged periods, as significant temperature fluctuations can compromise the viability of the samples.
    6. Prior to handing off to the courier, take note of the number of vials/tubes shipped and the time the courier left the Biobank. Note the following

upon arrival of the package: time of arrival and receipt at the designated center, temperature at arrival, number of vials/tubes received, and any compromised specimen (if there is any).

5. Update the database of samples sent and input all necessary information needed prior to further processing of samples. Monitor and assess the database once a week.

Representative Results

For the collection of samples during core needle biopsy, doing the procedure under ultrasound guidance had a huge impact on patient convenience and efficiency of workflow, as well as on the RNA integrity of the samples. If this is not done, the expected results might not be obtained, and patients must go back to the clinics to repeat the procedure, therefore prolonging sample collection and processing.

In addition to that, identification of tumor percentages per sample, which was done by the pathologist-in-charge, is also an important step to ensure the quality of the samples for extraction and minimize delays in data analysis. The tumor percentage is an estimation of the tumor composition of the breast tissue samples. With this, selecting tissue blocks for RNA extraction and sequencing will be easier, as only tumor blocks with high tumor percentages and normal tissue blocks without tumor tissue will be sent for extraction. In cases where there are no representative normal tissue blocks without tumor tissue, those with minimal tumor percentage are sent instead.

Samples were sent out for RNA extraction and sequencing after the pathologist had selected the samples based on the tumor percentage estimated by the pathologist. A detailed list of samples with suboptimal quality, along with their corresponding RNA integrity number (RIN) values and concentrations, is shown in **Table 1**. From the table provided, it can be observed that 2 samples with varying RIN values and concentration failed in the library preparation step during sequencing and were excluded in the final analysis. Factors that influence the quality of libraries produced include RIN value, sample concentration, and the time interval between sample collection and RNA extraction and sequencing.

To increase yield and achieve optimal results from RNA extraction and sequencing, resubmission of duplicates and sample pooling were done. Moreover, during specimen storage, it is important to ensure that the samples are stored in the -80 °C ultra-low freezer to prevent RNA degradation and maintain cell viability. When it comes to transport, similarly, multiple gel ice packs must be stored inside the ice box to prevent the samples from thawing easily.

Overall data on RNA extraction quality per batch of samples sent is presented in **Table 1.** The percentage was based on the number of samples with acceptable RNA quality and was recommended for RNA sequencing. From the table, an increasing trend in the percentage of samples with optimal RNA extraction quality can be observed. This can be attributed to increased personnel experience and proficiency with the procedures, as well as optimization of workflow. In particular, improvements were made to minimize cold ischemic time from removal of the breast from the patient up to the point of sample collection. Meanwhile, the decrease in RNA quality can be attributed to changes in personnel processing the samples. To ensure the quality of the samples that will be sent for extraction, personnel must be fully informed and well-trained before performing the transport of samples to the collaborators.

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After filtering out samples that did not pass all the quality control steps, samples with optimal quality were then subjected to RNA sequencing. Results show that all samples had great sequencing quality scores, where >90% of base calls had an accuracy of at least 99.9% (Q30). Further, out of 31 samples in the last sequencing run, more than 60% of the bases being sequenced passed Illumina's internal quality filtering procedure (%PF) and were able to produce >30 million reads after filtering. Post-filtering, the number of reads for all samples still exceeds the recommended 5-25 million reads for gene expression profiling experiments[8]. **Table 2** shows representative results on RNA sequencing quality.

Lastly, as mentioned, sample duplicates were also sent for culturing into organoids. In **Figure 6**, bright-field photos of 1-week old organoids generated from Stage III breast cancer mastectomy tissues after neoadjuvant chemotherapy are shown. The figure shows that due to the meticulous collection of samples and through ensuring that all steps in this protocol were employed, the cells were still viable for cell culture, as evidenced by the organoids that were derived post-chemotherapy. The effect of chemotherapy and response *in vitro* can be better visualized through the bright-field photos. These results hold the potential for the discovery of tailored treatment approaches and novel therapies for breast cancer, especially among Filipino patients.

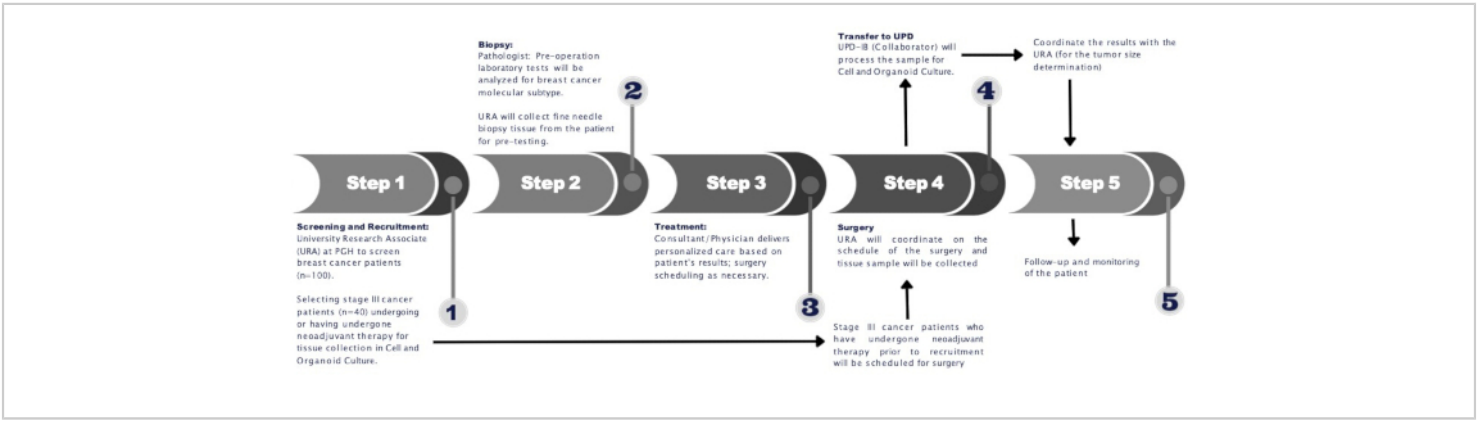


Figure 1: Diagrammatic workflow of the protocol. The roles that each personnel performs in this protocol are summarized in the figure. Please click here to view a larger version of this figure.

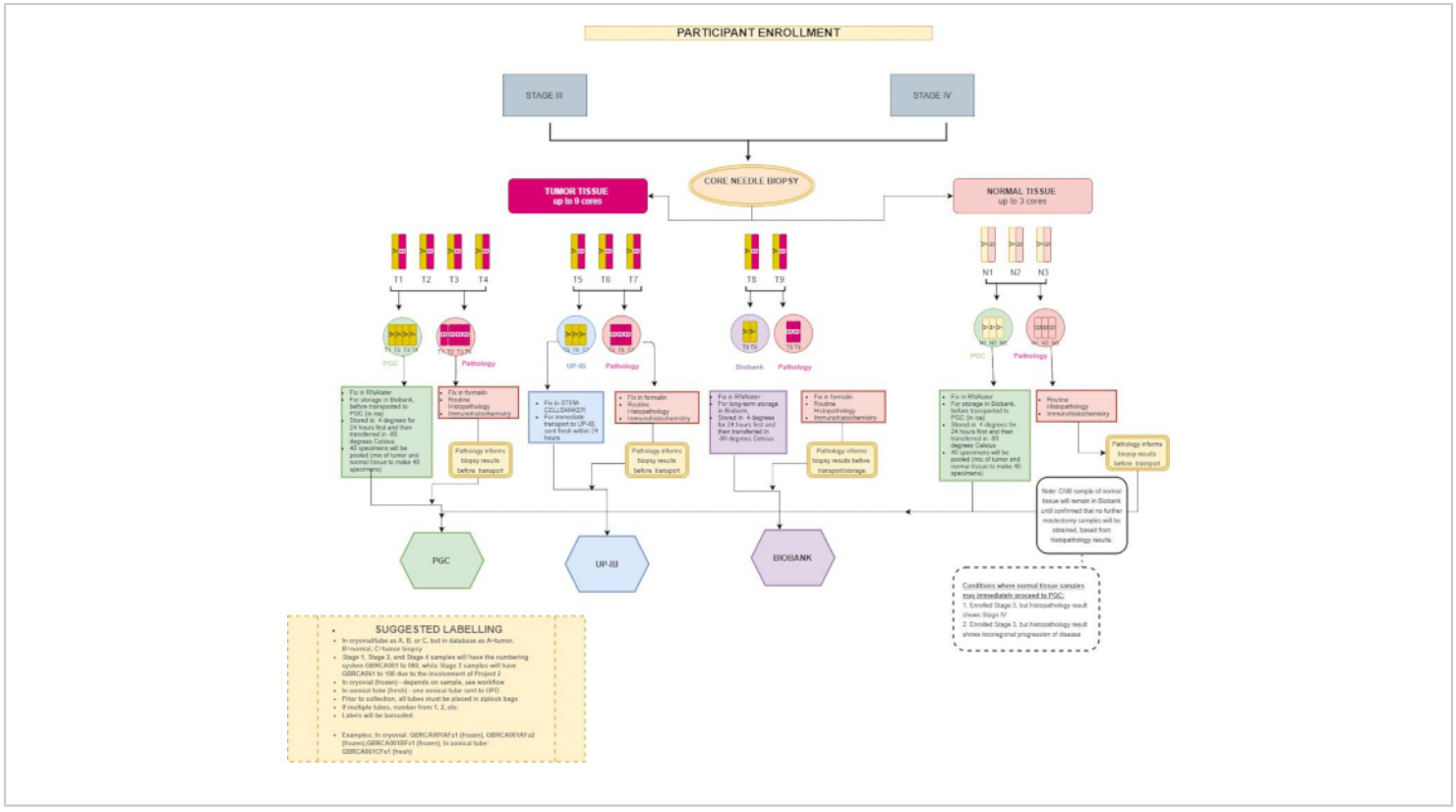


Figure 2: Workflow for core needle biopsy sample handling in stage III and IV breast cancer. This figure shows the sample processing workflow for core needle biopsies in advanced breast cancer. Tumor and normal tissues are divided for pathology, biobanking, and molecular analysis at the Philippine Genome Center (PGC), with RNA stabilization solution fixation and barcode-based labeling for traceability. See **Figure 3** for the mastectomy sample collection protocol. Please click here to view a larger version of this figure.

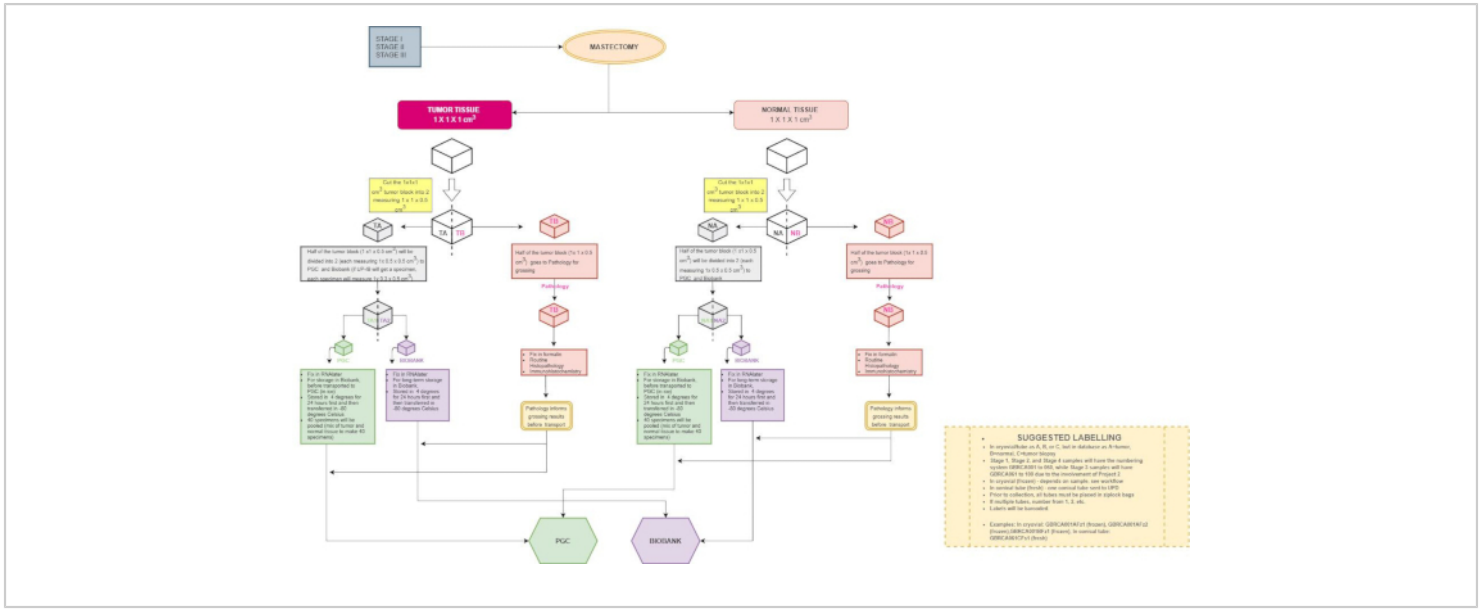


Figure 3: Workflow for tissue handling after mastectomy in Stage I-III breast cancer. This diagram outlines the protocol for collecting and processing tumor and normal tissues from the mastectomy. Samples are divided for pathology, molecular analysis, and long-term storage at the PGC and Biobank, with RNAlater fixation, controlled storage, and standardized labeling. Please click here to view a larger version of this figure.

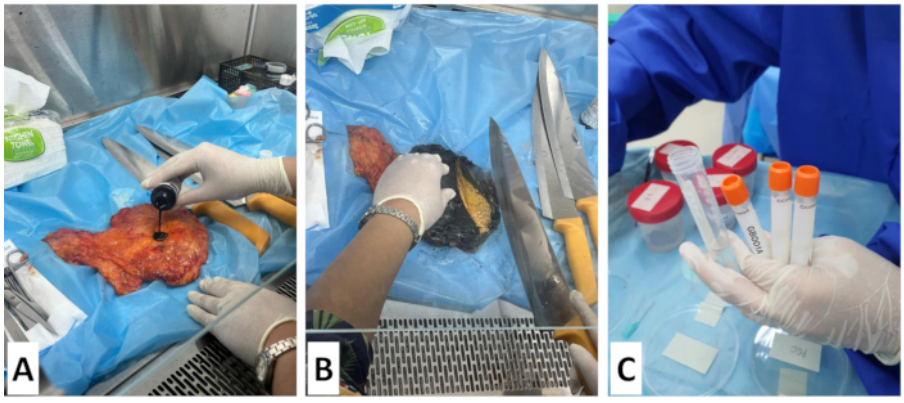


Figure 4: Sample collection during the mastectomy procedure. (**A**) The URA is in charge of collecting the breast specimen for grossing by the pathologist. The pathologist will stain the specimen with tissue ink for histopathological analysis. (**B**) The pathologist-in-charge will obtain the tumor and normal breast tissues, and the URA will cut the samples into two: one half of the tissue will be stored in ultra-low freezer for later use, and the other half will be sent to the IB for cell culture. (**C**) After cutting, tissues that will be used for genomic profiling will be placed in their respective and pre-labeled tubes, while tissues which will be used for routine histopathological analysis will be kept by the pathologist-in-charge. Please click here to view a larger version of this figure.



Figure 5: Placement of gel packs and resealable bag containing upright cryovials/conical tubes inside styrofoam box. After filling the ice box with wet ice, the resealable bag containing the bubble-wrapped cryovials/conical tubes and temperature logger must be sandwiched between gel packs to ensure that the sample will be kept cold during transport. Please click here to view a larger version of this figure.

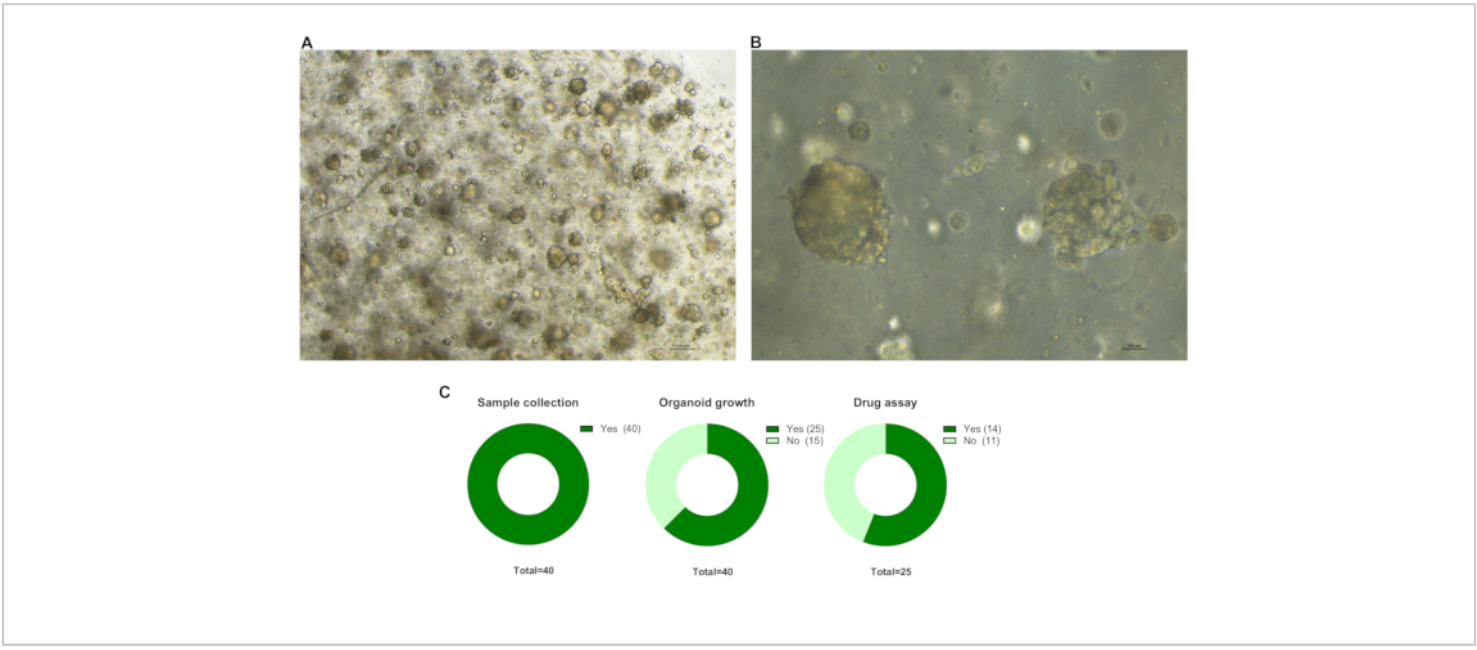


Figure 6: Organoids derived from breast cancer patients. Bright field photos of 1-week old organoids generated from Stage III mastectomy tissues after neoadjuvant therapy (**A**) 4x. (**B**) 20x. Scale bar = 100 µm. (**C**) Success rate in establishing organoids from patient samples and subsequent drug assay. Total samples collected from Stage III post-neoadjuvant mastectomies as of the period (left panel). Success rate in growing organoids from tissue samples (middle panel). Success rate in conducting drug assay from established organoids (right panel). Successful organoid cultures were labeled as yes, while failed organoid cultures were indicated as no. Please click here to view a larger version of this figure.

Table 1: Extracted tissue samples with suboptimal quality. The table presents the RNA extraction data of the samples that failed in the library preparation step during sequencing. These samples were supposed to be included in the RNA sequencing runs; however, due to sub-optimal quality and low concentration, good-quality libraries have not been generated for these samples. As a consequence, these samples have been excluded from the analysis. Please click here to download this Table.

Table 2: Sequence quality measurements. From left to right the columns indicate the sample name, % of bases with quality score > Q30 (99.9% accuracy), number of bases with score > Q30 in Mb (1 megabase = 1 million bases), number of reads in millions that are retained after pass filtering, % of bases that passed the filter. Samples were assigned with the laboratory's own label: "BC" and two digit sample number (e.g., BC01, BC21). Each sample has a corresponding "GB" code corresponding to the code assigned during specimen collection. Please click here to download this Table.

Discussion

The successful implementation of this protocol relies not only on technical precision but also on the coordination of clinical and research teams to ensure consistent sample quality and data integrity. This protocol incorporates several key procedures designed to maximize diagnostic accuracy and generate high-quality biospecimens suitable for downstream

molecular analyses. Key steps include ultrasound-guided core needle biopsy for obtaining both normal and tumor breast tissue, data collection on treatment response, and tumor percentage quantification.

Core needle biopsy (CNB) is recommended for the diagnosis of palpable breast masses suspicious for cancer. Based on the clinical practice guidelines for early breast cancer[9] published by the Philippine College of Surgeons (PCS), core needle biopsies are commonly used in the diagnosis of breast masses due to its advantages which include but are not limited to: increase in diagnostic accuracy; it can differentiate non-invasive from invasive cancer; and it can provide histological diagnosis and data on hormone receptor status and other prognostic markers from immunohistochemical staining. In this protocol, an ultrasound-guided CNB of patients is done to increase yield and ensure that the area of interest (tumor or normal tissue) is sampled adequately. Several studies support the use of ultrasound-guided CNB in the diagnosis of breast masses. Findings of a study conducted in the Middle East in 2020 include a 96.33% sensitivity and 75.10% specificity for malignancy from the 109 patients with ultrasound-guided CNB-detected malignant lesions[10]. Meanwhile, another study reported a high accuracy rate of 89.1% in pathological examination results from ultrasound-guided CNB in non-palpable breast masses[11]. The use of ultrasound-guided biopsy in this protocol helped increase the accuracy of diagnosis, minimize sampling errors, and reduced delays from repeat procedures by improving the likelihood of capturing tumor content in the initial biopsy and decreasing the number of invasive attempts needed. In this protocol, results show difficulty in extracting quality RNA from core needle biopsy samples from repeat procedures by improving the likelihood of capturing tumor content in the initial biopsy and decreasing the number of invasive attempts needed. This is usually due to low concentration and low RNA integrity value of the samples. This further highlights the need to conduct biopsies under ultrasound guidance.

Normal breast tissues in this study are collected alongside tumor specimens during core needle biopsy and surgical resection. This step is not usually done in routine diagnosis of breast cancer, but in this protocol, collection of normal tissues adjacent to breast tumors is critical to provide a control for RNA sequencing and evaluation of treatment response. Collection of normal tissues adjacent to the tumor will help provide relevant gene expression dataset for comparing tumor expression profiles and aid in the identification of molecular changes in early-stage breast cancer for better disease prognosis and possibly discover cancer prevention strategies[12,13]. In addition to that, in contrast to the procedure employed in the sampling site (PGH), additional cores are obtained in this protocol. This is done to ensure diagnostic accuracy and increase yield during RNA extraction.

Collection of data on treatment response is also an important step in this protocol. This is done to correlate the effect of neoadjuvant therapy to the tumor and gene expression profiles. Data that will be derived from this can be used for future studies exploring precision medicine.

Most importantly, pathologists play a crucial role in processing mastectomy specimens by accurately identifying and sampling both tumor and grossly normal tissue. The reason why the samples are halved and then 'paired' (one half sent for banking and sequencing, the other for routine histopathology) is to provide histopathologic confirmation that there is indeed invasive carcinoma in the ostensibly "tumor tissue", or that there is no malignancy in the ostensibly

"normal tissue". Pathologists estimate tumor percentage to optimize nucleic acid yield and select samples with the highest tumor content for RNA sequencing. This is similar to processes in anatomic pathology where the most suitable tissue block is selected for further testing, e.g., immunohistochemistry or molecular testing. Conversely, tissue blocks with the least tumor percentage (in cases where all tissue blocks have tumor in them), or without tumor, are submitted for sequencing as "normal tissue". This would minimize, but does not eliminate, uncertainty about the contents of the tissue submitted for molecular testing. Further, this is also essential to minimize cost and avoid delays in processing the samples collected. In case of sub-optimal results in RNA extraction and sequencing, the URA can decide which samples can replace the previously sent specimens based on the pre-identified percentages. The presence of sufficient tumor content is important to avoid missing genetic abnormalities during molecular testing. However, estimation of tumor cell percentage may be inaccurate and could potentially lead to misinterpretation of results[14].

Methods of a few studies on biobanking breast tissues have similarities to this protocol. A study conducted in India in 2021[15] focused on the evaluation of breast cancer profiles and correlation of stored biobank tissues with pertinent data filed in their database. Similar to the methods employed in the study, data on the patients' medical history and clinical records (Histopathological reports and data on neoadjuvant therapy) are also recorded in this protocol. However, the study differed in that the tissues were formalin-fixed paraffin-embedded (FFPE) and placed in tissue cabinets, in contrast to what was described in this protocol. Moreover, the study also focused on genetic counseling and testing of patients and their relatives to assess cancer risk and plan tailored surveillance and clinical management for the patients. Another study which dealt with multifocal breast carcinomas and DNA content[16] employed similar methods to this protocol. Both methods described the collection of tumoral and normal tissues and storage of fresh tissues in tubes at -80 °C for later transportation. The study differed in that it did not involve any patient who received neoadjuvant chemotherapy; it employed biopsy punch instead of ultrasound-guided core needle biopsy; and lastly, it focused on DNA extraction and genome-wide analysis. Both of the studies mentioned noted the role of pathologists in biobanking since they are responsible for evaluating the histological quality of the tissues and in routine pathological diagnosis.

To summarize, challenges encountered in the protocol mainly include patient recruitment, difficulty extracting quality RNA from core needle biopsy samples, and suboptimal RNA extraction quality for some samples. For patient recruitment, the problem is mainly due to a decrease in the number of patients visiting PGH for consultation during the pandemic's peak. Meanwhile, there is difficulty in extracting quality RNA from core needle biopsy samples due to insufficient volume of tissues for RNA extraction. Lastly, the substandard quality of RNA from previously processed samples was a result of an unoptimized workflow and set of procedures from sample collection to storage. Recommendations to improve these challenges include workflow optimization and pooling of samples, if necessary, to achieve optimal RNA extraction quality, especially for core needle biopsy samples.

Disclosures

The authors do not have any conflict of interest, whether financial, personal, or institutional, to disclose.

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