CORRELATIVE SCIENCE PROCEDURE MANUAL

1. Purpose

This document describes the procedures required for the collection, shipping, and processing of biospecimens from patients enrolled or registered who have consented to participate in A011202. This document also describes the procedures that will be followed subsequent to the receipt of biospecimens by the Alliance Biorepository (i.e. Siteman Cancer Center Tissue Procurement Core at Washington University), prior to their use for protocol-specified and future, unspecified correlative science research studies. This document should be used by staff involved with any aspect of the A011202 biospecimen collection, processing, and submission, including staff at satellite institutions.

2. Scope

This document applies to all biospecimens collected specifically for A011202 only. Please refer to the trial protocol-specific language for additional details regarding eligibility, participant enrollment, data submission, and specific procurement procedures. **Please ensure that you are reading the most updated version of this document. This document may experience minor updates, revisions, and clarifications independent of a formal protocol amendment. The most recent version of this document may be found on the Alliance website and CTSU.**

3. Definitions

Term	Definition
ABWUSTL	Alliance Biorepository at Washington University in St. Louis
FFPE	Formalin fixed, paraffin embedded

4. Contact Information

4.1 For questions and problems related to protocol administration, eligibility, patient registration, and data submission, relevant contact information is listed on protocol pages 1 and 2.

4.2 For information on using the BioMS system, please refer to the 'Help' links on the BioMS webpage to access the online user manual, FAQs, and training videos. To report technical problems, such as login issues or application errors, please contact: 1-855-55-BIOMS or bioms@alliancenctn.org. For assistance in using the application or questions or problems related to specific specimen logging, please contact: 1-855-55-BIOMS or bioms@alliancenctn.org.

4.3 For all other questions regarding biospecimen procurement and shipping procedures, please contact the Alliance Biorepository Program Manager: 1-314-747-4402 or alliance@email.wustl.edu.

5. Site Preparation

5.1 Please refer to A011202 protocol document for any specific requirements related to patient enrollment, registration, and regulatory compliance.

5.2 Please ensure that you have appropriate log on credentials and can successfully access the BioMS application. The BioMS application is used for both requesting biospecimen collection kits and for logging the collection and shipment of biospecimens to the Alliance Biorepository at Washington University. For training and assistance in using the application or questions or problems related to specific specimen logging, please contact: 1-855-55-BIOMS or bioms@alliancenctn.org.

5.3 Prior to collection of blood biospecimens, a biospecimen collection kit must be at the collection site. Please see section 7 for requesting biospecimen collection kits. Please allow at least 10 working days to receive the collection kit.

6. Collection Schema

The following biospecimens are to be collected at each of the time points. Please refer to individual collection kit instructions, biospecimen collection and processing methods, and specific shipping procedures below.

Time Point	Kit (Y/N)	Biospecimen	Quantity	Collection / Processing Method	Shipping	Recipient Lab	Notes
At Time of Registration to A011202 Biobanking	N	FFPE Tissue Block	4	FFPE tissue blocks (9.2)	Ambient	ABWUSTL	1, 2, 3
At Time of Registration to A011202 Biobanking	N	Unstained Tissue Slides	1 x 5 um AND 15 x 10 um	Unstained Slides (9.3)	Ambient	ABWUSTL	1, 2, 3
At Time of Registration to A011202 Biobanking	Y	Whole blood (Streck BCT)	3 x 10 ml	Plasma (10.1)	Ambient	ABWUSTL	1, 2
At Time of Registration to A011202 Biobanking	Y	Whole blood (KEDTA / purple top)	2 x 10 ml	Whole Blood (10.2)	Ambient	ABWUSTL	1, 2
5-6 Years After Surgery	Y	Whole blood (Streck BCT)	3 x 10 ml	Plasma (10.1)	Ambient	ABWUSTL	1, 4
5-6 Years After Surgery	Y	Whole blood (KEDTA / purple top)	2 x 10 ml	Whole Blood (10.2)	Ambient	ABWUSTL	1, 4
8 Years After End of RT	Y	Whole blood (Streck BCT)	3 x 10 ml	Plasma (10.1)	Ambient	ABWUSTL	1
8 Years After End of RT	Y	Whole blood (KEDTA / purple top)	2 x 10 ml	Whole Blood (10.2)	Ambient	ABWUSTL	1

Notes:

1. Collection is optional for patients but requires all sites offer to patients to consent. Please see protocol-specific consent documents.
2. See protocol **section 5.7** for information on when registration to A011202 Biobanking is to occur. If samples are not collected at this time point, the later time points (5-6 years after surgery, 8 years after discontinuation of RT) should still be collected.
3. Up to four (4) FFPE tissue blocks should be submitted at time of registration to A011202 Biobanking. Unstained slides will be accepted as alternative to block submission. A set of 16 unstained slides (1 x 5 micron charged, 15 x 10 micron non-charged) from each block should be submitted. If fewer than 16 unstained slides can be submitted, please prioritize the 1 x 5 micron slide and submit as many of the 10 micron slides as possible. **Block submission is strongly preferred.** See additional details in **section 9.2.**
4. Blood should be collected during routine clinical visit, 5-6 years after surgery.

7. Biospecimen Collection Kits

7.1 Blood Specimens

7.1.1 To facilitate the proper collection and shipping of whole blood specimens, biospecimen collection kits and materials will be provided. The cost of the kit and shipping the kit to the site will be paid for. The institution is expected to pay for shipping the kit with the biospecimens back to the Alliance Biorepository at Washington University in St. Louis via priority overnight shipping**.**

7.1.2 Kits will be sent via FedEx® Ground at no additional cost to the participating institutions. Kits will not be sent via rush delivery service unless the participating institution provides their own FedEx® account number or alternate billing number for express service. The study will not cover the cost for rush delivery of kits.

7.1.3 Kits should be requested at least 10 working days in advance of the anticipated collection date. As many as 3 kits can be requested at one time. Since the collection materials (vacutainer tubes) have expiration dates, do not request kits more than 90 days prior to their anticipated use. All kits must be requested by using the BioMS system.

7.1.4 Distributed kits will have a minimum shelf life of 90 days; unless precluded by the stability of a particular component.

7.1.5 Kit contents and specific instructions for use of the kit are provided in the kit box. Please return any unused collection materials with the kit.

7.1.6 Once a kit is received, do not discard the outer cardboard overwrap. The kit, containing biospecimens, is to be shipped back in the same box.

7.1.7 Please return all components of the kit, regardless of whether they have been used or not. Kits and kit components are recycled when possible, minimizing the kit cost.

7.1.8 Well in advance of collecting biospecimens, inspect the biospecimen collection kit to ensure that all components are present and not expired, particularly if the kit has been on site for longer than 30 days.

7.1.9 Note that individual kit components that are expired, damaged, or missing cannot be replaced. If a biospecimen collection component (e.g. vacutainer collection tube) is missing, damaged, or expired, the institution may substitute a like-kind collection tube from their own supply. However, note that some kit components are highly specialized (e.g. Streck BCT) and probably are not available at the institution. If substitutions are not available at the site, please order a complete, new kit. Please note in your request that you are replacing an expired or damaged kit.

7.1.10 Please return all kits that have expired or missing components. Return the ENTIRE kit using the cheapest possible shipping method at your expense. DO NOT DISCARD kits that have missing or expired components. Recycling kits keeps the cost of kit materials to a minimum. Please note that all outgoing and incoming kits are tracked, and sites that have requested many more kits than they have returned will be charged for non-returned kits.

7.1.11 Note that protocol requirements are based on blood volumes, not tube sizes. If the protocol requires the collection of 8 ml of whole blood, generally a 10 ml tube is provided in the kit for convenience. If desirable or necessary to collect 8 ml in 3 x 3 ml tubes (for example), that is permissible.

7.1.12 Note that all tubes must be filled. Incompletely filled tubes provide insufficient sample to conduct the planned analysis and will result in a request to re-draw the sample if possible.

7.2 Tissue Specimens

7.2.1 There is no independent "kit" for submission of paraffin blocks or slides.

7.2.2 Blocks and slides should be packaged to avoid breakage using a padded envelope or, preferably, a small Styrofoam container.

7.3 Please see Section 11 – Biospecimen Shipping for specific instructions on shipping to ABWUSTL.

8. Biospecimen Labeling and Tracking

8.1 All research biospecimens (vacutainer tubes, tissue bags) MUST be labeled with the participant study number, patient initials (Last, First, Middle), the date and time (if applicable) of collection and specimen type.

8.2 Surgical pathology tissue blocks should not be labeled in any manner. The institutional surgical pathology number (e.g. "S16-1234") and the individual block identifier (e.g. "A3") should be readable on the block. If tissue sections are being submitted instead of the block, each tissue section slide should be labeled with the patient study number, institutional surgical pathology number, the block identifier, section thickness, and the serial section number. Provide **a de-identified copy of the surgical pathology report**, labeled with the patient study number and trial, corresponding to the blocks or slides submitted to ABWUSTL. Please ensure the institutional surgical pathology number (SPN) and block ID that match the SPN on the physical label on the tissue are maintained on the surgical pathology report. See **section 9** for additional details on tissue submission.

8.3 Label all containers and vials with an indelible, solvent-resistant marker when they are at ambient temperature.

8.4 Do not affix any labels to vials, slides or tubes. Label the collection containers directly with the marking pen.

8.5 All biospecimens that are collected and sent to the Alliance Biorepository must be **logged and tracked in BioMS**. The BioMS system is a web-based application that tracks the collection and shipping of biospecimens. Once individual biospecimens are logged and 'shipped' in the BioMS system, a packing manifest will be created by the system. This manifest must be printed out and must accompany all biospecimen shipments. To become familiar with the BioMS system and for further information about training, access, and use, please contact the BioMS Help desk at: 1-855-55-BIOMS or bioms@alliancenctn.org.

8.6 In the event that BioMS cannot be accessed, please complete a BioMS Specimen Log and Shipping Manifest form which can be found here- **http://tinyurl.com/alliance-bioms-contingency**.

9. Tissue Collection

9.1 Overview.

9.1.1 Please refer to protocol-specific instructions for procedures related to actual tissue procurement from individual participants. The method for research tissue procurement (needle core biopsy, sampling of surgically resected tumor) is dependent upon the disease site and the individual patient.

9.1.2 When procuring tissue biospecimens by any method, when possible, avoid tissue that is grossly necrotic, hemorrhagic, fatty, or fibrous. If in doubt, briefly (1 min or less) place the tissue segment in a sterile specimen cup containing physiologic (normal) saline to rinse the tissue. Necrotic, hemorrhagic, and fatty tissue will generally dissolve or float on the surface while tumor and parenchymal tissue will remain intact and sink to the bottom of the cup.

9.2 Diagnostic Pathology Fixed Tissue Blocks

9.2.1 FFPE tissue blocks are to be submitted from:

a. surgical tissue from residual breast tumor (one block). If no residual disease in the breast, tissue from the tumor bed should be submitted.

b. surgical tissue from a positive lymph node (one block)

c. diagnostic core biopsy of breast tumor prior to chemotherapy

d. any FFPE block or representative slides from pre-chemotherapy lymph node biopsy (core or FNA), if available and feasible.

9.2.2 Any clinical surgical pathology block that is submitted for research studies will not be exhausted or rendered unsuitable for future diagnostic use. Any clinical surgical pathology block that is submitted will be returned within ten working days of written request, when needed for clinical management or clinical trial enrollment for a specific patient. Otherwise, all blocks will be returned to the submitting institution when the trial and correlative science study end points have been met.

9.2.3 In the event that an institution will not release tissue blocks, the institution may instead submit unstained slides. Please refer to **section 9.3.**

9.3 Unstained Slides from Diagnostic Fixed Tissue Blocks

9.3.1 In cases where an institution is unwilling or unable to submit tissue blocks, a set of 16 unstained slides from each block with adequate tumor cellularity may be sent as an alternative. If fewer than 16 unstained slides from a block can be submitted, please submit as many as possible. Please follow the procedures below for submitting unstained slides. If your pathology department is unwilling or unable to follow these tissue-sectioning instructions, please consider submission of tissue blocks, which can be cut at the Biorepository and returned to your institution at a later date.

# of slides	Section thickness	Slide type	Purpose
1	5 micron	Charged	H&E stained slide
15	10 micron	Non-Charged	DNA, RNA

9.3.2 Serial, tissue sections should be cut fresh from the appropriate formalin fixed, paraffin embedded tissue block.

9.3.3 Cut sections at 5 micron or 10 micron thickness onto glass slides (charged or non-charged) as indicated above.

9.3.4 Ensure that each slide is labeled with the patient study number, the institutional surgical pathology number and block ID, section thickness (5 um or 10 um), and the slide serial section number (1, 2, 3, etc.).

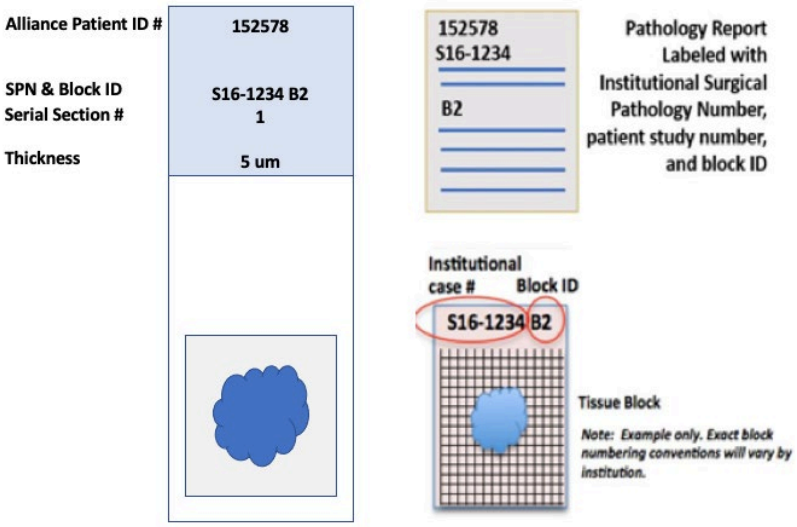
9.3.5 Do not label slides with adhesive labels. Write or print information on the textured surface of the slide with indelible, solvent-resistant ink.

9.3.6 No adhesives or other additives should be used in the water bath.

9.3.7 Mount only one tissue section per slide. Make certain sections are placed on the painted / textured side of the slide.

9.3.8 When placing the sections onto the slides, ensure that the tissue is placed on the bottom third of the slide. Ensure that each serial section from the block is placed in the same orientation on each slide.

9.3.9 See figure below for proper mounting and labeling.



9.3.10 Air dry slides for 12-24 hours prior to shipping. Do not oven dry slides.

9.3.11 Use slide mailers or a slide box to ship slides. Slides should not be touching each other. Ensure that slides from only one patient are placed in one slide mailer.

10. Blood Collection Methods

10.1 Plasma Nucleic Acid (Streck) Tube Processing

10.1.1 Collect 10 ml of blood into each of the Streck BCT tubes using standard venous phlebotomy. Invert tubes 10 times.

10.1.2 Store Streck tubes with whole blood at room temperature. Do not freeze or refrigerate the tubes. The tubes may be stored for up to 72 hours at ambient temperature before shipment. Ensure that the Streck tubes are shipped at ambient temperature to avoid freezing. **During warm weather months (i.e. June—August), a refrigerated pack (not frozen) should be included in the shipment to maintain ambient temperature. When shipping during other months of the year, a room temperature pack should be included in the shipment.**

10.2 Whole blood- EDTA tubes (no processing)

10.2.1 Collect whole blood by standard venous phlebotomy technique into each of the EDTA tubes. Invert tubes 10 times.

10.2.2 Store EDTA tubes with whole blood at 4 degrees Celsius (i.e. refrigerated) until shipping. Do not freeze the tubes. **Blood should be collected Monday—Thursday only. Due to required processing, the tubes MUST be received at the Biorepository within 24 hours of collection.** Ensure that the EDTA tubes are shipped at ambient temperature to avoid freezing. **During warm weather months (i.e. June—August), a refrigerated pack (not frozen) should be included in the shipment to maintain ambient temperature. When shipping during other months of the year, a room temperature pack should be included in the shipment.**

11. Biospecimen Shipping

11.1 Overview

11.1.1 Please see the Directions for Use (DFU) document that is included in each kit for specific directions on how to package and ship biospecimens.

11.1.2 Place the original, completed copy of the BioMS packing manifest in the shipment. If sending tissue, include a copy of the de-identified surgical pathology report, labeled with the patient study number. Do not send specimens without a completed BioMS Packing Manifest or substitute "BioMS Downtime Form." Biospecimens cannot be accepted without this completed form.

11.1.3 All biospecimens should be shipped within the time frames specified in **sections 10.1 and 10.2**. If collected biospecimens cannot be shipped within the specified time frame, (e.g. Friday – Saturday or Holiday collections), please contact the Alliance Biorepository Program Manager: 1-314-747-4402 for further instructions, at least 24 hours prior to anticipated collection.

11.1.4 **Do not ship on Friday, Saturday, Sunday or the day before a nationally recognized holiday.**

11.2 Shipping to ABWUSTL

11.2.1 Ship container for PRIORITY OVERNIGHT DELIVERY according to IATA guidelines and standard institutional policies and using the preferred vendor.

Ship to:
Alliance Biorepository
c/o Siteman Cancer Center Tissue Procurement Core
Washington Univ. School of Medicine
425 S. Euclid Ave.
Room 5120
St. Louis, MO
63110-1005
Phone: 314-454-7615

12. ABWUSTL Biospecimen Receipt and Quality Assurance Measures

12.1 Upon receipt, all biospecimens will be accessioned into the TPC informatics system, OpenSpecimen.

12.2 All biospecimens will be logged, associated, and tracked by the unique patient biopsy control number.

12.3 Each individual biospecimen will receive and be physically labeled with a unique biospecimen identifier, associated with the biopsy control number in the TPC informatics system.

12.4 Upon receipt, all physical biospecimens received will be reconciled with what is recorded on the BioMS packing manifest. Any discrepancies noted will be communicated to the Program Manager who will contact the submitting site for reconciliation.

12.5 Upon receipt, any biospecimen received that is not in appropriate physical condition (broken vials, frozen samples that are thawed, ambient samples that are frozen) will be reported to the Program Manager, who will contact the submitting site for reconciliation.

12.6 Frozen aliquoted biofluids will be stored under liquid nitrogen vapor.

12.7 EDTA tubes will undergo PBMC processing and cryopreservation. Resultant aliquots will be stored under liquid nitrogen vapor.

12.8 Plasma and buffy coat will be derived from Streck BCT tubes and stored under liquid nitrogen vapor.

12.9 All biospecimens will remain in storage until additional processing or review is requested in writing by the appropriate protocol PI.

13. Document History

Version	Description and Justification of Change	Author	Effective Date
1.4	Added info about kit shelf life and kit shipping. Clarified instructions regarding pathology report submission. Removed reference to blank waybill being provided	KAL	4/5/2023
1.3	Included provisions for shipping during warm weather months	PAA	07/30/2021
1.2	Updated biospecimen collection schedule	PAA	05/27/2021
1.1	Updated slide labeling diagram Corrected BioMS email address Fixed minor typos and grammatical errors	PAA	02/17/2021
1.0	New	PAA	01/15/2020