CORRELATIVE SCIENCE PROCEDURE MANUAL

1. Purpose

This document describes the procedures required for the collection, shipping, and processing of biospecimens that are being submitted for patients enrolled or registered on A211801. This document also describes the procedures that will be followed subsequent to the receipt of biospecimens by the Alliance Biorepository at Mayo Clinic (ABMAYO) 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 A211801 biospecimen collection, processing, and submission, including staff at satellite institutions.

2. Scope

This document applies to all biospecimens collected specifically for the A211801 clinical trial study. Please refer to the trial protocol-specific language for additional details regarding eligibility, participant enrollment, and data submission. **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
ABMAYO	Alliance Biorepository at Mayo Clinic
FFPE	Formalin fixed, paraffin embedded
IHC	Immunohistochemistry

4. Contact Information

4.1 For questions and problems related to protocol administration, eligibility, patient registration, and data submission, relevant contact information is in the A211801 Appendix for U.S. Investigators.

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:

ABMAYO

- Paraffin-embedded tissue samples:
  Amanda Sand
  Phone: 1-507-284-3559
  Email: sand.amanda@mayo.edu
  Alliance Inbox: NCCTGPATHOLOGY@mayo.edu
- Blood samples:
  Katie Halverson
  Phone: 1-507-293-7147
  Email: Halverson.katie@mayo.edu

5. Site Preparation

5.1 Please refer to A211801 Appendix for U.S. Investigators document for any specific requirements related to patient enrollment, data submission, registration, and regulatory compliance.

5.2 Please ensure that you have the appropriate log-in 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 Mayo Clinic. 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 whole blood specimens, 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 listed in the table below. Please refer to individual collection kit instructions, biospecimen collection and processing methods, and specific shipping procedures that are detailed in this manual.

Time Point	Kit (Y/N)	Biospecimen	Quantity	Collection / Processing Method	Shipping	Recipient	Notes
Mandatory for <u>all</u> patients registered to A211801							
Baseline	Y	Whole blood for serum	4 x 500 ul aliquots	Frozen serum (10.1)	Dry Ice	ABMAYO-BAP Freezer (11.3)	1
6 months after last dose of IP	N	Breast or BSO tissue	1 block **<u>OR</u>** 30 unstained slides (3-4 micron)	Fixed tissue block + H&E stained slide (9.2) **<u>OR</u>** Unstained fixed tissue slides + H&E stained slide (9.3)	Ambient	ABMAYO-FFPE (11.2)	1, 2
Tumor Diagnosis	N	FFPE Tissue	1 block **<u>OR</u>** 30 unstained slides (3-4 micron)	Fixed tissue block + H&E stained slide (9.2) **<u>OR</u>** Unstained fixed tissue slides + H&E stained slide (9.3)	Ambient	ABMAYO-FFPE (11.2)	1, 3
For patients consented to the optional sample collection study							
Baseline	Y	Whole blood (Streck BCT tube)	2 x 10 ml	Plasma for ctDNA and germline DNA (10.2)	Ambient	ABMAYO-BAP Freezer (11.3)	4
Baseline	Y	Whole blood for plasma	4 x 500 ul aliquots	Frozen plasma (10.3)	Dry Ice	ABMAYO-BAP Freezer (11.3)	4
Yearly visit (12, 24, 36, 48, 60 m)	Y	Whole blood (Streck BCT tube)	2 x 10 ml	Plasma for ctDNA and germline DNA (10.2)	Ambient	ABMAYO-BAP Freezer (11.3)	4
Yearly visit (12, 24, 36, 48, 60 m)	Y	Whole blood for serum	4 x 500 ul aliquots	Frozen serum (10.1)	Dry Ice	ABMAYO-BAP Freezer (11.3)	4
Yearly visit (12, 24, 36, 48, 60 m)	Y	Whole blood for plasma	4 x 500 ul aliquots	Frozen plasma (10.3)	Dry Ice	ABMAYO-BAP Freezer (11.3)	4
12 months after last dose of IP	Y	Whole blood for serum	4 x 500 ul aliquots	Frozen serum (10.1)	Dry Ice	ABMAYO-BAP Freezer (11.3)	4
12 months after last dose of IP	Y	Whole blood for plasma	4 x 500 ul aliquots	Frozen plasma (10.3)	Dry Ice	ABMAYO-BAP Freezer (11.3)	4

Tumor Diagnosis	Y	Whole blood (Streck BCT tube)	2 x 10 ml	Plasma for ctDNA and germline DNA (10.2)	Ambient	ABMAYO-BAP Freezer (11.3)	4
Tumor Diagnosis	Y	Whole blood for serum	4 x 500 ul aliquots	Frozen serum (10.1)	Dry Ice	ABMAYO-BAP Freezer (11.3)	4
Tumor Diagnosis	Y	Whole blood for plasma	4 x 500 ul aliquots	Frozen plasma (10.3)	Dry Ice	ABMAYO-BAP Freezer (11.3)	4

Notes:

1. Collection is mandatory for all patients registered to A211801.
2. A sample of breast tissue (if patients undergo risk reducing surgery), or bilateral salpingo-oophroectomy sample (if patients undergo prophylactic surgery) is requested, if available. Submission of a fixed tissue block is strongly preferred. If a block is not available, a set of 30 unstained slides (3-4 micron) is requested. If unable to submit 30 unstained slides, please submit as many as possible up to 30. **A H&E stained slide should accompany the block or unstained slides. If submitting unstained slides, the H&E stained slide should be from the same block from which the unstained slides were cut.**
3. A tumor tissue sample and corresponding histological report is required for any solid tumor, If applicable. Submission of a fixed tissue block is strongly preferred. If a block is not available, a set of 30 unstained slides (3-4 micron) is requested. If unable to submit 30 unstained slides, please submit as many as possible up to 30. **A H&E stained slide should accompany the block or unstained slides. If submitting unstained slides, the H&E stained slide should be from the same block from which the unstained slides were cut.**
4. Collection is optional for patients, but all sites are required to offer to patients to consent. Please see the U.S.-specific consent documents.

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 ABMAYO via priority overnight shipping. **NOTE: 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.**

7.1.2 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.

7.1.3 Kit contents and specific instructions for use of the kit are provided in the kit box.

7.1.4 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 onsite for longer than 30 days.

7.1.5 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.

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

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.

7.2.3 During warm weather months, paraffin blocks and slides should be shipped in an insulated container that contains a refrigerant pack, to avoid heat > 25 degrees C (77 degrees F) that may melt paraffin and damage the tissue specimens.

7.2.4 Please see **Section 11 – Biospecimen Shipping** for specific instructions on shipping to ABMAYO.

8. Biospecimen Labeling and Tracking

8.1 All research biospecimens, **with exception of** surgical pathology blocks and tissue slides, MUST be labeled with the Alliance study number (A211801), Alliance patient ID number, patient initials (Last, First, Middle), the date and time (if applicable) of collection and specimen type (e.g. serum, plasma). Specimen labels must match BioMS records.

8.2 Surgical pathology tissue recuts should be labeled according to institutional standards, including the surgical pathology number (e.g. "S16-1234") and the individual block identifier (e.g. "B2"). Provide **a de-identified copy of the surgical pathology report**, labeled with the patient study number, corresponding to the blocks or slides submitted. Usually, this is generated by obscuring all PHI (names and dates) with white-out or a black magic marker, labeling each page of the report with the Alliance patient ID, and photocopying the report. However, please make sure to **maintain the pathology accession numbers** so the submitted slides can be matched directly to the pathology report. **A copy of the de-identified pathology report is required for each and every 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 ABMAYO must be **logged and tracked in BioMS** under A211801. Do not send samples to the Biorepository in which the patient has not been registered onto the study. 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 A representative diagnostic block from breast tissue (if patients undergo risk reducing surgery), or bilateral salpingo-oophorectomy sample (if patients undergo prophylactic surgery) is requested from all patients registered to A211801. An additional block is requested from any solid tumor at time of tumor diagnosis, if applicable. **A H&E stained slide prepared from the block should also be submitted for tissue QA.**

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 Include a copy of a de-identified pathology report, labeled only with the patient study number with all block submissions.

9.3 Unstained Tissue Slides

9.3.1 In cases where an institution is unwilling or unable to submit the requested tissue blocks, sets of thirty (30) unstained tumor tissue slides can be submitted as an alternative. **An H&E stained slide should accompany unstained slides. The H&E stained slide should be from the same block from which the unstained slides were cut.** If fewer than 30 unstained slides can be submitted, please submit as many as possible (up to 30 slides). Please follow the procedures below for submitting unstained tissue slides. If your pathology department is unwilling or unable to follow these tissue-sectioning instructions, please consider submission of a tissue block, which can be cut at the Biorepository and returned to your institution at a later date.

# of slides	Section thickness	Slide type	Purpose
30	3-4 microns	Positively charged	IHC

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 3-4 micron thickness as indicated onto positively charged slides.

9.3.4 Ensure that each slide is labeled with the Alliance study number (A211801), Alliance patient ID number, surgical pathology number (SPN) and institutional block ID, slide serial section number (1, 2, 3, etc.), and thickness (3-4 microns). Please make sure to **maintain the pathology accession numbers** so the submitted slides can be matched directly to the pathology report.

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.

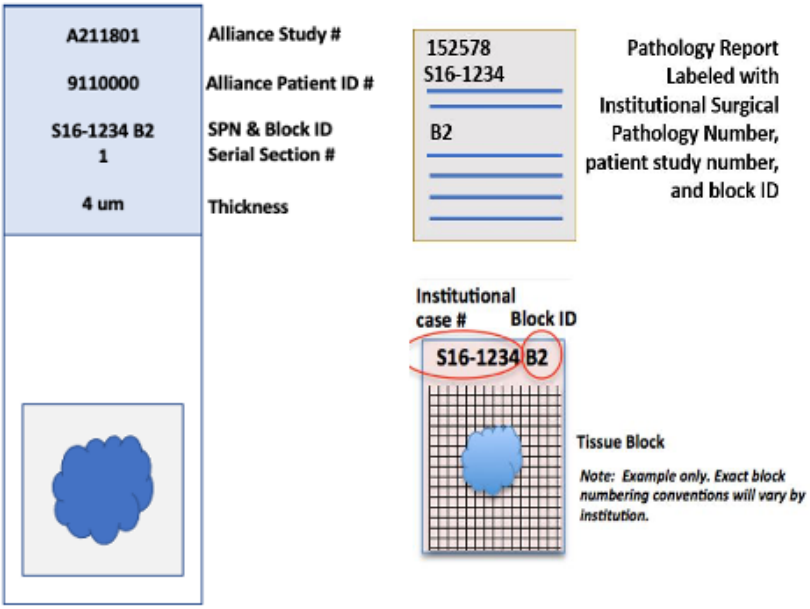
9.3.7 Mount only one tissue section per slide. Make certain sections are placed on the painted / textured side of the slide.

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 unstained 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 Serum Processing

10.1.1 Collect whole blood by standard venous phlebotomy technique into the red top (plain glass with clot activator) tube. Do not collect whole blood into a "tiger top" / "SST" / "gel tube." Invert tube 10 times

10.1.2 Allow blood to clot for 30 minutes.

10.1.3 Label 4 cryovials as instructed in **section 8**. Make certain each vial is labeled completely and identically.

10.1.4 Spin blood in vacutainer tube at 4 degrees in a clinical centrifuge using standard programming for serum separation. Usually this is 1200 xG (actual speed will depend upon the centrifuge) for 10 minutes.

10.1.5 Carefully remove 2 ml of serum (without touching the clot layer) and aliquot 500 ul into each of 4 labeled cryovials.

10.1.6 Freeze serum containing cryovials on dry ice or a -70 to -90 degree Celsius ultralow freezer. Store at -70 to -90 degrees until ready for shipment on dry ice.

10.2 Whole blood for Plasma for ctDNA and germline DNA (Streck Tube- no processing)

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

10.2.2 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. If blood must be collected on Friday, it can be shipped on Friday for Saturday delivery. Ensure that the Streck tubes are shipped at ambient temperature to avoid freezing.

10.3 Plasma Processing

10.3.1 Collect whole blood by standard venous phlebotomy technique into the purple top (EDTA) tube. Invert tube 10 times.

10.3.2 Within 30 minutes of collection, spin the vacutainer tube at room temperature in a clinical centrifuge at 1200 xG for 15 minutes.

10.3.3 Carefully remove the plasma layer (~3 ml), without touching the white, buffy coat layer, and transfer to a new 15 ml conical centrifuge tube.

10.3.4 Spin the centrifuge tube containing plasma at room temperature in a clinical centrifuge at 1200 xG for 15 minutes.

10.3.5 Label 4 cryovials as instructed in **section 8.** Make certain each vial is labeled completely and identically.

10.3.6 Carefully remove 2 ml of plasma (without touching the pellet) and aliquot 500 ul into each of the labeled cryovials.

10.3.7 Freeze plasma containing cryovials on dry ice or a -70 to -90 degree Celsius ultralow freezer. Store at -70 to -90 degrees C until ready for shipment on dry ice.

11. Biospecimen Shipping

11.1 Overview

11.1.1 Please see the instruction 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 de-identified copy of the surgical pathology report labeled with the Alliance patient ID 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 collected on the study should be shipped within the time frames specified in **sections 9 and 10**. If patient or physician schedules do not allow biospecimens to be shipped within the time frame specified, please contact appropriate personnel listed in **section 4.3** for further instructions, at least 24 hours prior to anticipated collection.

11.2 Shipping to ABMAYO- FFPE Tissue

11.2.1 Ship container for PRIORITY OVERNIGHT DELIVERY according to IATA guidelines and standard institutional policies and using the preferred vendor. **Ship specimens to ABMAYO on Monday through Friday only. Do not ship on Saturdays or on the day before a national holiday.**

Ship to:

Alliance Biorepository at Mayo Clinic- FFPE Tissue
Attn: PC Office (Study A071801)
RO-FF-03-24-CC/NW Clinic
200 First Street Southwest
Rochester, MN 55905
Phone: 507-266-0724

11.3 Shipping to ABMAYO- BAP Freezer (for Blood Samples)

11.3.1 Ship container for PRIORITY OVERNIGHT DELIVERY according to IATA guidelines and standard institutional policies and using the preferred vendor. **Ship specimens to ABMAYO on Monday through Friday only. Do not ship on Saturdays or on the day before a national holiday.**

Ship to:

Alliance Biorepository at Mayo Clinic- BAP Freezer
ST-SL-16
150 Third Street SW
Rochester, MN 55902
Phone: 507-538-0602

12. ABMAYO Biospecimen Receipt and Quality Assurance Measures

12.1 All biospecimens sent to ABMAYO will be accessioned into the informatics system BioMS, at ABMAYO Research Laboratory Information Management System (RLIMS) which interfaces with BioMS.

12.2 All biospecimens received at the ABMAYO will be logged, associated, and tracked by the patient study ID number.

12.3 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 submitting site for reconciliation.

12.4 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.

12.5 All biospecimens submitted to ABMAYO will remain in storage until exhausted. In the event the patient withdraws consent, the specimens will be disposed/returned according to Alliance Policy and Procedure Chapter 11.

13. Document History

Version	Description and Justification of Change	Author	Effective Date
1.2	Updated contact phone number for Katie Halverson	PAA	02/22/2022
1.1	Updated contact info for Mayo	PAA	08/12/2021
1.0	New	PAA	04/09/2021