# CORRELATIVE SCIENCE PROCEDURE MANUAL

## 1. Purpose

This document describes the procedures required for the collection, shipping, and processing of biospecimens from all patients enrolled or registered on A092001. 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 A092001 biospecimen collection, processing, and submission; including staff at satellite institutions.

## 2. Scope

This document applies to all biospecimens collected specifically for A092001 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 |
| H&E | Hematoxylin and Eosin |
| WSI | Whole Slide Image |

## 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 A092001 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 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.

**5.4** Identify a reliable source of dry ice for freezing and shipping biospecimens and a -70 to -90 degree Celsius freezer ("ultralow") in which frozen biospecimens may be stored prior to shipment.

## 6. Collection Schema

The following biospecimens are to be collected at each of the time points below. 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 | Notes |
|---|---|---|---|---|---|---|
| **Mandatory for all patients registered to A092001** | | | | | | |
| Baseline ≤ 28 days after registration | Y | Whole blood for serum | 3 x 1 ml aliquots | Frozen serum (10.1) | Dry Ice | 1 |
| Baseline ≤ 28 days after registration | N | Local diagnostic slides for central pathology review | Case dependent | Local Diagnostic Slides (9.2) | Ambient | 1, 2, 3 |
| Baseline ≤ 28 days after registration | N | Scanned slide images | Case dependent | Scanned slide images (9.3) | N/A | 1, 2, 3 |
| | | | | | | |
| Cycle 2 Day 1 | Y | Whole blood for serum | 3 x 1 ml aliquots | Frozen serum (10.1) | Dry Ice | 1 |
| | | | | | | |
| Cycle 3 Day 1 | Y | Whole blood for serum | 3 x 1 ml aliquots | Frozen serum (10.1) | Dry Ice | 1 |
| | | | | | | |
| Cycle 4 Day 1 | Y | Whole blood for serum | 3 x 1 ml aliquots | Frozen serum (10.1) | Dry Ice | 1 |
| **For patients registered to A092001 biobanking, submit the following:** | | | | | | |
| Baseline ≤ 28 days after registration | Y | Whole blood (Streck BCT) | 2 x 10 ml | Plasma for cfDNA (10.2) | Ambient | 4 |
| Baseline ≤ 28 days after registration | Y | Whole blood (EDTA) | 2 x 10 ml | Whole blood- EDTA tubes (10.3) | Ambient | 4, 5 |
| Baseline ≤ 28 days after registration | Y | Whole blood for plasma | 6 x 1 ml aliquots | Frozen plasma (10.4) | Dry Ice | 4 |
| Baseline ≤ 28 days after registration | N | Fixed tumor tissue block | 1 | Fixed tissue blocks (9.4) | Ambient | 4 |
| | | | | | | |
| Cycle 2 Day 1 | Y | Whole blood (EDTA) | 2 x 10 ml | Whole blood- EDTA tubes (10.3) | Ambient | 4, 5 |
| | | | | | | |
| Cycle 3 Day 1 | Y | Whole blood (Streck BCT) | 2 x 10 ml | Plasma for cfDNA (10.2) | Ambient | 4 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Cycle 3 Day 1 | Y | Whole blood (EDTA) | 2 x 10 ml | Whole blood- EDTA tubes (10.3) | Ambient | 4, 5 |
| Cycle 3 Day 1 | Y | Whole blood for plasma | 6 x 1 ml aliquots | Frozen plasma (10.4) | Dry Ice | 4 |
| | | | | | | |
| Cycle 4 Day 1 | Y | Whole blood (Streck BCT) | 2 x 10 ml | Plasma for cfDNA (10.2) | Ambient | 4 |
| Cycle 4 Day 1 | Y | Whole blood (EDTA) | 2 x 10 ml | Whole blood- EDTA tubes (10.3) | Ambient | 4, 5 |
| Cycle 4 Day 1 | Y | Whole blood for plasma | 6 x 1 ml aliquots | Frozen plasma (10.4) | Dry Ice | 4 |
| | | | | | | |
| At Surgery | N | Fixed tumor tissue block | 1 | Fixed tissue blocks (9.4) | Ambient | 4 |

**Notes:**

1. Collection is mandatory for all patients registered to A092001.
2. All slides including a hematoxylin and eosin (H&E) stained slide and any performed immunostains from diagnostic tumor tissue should be submitted for central pathology review. If diagnostic slides cannot be submitted, whole slide image scan files of the stained slides in .SVS file format may be uploaded digitally to the Biorepository.
3. If submitted tissue is not sufficient for review, additional tissue in the form of fixed tissue blocks or unstained slides will be requested. The number of unstained slides requested will be determined by the particular case and may vary from patient to patient.
4. All participating institutions must ask patients for their consent to participate in the banking of their specimens for future correlative studies, although patient participation is optional.
5. Whole blood (EDTA) 2 x 10 ml for PBMC isolation and cryopreservation at the Biorepository.

## 7. Biospecimen Collection Kits

**7.1** To facilitate the proper collection and shipping of all biospecimens, 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.

**7.2** Kits should be requested at least 10 working days in advance of the anticipated collection date. As many as 2 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.

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

**7.4** 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.

**7.5** 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.

**7.6** 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.

**7.7** Note that individual kit components that are expired, damaged, or missing cannot be replaced. The remedy is to order a complete, new kit. Please note in your request that you are replacing an expired or damaged kit.

**7.8** 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 out-going and incoming kits are tracked, and sites that have requested many more kits than they have returned will be charged for non-returned kits.

**7.9** 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 while some kit components are generic (i.e. EDTA tubes), others are highly specialized (i.e. Streck BCT) and probably are not available at the institution.

**7.10** 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.

**7.11** Because paraffin blocks or slides cut from such blocks may be requisitioned and received from the surgical pathology department at a different time than the day of procurement for other biospecimens, paraffin blocks or cut slides may be sent independently of other biospecimens using the following guidelines:

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

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

**7.11.3** 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.

**7.11.4** Blocks and slides may be shipped for standard overnight delivery according to institutional policies and using the preferred vendor.

**7.12** 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, cryovials, and 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 (i.e. plasma, serum).

**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. Provide **a de-identified copy of the surgical pathology report**, labeled with the patient study number, corresponding to the blocks or slides submitted. Please ensure the institutional surgical pathology number and block ID are maintained on the surgical pathology report. See **section 9** for additional details.

**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.

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, or tumor 'debulking') 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 Local Diagnostic Stained Tissue Slides for Central Pathology Review

**9.2.1** Submission of all local diagnostic slides from tumor tissue is required for retrospective central pathology review to confirm local diagnosis. Sites should submit a H&E stained slide and any others produced for histopathologic diagnosis to the Biorepository.

**9.2.2** Any stained slide submitted must be labeled with Alliance participant study number, institutional surgical pathology number, the block identifier, and the stain type. Provide **a de-identified copy of the surgical pathology report(s)**, labeled with the patient study number, corresponding to the slides submitted. Please ensure the institutional surgical pathology number and block ID are maintained on the surgical pathology report(s).

**9.2.3** Submitted slides will be scanned at the Alliance Biorepository and slides will be returned to the site within 14 days.

**9.2.4** De-identified, whole slide image scans will be stored, distributed, and used by approved investigators for future correlative studies involving histopathology image analysis.

**9.2.5** In the event that an institution will not release diagnostic stained tissue slides, the institution may instead submit scanned whole slide images (see **section 9.3**).

### 9.3 Scanned Slide Images

**9.3.1** As an alternate to submitting stained tissue slides, sites may instead submit a whole slide image scan (WSI) at 40X magnification of a H&E stained slide and any others produced for histopathologic diagnosis, in .SVS file format.

**9.3.2** Please contact the BioMS Helpdesk via email (bioms@alliancenctn.org) for further instructions on appropriate image labeling, file naming conventions, and how to submit digital pathology image files.

**9.3.3** Scanned images should not contain PHI (patient name, date of birth).

**9.3.4** De-identified, whole slide image scans will be stored, distributed, and used by approved investigators for future correlative studies involving histopathology image analysis.

### 9.4 Diagnostic Pathology Fixed Tissue Blocks.

**9.4.1** This protocol requests submission of one representative, diagnostic pathology, formalin fixed paraffin embedded block at time of patient registration and an additional formalin fixed paraffin embedded block at time of surgery.

**9.4.2** 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.

## 10. Blood Collection Methods

### 10.1 Serum Processing

**10.1.1** Using an indelible, solvent resistant marker, label cryovials as indicated in **section 8.**

**10.1.2** If using a non-refrigerated centrifuge, pre-chill the rotor or swinging buckets for at least 1 hour prior to sample processing in a refrigerator set at 2 to 8 degrees. Do not place in the freezer.

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

**10.1.4** Allow blood to clot for 30 min at room temperature with tube in upright position.

**10.1.5** Spin blood in vacutainer tube using pre-chilled rotors / swinging buckets in non-refrigerated centrifuge or at 4 degrees in a refrigerated clinical centrifuge at 1700 x G for 10 min.

**10.1.6** Carefully remove serum (without touching the clot layer) and aliquot 1 ml into each of the labeled cryovials.

**10.1.7** Freeze serum containing cryovials upright 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. If -70 to -90 degree C ultralow freezer is not available, serum may be stored in a -20 degree C freezer. The blood samples must be processed to serum and placed in freezer within 1 hour of collection.

### 10.2 Plasma Nucleic Acid (Streck) Tube 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. 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.**

### 10.3 Whole blood (EDTA- no processing)

**10.3.1** Collect 10 ml of whole blood by standard venous phlebotomy technique into each of the 2 EDTA tubes. Invert tubes 10 times.

**10.3.2** 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.**

### 10.4 Plasma Processing

**10.4.1** Collect 10 ml of whole blood by standard venous phlebotomy technique into each of the 2 purple top (EDTA) tubes. Invert tubes 10 times.

**10.4.2** Within 30 minutes of collection, spin the vacutainer tubes at room temperature in a clinical centrifuge at 2500 xG for 15 minutes.

**10.4.3** Carefully remove the plasma layer (~3 ml from each 10 ml EDTA tube), without touching the white, buffy coat layers, and transfer to new, 15 ml conical centrifuge tubes.

**10.4.4** Spin the centrifuge tubes containing plasma at room temperature in a clinical centrifuge at 2500 xG for 15 minutes.

**10.4.5** Label 3 cryovials for each 10 ml of whole blood collected, as instructed in **section 8**. Make certain each vial is labeled completely and identically.

**10.4.6** Carefully remove plasma without touching the pellet and divide into 1 ml labeled cryovials. Each 10 ml EDTA tube should yield 3 x 1 ml aliquots of plasma.

**10.4.7** 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 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. 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 frame specified in **sections 9** and **10** above. 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 or alliance@email.wustl.edu 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.**

**11.1.5** Ship container for PRIORITY OVERNIGHT DELIVERY according to IATA guidelines and standard institutional policies and using the preferred vendor. A blank FedEx Air Bill is provided with the kit for convenience.

Ship to:

**Alliance Biorepository at Washington University in St. Louis**
**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. 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** Aliquoted biofluids will be stored under liquid nitrogen vapor.

**12.7** 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 |
|---|---|---|---|
| 1.1 | Updated timepoint name to match protocol | PAA | 07/29/2021 |
| 1.0 | New | PAA | 06/24/2021 |