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 A091802. 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) or the Tissue Qualification Laboratory at MD Anderson, 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 A091802 biospecimen collection, processing, and submission; including staff at satellite institutions.

2. Scope

This document applies to all biospecimens collected specifically for A091802 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: alliance@email.wustl.edu or 1-314-747-4402.

5. Site Preparation

5.1 Please refer to A091802 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 and to the Tissue Qualification Laboratory at MD Anderson. 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.

5.4 Please confirm that your institutional pathology department will be willing to submit tissue section slides from a diagnostic, archival tissue block at the required time point designated in this document and in the trial protocol. If adequate archival tissue is not available, a new biopsy will be required. An institution whose pathology department is unwilling to comply with these requirements should not enroll patients to this study.

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
Mandatory for all patients registered to A091802							
≤ 14 days of pre-registration	N	Unstained tumor tissue slides	5	Fixed tissue slides for PD-L1 (9.2)	Ambient	MD Anderson	1
Research biopsy for patients with inadequate archival tissue							
≤ 14 days of pre-registration	N	Unstained tumor tissue slides	5	Research biopsy (9.3)	Ambient	MD Anderson	1
A091802 Biobanking							
Baseline ≤ 28 days of registration	N	Fixed tissue block	1	Fixed tissue block (9.4)	Ambient	ABWUSTL	2,3
Baseline ≤ 28 days of registration	N	Unstained tumor tissue slides	15	Fixed tissue slides (9.5)	Ambient	ABWUSTL	2,3

Baseline ≤ 28 days of registration	Y	Whole blood (EDTA tubes)	3 x 10 ml	Whole blood- EDTA (10.1)	Ambient	ABWUSTL	2
Baseline ≤ 28 days of registration	Y	Whole blood (Streck BCT tubes)	2 x 8 ml	Plasma for cfDNA (10.2)	Ambient	ABWUSTL	2
Cycle 4 Day 1	Y	Whole blood (EDTA tubes)	3 x 10 ml	Whole blood- EDTA (10.1)	Ambient	ABWUSTL	2
Cycle 4 Day 1	Y	Whole blood (Streck BCT tubes)	2 x 8 ml	Plasma for cfDNA (10.2)	Ambient	ABWUSTL	2
Cycle 7 Day 1	Y	Whole blood (EDTA tubes)	3 x 10 ml	Whole blood- EDTA (10.1)	Ambient	ABWUSTL	2
Cycle 7 Day 1	Y	Whole blood (Streck BCT tubes)	2 x 8 ml	Plasma for cfDNA (10.2)	Ambient	ABWUSTL	2
At Progression	Y	Whole blood (EDTA tubes)	3 x 10 ml	Whole blood- EDTA (10.1)	Ambient	ABWUSTL	2
At Progression	Y	Whole blood (Streck BCT tubes)	2 x 8 ml	Plasma for cfDNA (10.2)	Ambient	ABWUSTL	2
Completion of study treatment	Y	Whole blood (EDTA tubes)	3 x 10 ml	Whole blood- EDTA (10.1)	Ambient	ABWUSTL	2
Completion of study treatment	Y	Whole blood (Streck BCT tubes)	2 x 8 ml	Plasma for cfDNA (10.2)	Ambient	ABWUSTL	2

Notes:

1. Five (5) unstained tumor tissue slides (5 micron thickness, charged) must be submitted for all patients registered to A091802. If archival tissue is not available or sufficient for submission, a new biopsy will be required. Slides will be submitted directly to the Tissue Qualification Laboratory at MD Anderson. For additional details, please refer to **sections 9.2** and **9.3**. Please see study funding sheet regarding site reimbursement for this research biopsy.
2. Collection is optional for patients but requires all sites offer to patients to consent. Please see protocol-specific consent documents.

3. A paraffin tissue block from the skin or metastatic site should be submitted for patients opting in to A091802 biobanking. If a site cannot send a block, ten (10) unstained slides (10 micron thickness, uncharged) **AND** five (5) unstained slides (5 micron thickness, charged) from such a block should be submitted. If archival tissue is not available and patient has undergone new biopsy to fulfill requirements for mandatory tissue submission for PD-L1 analysis, the re-embedded core biopsy block should be submitted for biobank for consented patients. Please see study funding sheet regarding site reimbursement for this research biopsy.

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 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.

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

7.1.4 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.

7.1.5 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.

7.1.6 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.

7.1.7 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).

7.1.8 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 in coming kits are tracked, and sites that have requested many more kits than they have returned will be charged for non-returned kits.

7.1.9 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 (e.g. Streck BCT) and probably are not available at the institution.

7.1.10 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.

7.2 Tissue Specimens

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

7.2.2 Paraffin 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 block and unstained 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 ABWUSTL and MD Anderson.

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), and the date and time (if applicable) of collection.

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 and the block identifier. Provide **a de-identified copy of the surgical pathology report**, labeled only with the patient study number and institutional surgical pathology number, corresponding to the blocks or slides submitted. A copy of the "**A091802 Central PD-L1 Testing Submission Form" should be submitted with unstained slides submitted to the Tissue Qualification Laboratory at MD Anderson.** See **section 9.2** 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 or to MD Anderson 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 Unstained Slides from Diagnostic Fixed Tissue Block for PD-L1 Analysis

9.2.1 Five (5) unstained sections from a diagnostic tissue block from skin or other metastatic site are required for PD-L1 analysis. Please follow the procedures below for submitting unstained slides. For this mandatory submission, please download and complete the "**A091802 Central PD-L1 Testing Submission Form"** from the Alliance or CTSU websites. **This completed result form is REQUIRED to be submitted for PD-L1 analysis.** Sites should expect to receive the results within **5 days** after receipt of adequate tissue slides and appropriate paperwork.

# of slides	Section thickness	Slide type	Purpose
5	5 microns	Charged	PD-L1 analysis

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

9.2.3 Cut sections at 5 micron thickness as indicated onto charged slides.

9.2.4 Ensure that each slide is labeled with the patient study number, the institutional Surgical Pathology number and block ID, and the slide serial section number (1, 2, 3, etc.).

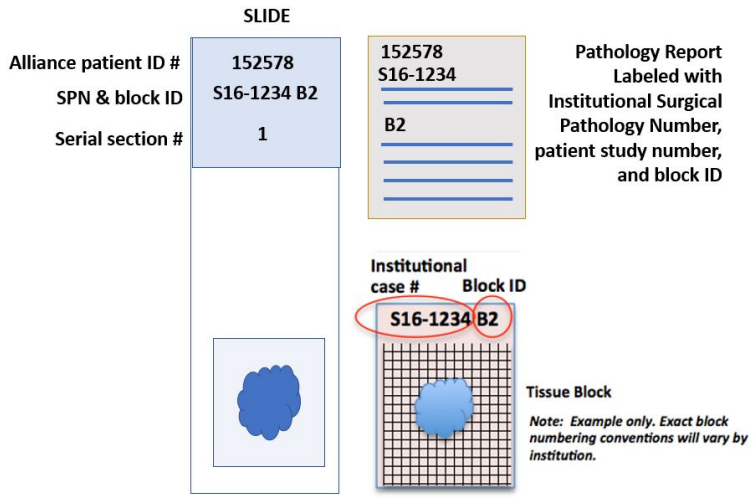
9.2.5 Do not label slides with adhesive labels. Write or print information on the textured surface of the slide with indelible, solvent-resistant ink.

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

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

9.2.8 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.

9.2.9 See figure below for proper mounting and labeling.



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

9.2.11 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.

9.2.12 Include a copy of a de-identified pathology report, labeled only with the patient study number and institutional surgical pathology number with all slide submissions.

9.3 Unstained Slides from Research Biopsy for PD-L1 Analysis

9.3.1 In cases where archival diagnostic tissue is not available or not sufficient for submission, a research biopsy should be performed to collect tissue. Please follow institutional procedures to ensure research biopsy tissues are obtained from the safest / most accessible site and preferably not a sclerotic bone lesion. A core biopsy is required; FNAs are not accepted.

9.3.2 Unstained slides should be prepared from biopsy tissue following guidelines outlined in **section 9.2**.

9.4 Diagnostic Pathology Fixed Tissue Blocks.

9.4.1 For patients who consent to A091802 biobanking for future research, a representative diagnostic block from the skin or metastatic site should be submitted, if applicable. This can be the archival block from which slides were submitted for PD-L1 analysis or a re-embedded core biopsy block for those patients who undergo new biopsy.

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.

9.4.3 In the event that an institution will not release a tumor tissue block, the institution may instead submit tissue sections, mounted and unstained to glass slides.

9.5 Unstained Slides from Diagnostic Fixed Tissue Blocks for Biobanking

9.5.1 In cases where institutions are unable or unwilling to submit the requested tissue block, a set of 15 unstained tissue slides may be sent as an alternative. 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
10	10 micron	Non-Charged	DNA / RNA
5	5 micron	Charged	DNA / RNA

9.5.2 Follow sectioning and labeling instructions outlined in section 9.2 and in the table above.

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

9.5.4 If archival tissue is not available and patient has undergone new biopsy to fulfill requirements for mandatory tissue submission for PD-L1 analysis (see **section 9.3**), the re-embedded core biopsy block should be submitted for biobank for consented patients.

10. Blood Collection Methods

10.1 Whole Blood- EDTA tubes (no processing)

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

10.1.2 Store EDTA tubes with whole blood at 4 degrees Celsius (i.e. refrigerated) until shipping. Do not freeze the tubes. The tubes must be shipped within 24 hours of collection (e.g. Friday—Saturday or holiday collections are not allowed). 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.**

10.2 Plasma Nucleic Acid (Streck) Tube Processing

10.2.1 Collect 8 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.**

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 and institutional surgical pathology 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 frame specified in **sections 9-10**. 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.**

11.2 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. A blank FedEx Air Bill is provided with the kit for convenience.

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

11.3 Shipping to MD Anderson

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

Ship to:

UTMDACC- TQL Clinical Trials
1515 Holcombe Blvd.
Rm G1.3598
Houston, TX 77030
Phone: 713-745-4903

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, 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 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
3.0	Updated address for MD Anderson Added provisions for shipping during warm weather months Aligned time point names with study protocol Corrected minor typos and grammatical errors	PAA	06/03/2022
2.0	Removed signature page Corrected email addresses Removed reference to waybill for MDA Fixed minor typos and grammatical errors	PAA	09/28/2020
1.0	New	PAA	04/24/2019