

ALLIANCE FOR CLINICAL TRIALS IN ONCOLOGY	CORRELATIVE SCIENCE PROCEDURE MANUAL Biospecimen Collection for Integration of immunotherapy into adjuvant therapy for resected NSCLC: ALCHEMIST Chemo-IO Short Title- A081801 (ALCHEMIST)	Version No: 3.0	Effective Date: 03/04/2022
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CORRELATIVE SCIENCE PROCEDURE MANUAL

1. Purpose

This document describes the procedures required for the collection, shipping, and processing of biospecimens from patients who have consented to participate in A081801 biobanking. 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 BCR (i.e. Biospecimen Core Resource at Nationwide Children’s Hospital), 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 A081801 biospecimen collection, processing, and submission, including staff at satellite institutions.

2. Scope

This document applies to all biospecimens collected specifically for A081801 biobanking 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
BCR	Biospecimen Core Resource at Nationwide Children’s Hospital
FFPE	Formalin fixed, paraffin embedded

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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 for specimens shipped to ABWUSTL, please contact the Alliance Biorepository Program Manager: 1-314-747-4402 or alliance@email.wustl.edu.
- 4.4** For all other questions regarding biospecimen procurement and shipping procedures for specimens shipped to BCR, please contact: 1-614-355-2919 or alchbcr@nationwidechildrens.org.

5. Site Preparation

- 5.1** Please refer to A081801 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 BCR or 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.

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5.3 Prior to collection of blood biospecimens in Streck BCT tubes, 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.

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Time Point	Kit (Y/N)	Biospecimen	Quantity	Collection / Processing Method	Shipping	Recipient Lab	Notes
A081801 Biobanking							
After registration, prior to therapy	N	FFPE Tumor Tissue Block	1 block from primary tumor tissue block OR 11 slides (if block is unavailable)	FFPE tissue block (9.2) OR 1 (one) 5 micron charged unstained slide AND 10 (ten), 10 micron non-charged unstained slides (9.3)	Ambient	ABWUSTL	1, 2
After registration, prior to therapy	N	Whole blood (EDTA)	1 x 10 ml	Whole Blood- EDTA (10.1)	Ambient	ABWUSTL	1
After registration, prior to therapy	Y	Whole blood (Streck BCT)	4 x 8.5 ml	Plasma for ctDNA (10.2)	Ambient	BCR	1
At the end of initial chemotherapy	Y	Whole blood (Streck BCT)	4 x 8.5 ml	Plasma for ctDNA (10.2)	Ambient	BCR	1, 4

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Every 6 months from the end of chemotherapy for a total of 3 years post randomization	Y	Whole blood (Streck BCT)	2 x 8.5 ml	Plasma for ctDNA (10.2)	Ambient	BCR	1
At recurrence	Y	Whole blood (Streck BCT)	2 x 8.5 ml	Plasma for ctDNA (10.2)	Ambient	BCR	1, 3

Notes:

- Collection is optional for patients but requires all sites offer to patients to consent. Please see protocol-specific consent documents.
- One paraffin tissue block from the primary tumor should be submitted for patients opting in for A081801 biobanking. Unstained slides will be accepted as alternative to block submission. A set of 11 unstained slides (1 x 5 micron charged, 10 x 10 micron non-charged) from a single block should be submitted. If fewer than 11 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. THIS TISSUE SUBMISSION IS IN ADDITION TO THE TISSUE BEING SUBMITTED ON A151216.**
- Recurrence samples may be collected and submitted up to 3 months after recurrence. This sample is to be collected prior to initiating subsequent treatment.
- Specimen should be collected 1-4 weeks following the last infusion of doublet chemotherapy.

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7. Biospecimen Collection Kits

7.1 Blood Specimens

- 7.1.1** To facilitate the proper collection and shipping of whole blood specimens in Streck BCT tubes, biospecimen collection kits and materials will be provided by BCR. **Please note, kits are not available for EDTA tubes to be sent to ABWUSTL. Please use your local supply for the EDTA whole blood collection and shipping.**
- 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. **If a kit is needed urgently, overnight shipping is available. A FedEx account number must be provided by the site for all urgent requests. The FedEx account number must be entered into BioMS at the time the kit request is placed.**
- 7.1.3** 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.
- 7.1.4** 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.

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7.1.7 If a biospecimen collection component (e.g. vacutainer collection tube) is missing, damaged, or expired, please contact BCR via email at: alchbcr@nationwidechildrens.org to request replacement components. Replacement components can also be requested via BioMS by entering a new kit request with a comment that additional materials are needed.

7.1.8 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 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.

7.1.10 When shipping to ABWUSTL, 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. Please follow instructions provided within the kit box for specimens submitted to BCR.

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.

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

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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, the section thickness, and the serial section number. Provide a **de-identified copy of the surgical pathology report**, labeled with the patient study number, corresponding to the blocks or slides submitted to ABWUSTL. 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 ABWUSTL or to BCR 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.

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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.

9.1.3 Tissue for A081801 biobanking is to be submitted in addition to the tissue submitted for A151216.

9.2 Diagnostic Pathology Fixed Tissue Blocks

9.2.1 For patients who consent to A081801 biobanking, one representative diagnostic block from the primary tumor should be submitted after registration but prior to therapy.

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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 11 unstained slides from each block with adequate tumor cellularity may be sent as an alternative. If fewer than 11 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.

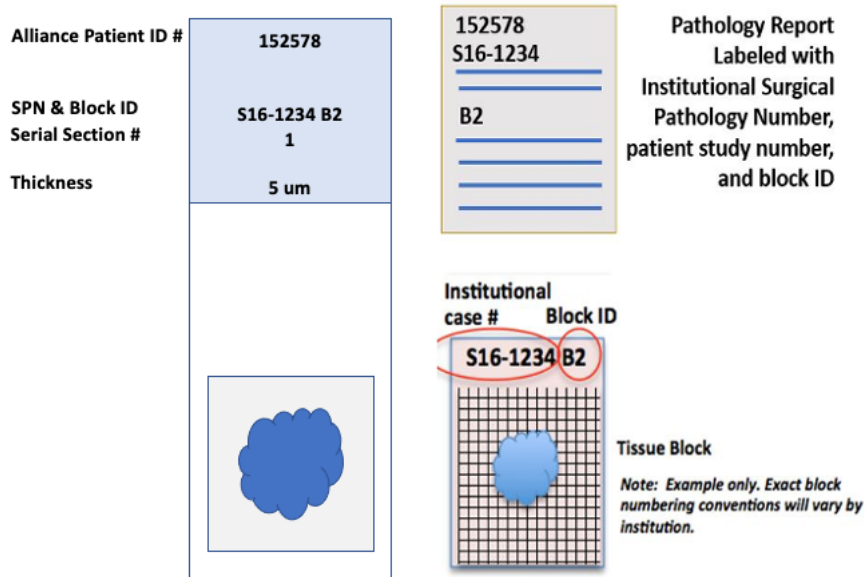
# of slides	Section thickness	Slide type	Purpose
1	5 micron	Charged	H&E stained slide
10	10 micron	Non-Charged	DNA for Whole Exome Sequencing

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

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- 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 (i.e. 5 um, 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.

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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.

9.3.12 Include a copy of the **de-identified pathology report with all slide submissions.**

10. Blood Collection Methods

10.1 Whole blood- EDTA tube (no processing)

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

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10.1.2 Store EDTA tube with whole blood at 4 degrees Celsius (i.e. refrigerated) until shipping. Do not freeze the tube. **The tube may be stored for up to 72 hours at refrigerated temperature before shipment (i.e. if blood must be collected on Friday, it should be stored at 4 degrees Celsius over the weekend until Monday shipment).** Ensure that the EDTA tube is 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 8.5 ml of blood into each of the Streck BCT tubes using standard venous phlebotomy technique. Invert tubes 10 times.

10.2.2 Store Streck tubes with whole blood at ambient temperature. Do not freeze or refrigerate the tubes. **The tubes may be stored for up to 72 hours at ambient temperature before shipment.**

11. Biospecimen Shipping

11.1 Overview

11.1.1 Streck BCT tubes should be shipped at ambient temperature with the packs included in the biospecimen collection kits.

11.1.2 EDTA tubes should be packaged to avoid breakage using a padded envelope, or preferably, a small Styrofoam container. **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.**

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- 11.1.3** 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.
- 11.1.4** If collected biospecimens cannot be shipped within the time frames specified in **sections 9-10**, 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.
- 11.1.5** **ABWUSTL: Do not ship on Friday, Saturday, Sunday or the day before a nationally recognized holiday. ABWUSTL is NOT staffed on the weekend and cannot accept Saturday deliveries.**
- 11.1.6** **BCR: Do not ship on Saturday or on the day before a nationally recognized holiday. BCR is staffed on the weekend and can accept Saturday deliveries.**

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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. Sites are expected to pay for shipping of specimens to ABWUSTL.

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 BCR

11.3.1 Airbills may be requested for shipments to BCR by contacting: ALCHBCR@nationwidechildrens.org.

11.3.2 Ship container according to IATA guidelines and standard institutional policies, using the airbill provided.

Ship to:

**Biospecimen Core Resource
Nationwide Children's Hospital
700 Children's Dr., WA1340
Columbus, OH 43205
Phone: 614-355-2919**

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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** Fixed tissue blocks and unstained slides will be stored at ambient temperature.
- 12.8** All biospecimens will remain in storage until additional processing or review is requested in writing by the appropriate protocol PI.

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13. Document History

Version	Description and Justification of Change	Author	Effective Date
3.0	Removed ST1 and changed to Biobanking Added statement to clarify tissue submission for A081801 vs A151216	PAA	03/04/2022
2.1	Clarified timepoint name Included provisions for shipping during warm weather months to ABWUSTL	PAA	09/07/2021
2.0	Updated biospecimen collection schema to remove "After Chemotherapy" time point.	PAA	10/31/2020
1.2	Updated hyperlinks Minor grammatical corrections	PAA	06/25/2020
1.1	Collection schema update, change every 3 months to every 6 months	GLS	06/01/2020
1.0	New	PAA	02/12/2020