CORRELATIVE SCIENCE PROCEDURE MANUAL

AFT-46: CHIO3 TRIAL: CHEMOTHERAPY COMBINED WITH IMMUNE CHECKPOINT INHIBITOR FOR OPERABLE STAGE IIIA/B NON-SMALL CELL LUNG CANCER

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AFT-46 Correlative Science Procedure Manual Signature Page

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1. PURPOSE

This document describes the procedures required for the collection, shipping, and processing of biospecimens from all patients enrolled or registered on AFT-46. This document also describes the procedures that will be followed subsequent to the receipt of biospecimens by the AFT Biorepository (AFB) (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 AFT-46 study biospecimen collection, processing, and submission, including staff at satellite institutions.

2. SCOPE

This document applies to all biospecimens collected specifically for the AFT-46 study only. Please refer to the study 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 can be found on the AFT BioMS web site which is accessible via the AFT portal: (https://alliancefoundationtrials.org)

3. CONTACT INFORMATION

3.1. Please send any questions and problems related to protocol administration, eligibility, patient registration, and data submission, to the AFT Project Manager at: AFT46@alliancefoundationtrials.org or 1-617-525-8493.

3.2. For specific questions about kits or shipments, please contact the Siteman Cancer Center Tissue Procurement Core at: 1-314-454-7615 or tbank@wudosis.wustl.edu.

3.3. For questions about using the AFT BioMS web application for ordering kits, or registering and shipping biospecimens, please contact: 1-855-642-4667 or aftbiomshelp@email.wustl.edu

3.4. For any other questions about biospecimen procurement and shipping procedures, please contact the AFB Program Manager at: 1-314-747-4402 or afbhelp@email.wustl.edu

4. SITE PREPARATION

4.1. Please refer to the AFT-46 protocol for any specific requirements related to patient enrollment, registration, and regulatory compliance.

4.2. The AFT BioMS application is used for both requesting biospecimen collection kits and for logging the collection and shipment of biospecimens to the AFT Biorepository. For training and assistance in using the AFT BioMS application, please see the AFT BioMS web site which is accessible via the AFT portal: https://alliancefoundationtrials.org or contact the AFT BioMS Help desk at: aftbiomshelp@email.wustl.edu or 1-855-642-4667.

4.3. Prior to collection of biospecimens, a biospecimen collection kit must be at the collection site. Please see section 6 for requesting biospecimen collection kits. Please allow at least 10 working days to receive the collection kit.

4.4. Please confirm that your institutional pathology department will release a tumor tissue paraffin block **OR** one unstained slide (5 um) **AND** 5-10 tissue sections (10 um) at the required time

points designated in this document and in the study protocol. An institution whose pathology department is unwilling to comply with tumor tissue submission should not enroll patients to this study.

5. COLLECTION SCHEMA

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

Time Point	Biospecimen	Quantity	Collection / Processing Method	Shipping	Notes
Screening	Fixed tissue block	1	Fixed tissue blocks (8.1)	Ambient	1, 2
Screening	Unstained tissue slide **AND** Tissue sections	1 (5 um) unstained slide **AND** 5-10 (10 um) tissue sections	Fixed tissue slide (8.2) **AND** Tissue sections (8.3)	Ambient	1, 2
Screening	Whole blood (EDTA tube)	3 x 10 ml	Whole blood (9.1)	Ambient	1, 3
Screening	Whole blood (BCT tube)	2 x 8 ml	Plasma for cfDNA (9.2)	Ambient	1
Pre-Surgery	Whole blood (EDTA tube)	3 x 10 ml	Whole blood (9.1)	Ambient	3
Pre-Surgery	Whole blood (BCT tube)	2 x 8 ml	Plasma for cfDNA (9.2)	Ambient	
Surgery	Fixed tissue block	1	Fixed tissue blocks (8.1)	Ambient	2, 4
Surgery	Unstained tissue slide **AND** Tissue sections	1 (5 um) unstained slide **AND** 5-10 (10 um) tissue sections	Fixed tissue slide (8.2) **AND** Tissue sections (8.3)	Ambient	2, 4
EOT	Whole blood (EDTA tube)	3 x 10 ml	Whole blood (9.1)	Ambient	3, 5
EOT	Whole blood (BCT tube)	2 x 8 ml	Plasma for cfDNA (9.2)	Ambient	5
Progression	Fixed tissue block	1	Fixed tissue blocks (8.1)	Ambient	2, 6
Progression	Unstained tissue slide **AND** Tissue sections	1 (5 um) unstained slide **AND** 5-10 (10 um) tissue sections	Fixed tissue slide (8.2) **AND** Tissue sections (8.3)	Ambient	2, 6

NOTES:

1. Screening specimens should be submitted within 28 days of patient registration.

2. Submission of fixed tissue block is **strongly preferred**. If institutional policy prohibits release of a fixed tissue block, 1 unstained tissue slide **AND** 5-10 (10 um) tissue sections must be submitted.
3. Whole blood (EDTA) for PBMC isolation and cryopreservation at the Biorepository.
4. Surgery must take place 4-8 weeks after completion of induction therapy unless a delay is approved by the study co-chair. Tissue should be submitted to AFB within 12 weeks of surgery.
5. If the patient completes all treatment per protocol, the end of treatment visit must take place 30-45 days after completion of adjuvant therapy. If the patient does not complete treatment per protocol, the end of treatment visit must take place as soon as possible after treatment is discontinued.
6. Biopsy at progression is optional but may be done as standard of care. If collected, tissue should be submitted to AFB.

6. BIOSPECIMEN COLLECTION KITS

6.1. 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 of the kit to the site will be covered by AFT. A pre-paid FedEx waybill is provided for shipping the kit with the biospecimens back to the AFB at Washington University in St. Louis via priority overnight shipping.

6.2. 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 many of 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 using the AFT BioMS system.**

6.3. Kit contents and specific instructions for use of the kit are provided in the kit box and are available for download on the AFT BioMS webpage which is accessible via the AFT portal: https://alliancefoundationtrials.org. **Please return any used collection materials with the kit**.

6.4. 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.

6.5. 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.

6.6. 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.

6.7. 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).

6.8. Please return all kits that have expired or missing components. Return the ENTIRE kit using the pre-paid FedEx waybill provided. 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.

6.9. 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 (EDTA tubes) others (i.e. Streck BCT tubes) are highly specialized and probably are not available at the institution.

6.9.1. 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.

6.10. Because paraffin blocks and sections 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 or sections may be sent independently of other biospecimens using the following guidelines:

6.10.1. There is no independent "kit" for submission of paraffin blocks, slides, or sections.

6.10.2. Tissue should be packaged to avoid breakage using a padded envelope or, preferably, a small Styrofoam cooler.

6.10.3. During warm weather months, paraffin block, sections, 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.

6.10.4. Fixed tissue may be shipped for standard overnight delivery according to institutional policies.

6.11. Please see **Section 10 – Biospecimen Shipping** for specific instructions on packaging biospecimens into the shipping kit for shipment to AFB.

7. BIOSPECIMEN LABELING AND TRACKING

7.1. All research biospecimens (vacutainer tubes, cryovials, and tissue bags) MUST be labeled with the participant study number and patient initials (Last, First, Middle). Blood tubes should additionally be labeled with the date and time of collection.

7.2. 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 an unstained tissue slide and sections are being submitted instead of a block, the tissue section slide or cryovial should be labeled with the patient study number, institutional surgical pathology number, and the block identifier. Provide a **de-identified** copy of the surgical pathology report, labeled only with the participant study number, corresponding to the block, slide, or sections submitted. Please ensure the institutional surgical pathology number and block ID are maintained on the surgical pathology report. See **section 8** for additional details.

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

7.4. Do not affix any labels to vials or tubes. Label the collection containers directly with a marking pen.

7.5. All biospecimens that are collected and sent to the AFT biorepository (AFB) **must be logged and tracked in AFT BioMS**. The AFT BioMS system is a web-based application that tracks the collection and shipping of biospecimens. Once individual biospecimens are logged and 'shipped' in the AFT 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 AFT BioMS system and for further information about training, access, and use, please contact the AFT BioMS Help desk at: 1-855-642-4667 or aftbiomshelp@email.wustl.edu. Note that the AFT BioMS system is similar to but independent of the NCI BioMS application, which is used to manage biospecimens collected on NCI NCTN Alliance trials.

7.6. In the event that AFT BioMS cannot be accessed, please complete an AFT BioMS specimen manifest form, which can found here- https://cbmiapps.wustl.edu/confluence/display/AB/Forms

8. TISSUE COLLECTION METHODS

8.1. Diagnostic Pathology Fixed Tissue Blocks

8.1.1. This protocol requires submission of ONE representative, diagnostic pathology, formalin fixed paraffin embedded (FFPE) tumor tissue block at the time of patient enrollment. Additionally, if available, one FFPE tumor tissue block is requested at time of surgery and one FFPE tumor tissue block is requested at time of progression.

8.1.2. Blocks submitted must contain at least 10% neoplastic cellularity, be representative of the primary histopathology diagnosis, and have sufficient cross sectional area (e.g. at least 4 mm^2) and thickness (e.g. 100 um) so that tissue sections may be cut without exhausting the tissue.

8.1.3. 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 correlative science study end points have been met.

8.1.4. In the event that institutional policy prohibits release of tissue blocks, the institution must inform AFT and submit their site SOP or policy for review. Once reviewed and approved by AFT, the institution may instead submit one unstained slide **AND** a set of 5-10 (10 um) tissue sections at each of the time points where tissue is requested (see **sections 8.2** and **8.3**).

8.2. Unstained Slide from Diagnostic Fixed Tissue Block

8.2.1. In cases where institutional policy prohibits release of tissue blocks, after AFT approval, one unstained slide **AND** 5-10 (10 um) tissue sections may be submitted at each study time point where tissue is requested. See section **8.3** for instructions on submitting tissue sections.

8.2.2. Please follow the procedures below for submitting the unstained tissue slide.

# of slides	Section thickness	Slide type	Purpose
1	5 micron	Positively Charged	H&E stain for pathology review

8.2.3. The tissue section should be **cut fresh** from the appropriate formalin fixed, paraffin embedded tissue block.

8.2.4. Cut section at 5 micron thickness as indicated onto positively charged slide.

8.2.5. Ensure that the slide is labeled with the patient study number, the institutional surgical pathology number and block ID.

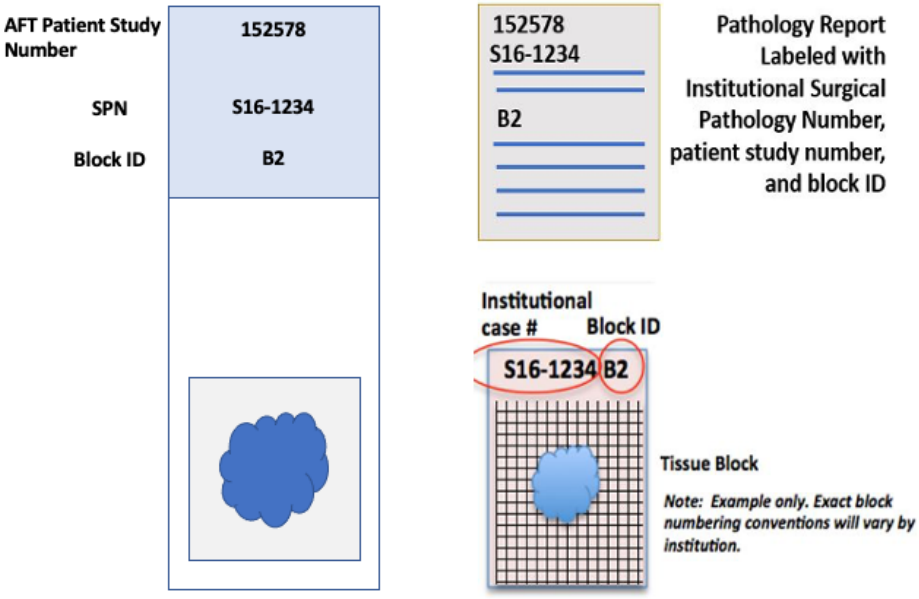
8.2.6. Do not label slide with adhesive label. Write or print information on the textured surface of the slide with indelible, solvent-resistant ink.

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

8.2.8. Mount only one tissue section on the slide. Make certain the section is placed on the painted / textured side of the slide.

8.2.9. When placing the section onto the slide, ensure that the tissue is placed on the bottom third of the slide.

8.2.10. See figure below for proper mounting and labeling.



8.2.11. Air dry the slide for 12-24 hours prior to shipping. Do not oven dry the slide.

8.2.12. Use slide mailer or a slide box to ship the slide. Ensure that slides from only one patient are placed in one slide mailer or slide box.

8.2.13. Include a copy of a de-identified pathology report with all slide submissions.

8.3. Tissue Sections

8.3.1. In cases where institutional policy prohibits release of a tissue block, a single unstained slide **AND** serial tissue sections from the same block should be submitted. Please refer to section **8.2** for unstained slide submission.

8.3.2. Cut 5-10 (10 um) serial paraffin tissue sections. Place the tissue sections directly into the cryovial provided or equivalent container. Do not float the tissue sections in a water bath. Label the tube of tissue following the guidelines outlined in **section 7.2**.

9. BLOOD COLLECTION METHODS

9.1. Whole blood (EDTA Tube- no processing)

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

9.1.2. Store EDTA tubes with whole blood at 4 degrees Celsius (i.e. refrigerated) until shipping. Do not freeze the tubes. 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.**

9.1.3. **Blood should only be collected on Monday—Thursday. Do not collect on Friday, Saturday, Sunday, or on the day before a national holiday.**

9.1.4. **EDTA tubes should be shipped on the same day that they are collected. Blood MUST be received at the Biorepository within 24 hours of collection due to required processing.**

9.2. Plasma Nucleic Acid (Streck) Tube Processing

9.2.1. Collect 8 ml of blood into each of the Streck BCT tubes using standard venous phlebotomy. Invert tubes 10 times.

9.2.2. 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. BIOSPECIMEN SHIPPING

10.1. All biospecimens should be shipped within the time frames specified in **sections 8** and **9**. If collected biospecimens cannot be shipped within the specified time frame (e.g. Friday – Saturday or Holiday collections), please contact the AFB Program Manager via phone (1-314-747-4402) or email (afbhelp@email.wustl.edu) for further instructions, at least 24 hours prior to anticipated collection

10.2. Please see the Directions for Use (DFU) document that is included in each kit, for specific directions on how to package and ship biospecimens. The DFU document is also available for download on the AFT BioMS webpage which is accessible via the AFT portal: (https://alliancefoundationtrials.org)

10.3. Place the original, completed copy of the AFT BioMS packing manifest in the kit. If sending tissue, a copy of the de-identified surgical pathology report, labeled with the patient study number should also be included. **Do not send specimens without a completed AFT BioMS**

packing manifest or substitute "AFT BioMS Downtime Form." Biospecimens cannot be accepted without this completed form.

10.4. **<u>Do not ship on Friday, Saturday, Sunday, or on the day before a nationally recognized holiday.</u>**

10.5. Ship container for PRIORITY OVERNIGHT DELIVERY using the pre-paid FedEx waybill provided within the specimen collection kit. Ship to:

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

11. AFB BIOSPECIMEN RECEIPT AND QUALITY ASSURANCE MEASURES

11.1. All biospecimens will be shipped to and received by the AFT Biorepository at the Siteman Cancer Center Tissue Procurement Core (TPC), Washington University in St. Louis, a CAP-accredited biorepository.

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

11.3. 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.

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

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

11.6. Aliquoted biofluids will be stored under liquid nitrogen vapor.

11.7. All biospecimens will remain in storage until additional processing or review is requested in writing by the appropriate protocol principal investigator.

12. DOCUMENT HISTORY

VERSION	DESCRIPTION AND JUSTIFICATION OF CHANGE	AUTHOR	EFFECTIVE DATE
2.0	Added unstained slide + tissue sections as block alternative	PAA	30-July-2021
1.0	New	PAA	08-July-2020