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 AFT-31. This document also describes the procedures that will be followed subsequent to the receipt of biospecimens by the AFT 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 AFT-31 biospecimen collection, processing, and submission; including staff at satellite institutions.

2. Scope

This document applies to all biospecimens collected specifically for AFT-31 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 AFT.BioMS web page located at:**
https://cbmiapps.wustl.edu/confluence/display/AB/AFT+BioMS+Public+Home

3. Definitions

Term	Definition
AFB	Alliance Foundation Biorepository
SCC TPC	Siteman Cancer Center Tissue Procurement Core Shared Resource
FFPE	Formalin fixed, paraffin embedded

4. Contact Information

4.1 For questions and problems related to protocol administration, eligibility, patient registration, and data submission, please contact the AFT Protocol Manager at: **(617) 525-7734.**

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

4.3 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@bmi.wustl.edu.

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

5. Site Preparation

5.1 Please refer to the disease-specific 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 AFT.BioMS application (https://aftbioms.wustl.edu/aft). 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

https://cbmiapps.wustl.edu/confluence/display/AB/AFT+BioMS+Home

or contact the AFT.BioMS Help desk at: aftbiomshelp@bmi.wustl.edu or 1-855-642-4667.

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 Please confirm that your institutional pathology department will release a tumor tissue paraffin block or will be willing to submit tissue section slides from such a block, at each required time point designated in this document and in the trial protocol. An institution whose pathology department is unwilling to comply with tumor block or slide submission 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 Type	Biospecimen	Quantity	Collection / Processing Method	Shipping	Notes
Screen	A	Fixed tumor tissue block	1	Fixed tissue blocks (9.2)	Ambient	1
Screen	A	Unstained tumor tissue slides	5	Fixed tissue slides (9.3)	Ambient	1, 2
Screen	A	Unstained tumor tissue slides	15	Fixed tissue slides (9.3)	Ambient	1, 2
Screen	A	Whole blood (EDTA tube)	10 ml	Whole blood (10.5)	Ambient	
Screen	A	Whole blood (BCT tube)	2 x 8 ml	Plasma for cfDNA (10.4)	Ambient	
1st Restage	A	Whole blood (BCT tube)	2 x 8 ml	Plasma for cfDNA (10.4)	Ambient	
Progression	A	Fixed tumor tissue block	1	Fixed tissue blocks (9.2)	Ambient	1
Progression	A	Unstained tumor tissue slides	5	Fixed tissue slides (9.3)	Ambient	1, 2
Progression	A	Unstained tumor tissue slides	15	Fixed tissue slides (9.3)	Ambient	1, 2
Progression	A	Whole blood (BCT tube)	2 x 8 ml	Plasma for cfDNA (10.4)	Ambient	

Notes:

1. Either a representative tumor tissue block **OR** 5+15 (20 total) unstained tissue section slides from such a block must be submitted. Block submission is strongly preferred.
2. Cut 5 slides for PDL-1 immunostaining and 15 additional slides for nucleic acid extraction and analyses. See details in section 9.3

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 AFT 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 many of the collection materials (vacutainer tubes) have expiration dates, do not request kits more than 60 days prior to their anticipated use. **All kits must be requested by using the AFT.BioMS system.**

7.3 This protocol uses a single kit ("A") for biospecimen collection at all time points. Kit contents and specific instruction for use of the kit are provided in the kit box (see Attachment A). **Please return any used collection materials with the kit.**

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 (e.g. glass slides), 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 in coming kits are tracked, and sites that have requested many more kits than they have returned will be charged for non-returned kits.

7.9 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 are highly specialized (Streck BCT tubes) and probably are not available at the institution.

7.9.1 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.

7.10 Please see **Section 11 – Biospecimen Shipping** for specific instructions on packaging biospecimens into the shipping kit for shipment to the biorepository.

8. Biospecimen Labeling and Tracking

8.1 All research biospecimens (vacutainer tubes, cryovials, formalin vial, cryomolds 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.

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 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 blocks or slides submitted. See section 9.3 for additional details.

8.3 Label all contains and vials with an indelible, solvent-resistant marker when they are at ambient temperature. Do not try to label frozen vials.

8.4 Do not affix any labels to vials, slides, tubes, or cryomolds. Label the collection containers directly with a marking pen.

8.5 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 to the AFB. 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@bmi.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.

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

9. Tissue Collection Methods

9.1 Overview.

9.2 Diagnostic Pathology Fixed Tissue Blocks.

9.2.1 This protocol requires submission of ONE representative, diagnostic pathology, formalin fixed paraffin embedded tumor tissue block.

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 a tumor tissue block, the institution may instead submit tissue sections, mounted and unstained to glass slides.

9.3 Unstained Slides from Diagnostic Fixed Tissue Blocks

9.3.1 In cases where institutions are unable or unwilling to submit the requested tissue block, a set of 20 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
5	4 micron	Charged, ProbeOn Plus	PDL-1 immunostaining
15	10 micron	Uncharged	Nucleic acid extraction

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 4 or 10 micron thickness as indicated.

9.3.4 For PDL-1 immunostaining slides, cut sections onto Fisher ProbeOn Plus slides, which are provided in the kit.

9.3.5 For all other slides, cut sections to uncharged, plain glass slides.

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

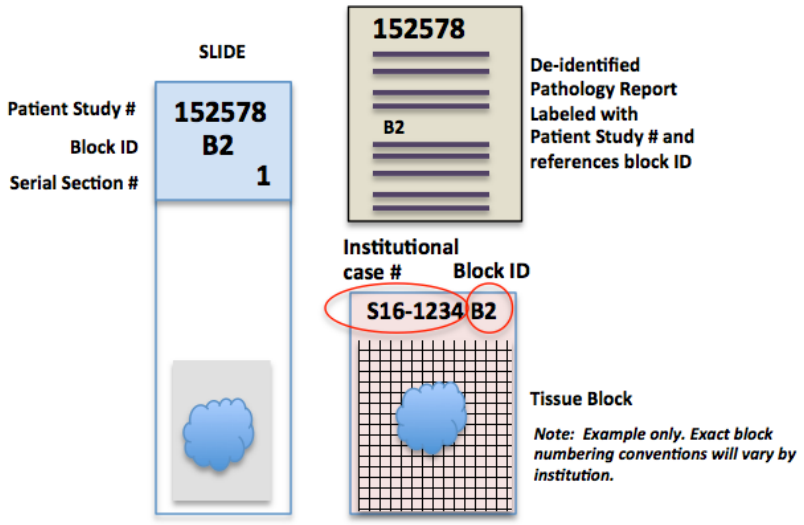
9.3.7 Do not label slides with adhesive labels. Write or print information on the textured surface of the slide with indelible, solvent-resistant ink.

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

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

9.3.10 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.

9.3.11 See figure below for proper mounting and labeling.



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

9.3.13 Use the slide boxes provided in the kit for shipping slides. Slides should not be touching each other. Ensure that slides from only one patient are placed in one slide mailer.

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

10. Blood Collection Methods

10.1 Serum Processing (not applicable for this trial)

10.2 Plasma Processing (not applicable for this trial)

10.3 "Buffy Coat" (White Blood Cell) Processing (not applicable for this trial)

10.4 Plasma Nucleic Acid (Streck) Tube Processing

10.4.1 Collect 8 ml of blood into the Streck BCT tube using standard venous phlebotomy. Invert tube 10 times.

10.4.2 Store Streck tube with whole blood at room temperature. Do not freeze or refrigerate the tube. The tube may be stored for up to 72 hours at ambient temperature before shipment. Ensure that the Streck tube is shipped at ambient temperature to avoid freezing.

10.5 Whole blood (EDTA Tube- no processing)

10.5.1 Collect 10 ml of blood into the KEDTA tube using standard venous phlebotomy. Invert tube 10 times.

10.5.2 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.

11. Biospecimen Shipping

11.1 All biospecimens should be shipped on the same day that they are collected (Monday – Thursday). Biospecimens must be received by the AFB within 24 hours of collection. If collected biospecimens cannot be shipped on the same day that they are collected (e.g. Friday – Saturday or Holiday collections), please contact the AFB Program Manager at 1-314-747-4402 for further instructions, at least 24 hours prior to anticipated collection.

11.2 Please see the Directions for Use (DFU) document that is included in each kit, for specific directions on how to package and ship biospecimens.

11.3 Place the original, completed copy of the AFT.BioMS Packing Manifest and a copy of the de-identified surgical pathology report, labeled with the patient study number in the kit. **Do not send specimens without a completed AFT.BioMS Packing Manifest or substitute "AFT.BioMS Downtime Form". Biospecimens can not be accepted without this completed form.**

11.4 Close the cardboard carton overwrap and secure lid with packaging tape; one piece on each side and two pieces over the front flap.

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

AFT 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

11.6 **Do not ship on Friday, Saturday, Sunday or day before a nationally recognized holiday.**

11.7 Because paraffin tissue 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:

11.7.1 There is no independent "kit" for the submission of paraffin blocks or slides. Use the slide mailers provided in "Kit A" to store and ship slides, if required.

11.7.2 Block and slides should be packaged to avoid breakage using a padded envelope or, preferably, a small Styrofoam container.

11.7.3 During warm weather months, paraffin slides and blocks 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 blocks and slides.

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

12. Biospecimen Receipt and Quality Assurance Measures

12.1 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.

12.2 All biospecimens will be accessioned into the TPC informatics system, OpenSpecimen.

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

12.4 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.

12.5 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 Program Manager who will contact the submitting site for reconciliation.

12.6 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.

12.7 Frozen tissues and aliquoted biofluids will be stored under liquid nitrogen vapor.

12.8 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 collection schema	MAW	12/23/2016
1.0	New	MAW	N/A

14. Signature Page

Signatures of the following individuals indicate review and acceptance of the attached document version:

Name	Role	Signature	Date
Mark A Watson, MD, PhD	Director, AFB		12/28/2016
Patsy Alldredge, MS	AFB Program Manager		12/28/2016
Brian Goetz	SCC TPC Laboratory Manager		
Neal Ready, MD PhD	Protocol PI		
Andrew Morrison	AFT Operations		