

ALLIANCE FOR CLINICAL TRIALS IN ONCOLOGY	CORRELATIVE SCIENCE PROCEDURE MANUAL Biospecimen Collection for Duration of Immune Checkpoint Therapy in Locally Advanced or Metastatic Urothelial Carcinoma: A Randomized Phase 3 Non-Inferiority Trial Short Title- A031901	Version No: 2.0	Effective Date: 06/01/2021
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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 enrolled or registered who have consented to participate in A031901. 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 A031901 biospecimen collection, processing, and submission, including staff at satellite institutions.

2. Scope

This document applies to all biospecimens collected specifically for A031901 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
ABWU STL	Alliance Biorepository at Washington University in St. Louis
FFPE	Formalin Fixed, Paraffin Embedded
IHC	Immunohistochemistry

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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, please contact the Alliance Biorepository Program Manager: 1-314-747-4402 or alliance@email.wustl.edu.

5. Site Preparation

- 5.1** Please refer to A031901 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 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.

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6. Collection Schema

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

Time Point	Kit (Y/N)	Biospecimen	Quantity	Collection / Processing Method	Shipping	Notes
For patients who are registered to A031901-ST1						
After Registration	N	Fixed tumor tissue block	2 cores with \geq 0.2cm region of tumor and \geq 20% tumor content	Fixed tissue block (9.2)	Ambient	1, 2
After Registration	N	Unstained tissue slides	10 (5 μ m) slides AND 20 (10 μ m) slides	Fixed tissue slides (9.3)	Ambient	1, 2
After Registration	N	Whole blood for plasma	6 x 1 ml aliquots	Frozen plasma (10.1)	Dry Ice	1, 3
After Registration	N	Whole blood for "buffy coat"	4 aliquots	"Buffy Coat" (10.2)	Dry Ice	1, 3
For patients who are registered to A031901-ST2						
Week 12	N	Whole blood for plasma	6 x 1 ml aliquots	Frozen plasma (10.1)	Dry Ice	1, 3
Week 12	N	Whole blood for "buffy coat"	4 aliquots	"Buffy Coat" (10.2)	Dry Ice	1, 3
For patients who are registered to A031901-ST3						
At Progression	N	Whole blood for plasma	6 x 1 ml aliquots	Frozen plasma (10.1)	Dry Ice	1, 3
At Progression	N	Whole blood for "buffy coat"	4 aliquots	"Buffy Coat" (10.2)	Dry Ice	1, 3

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Notes:

1. Collection is optional for patients but requires all sites offer patients to consent. Please see protocol-specific consent documents.
2. Submission of archival tumor tissue blocks with at least 2 cores with ≥ 0.2 cm region of tumor and $\geq 20\%$ tumor content by morphologic evaluation **OR** unstained tumor tissue FFPE slides (10 x 5 um **AND** 20 x 10 um) is requested. **Block submission highly preferred.** If submitting slides and tissue is limited, please prioritize submission of 5 um slides and submit as many at 10 um slides as possible.
3. Peripheral blood (EDTA) 2 x 10 ml to be processed for plasma (6 x 1 ml aliquots) and “buffy coat,” frozen on site and shipped on dry ice.

7. Biospecimen Collection Kits

7.1 Blood Specimens

7.1.1 There are no “kits” provided for submission of blood specimens for this study. Sites are responsible for acquiring materials for collection and shipping of biospecimens to the Biorepository.

7.2 Tissue Specimens

7.2.1 There are no “kits” provided for submission of paraffin blocks or slides for this study.

7.2.2 Blocks and slides should be packaged to avoid breakage using a padded envelope or, preferably, a small Styrofoam cooler.

7.2.3 During warm weather months, 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.

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7.3 Please see section 11- Biospecimen Shipping for specific instructions on shipping to ABWUSTL.

8. Biospecimen Labeling and Tracking

- 8.1** All research biospecimens (cryovials, 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, buffy coat).
- 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, serial section number, and section thickness. Provide **a de-identified copy of the surgical pathology report**, labeled with the patient study number, corresponding to the blocks or slide 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.

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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@alliancencn.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.

9.1.3 Include a copy of the **de-identified pathology report with all tissue submission**.

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9.2 Diagnostic Pathology Fixed Tissue Block

- 9.2.1** This protocol requests submission of one representative, diagnostic pathology, formalin fixed paraffin embedded tumor tissue block. The archival tumor tissue block should contain at least 2 cores with ≥ 0.2 cm region of tumor and $\geq 20\%$ tumor content by morphologic evaluation.
- 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 tissue block, the institution may instead submit tissue sections, mounted and unstained to glass slides.

9.3 Unstained Slides from Diagnostic Fixed Tissue Block

- 9.3.1** In cases where institutions are unable or unwilling to submit the requested tissue block, a set of 30 unstained slides may be submitted. If tissue is limited, please prioritize submission of slides cut at 5 micron and submit as many slides cut at 10 micron as possible. 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.

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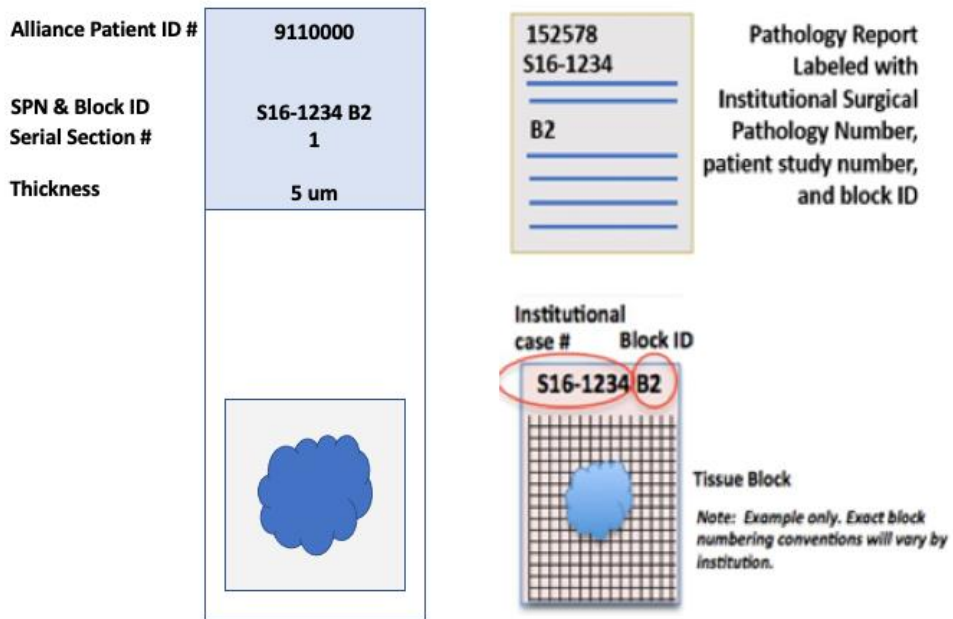
# of cores	Length of tumor region	Tumor content	Purpose
2	≥ 0.2cm	≥ 20% tumor	IHC, DNA, RNA

# of slides	Section thickness	Slide type	Purpose
10	5 micron	Positively Charged	IHC
20	10 micron	Positively Charged	DNA, RNA

- 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 5 or 10 micron thickness as indicated onto positively charged slides.
- 9.3.4** Ensure that each slide is labeled with the patient study number, the institutional surgical pathology number and block ID, the slide serial section number (1, 2, 3, etc.), and section thickness.
- 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.

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9.3.9 See figure below for proper mounting and labeling.



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 a **de-identified** pathology report with all slide submissions.

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10. Blood Collection Methods

10.1 Plasma Processing

- 10.1.1** Collect whole blood by standard venous phlebotomy technique into the purple top (EDTA) tubes. Invert tubes 10 times.
- 10.1.2** Within 30 (preferred) to 120 minutes of collection, spin the vacutainer tubes at room temperature in a clinical centrifuge at 2500 xG for 15 minutes. Make sure that the caps are secured tightly on the tubes.
- 10.1.3** Carefully remove the plasma layer from each tube (~3 ml), without touching the white, buffy coat layer, and transfer to new 15 ml conical centrifuge tubes. Keep the vacutainer tubes containing the white, buffy coat layer for white blood cell isolation (**section 10.2**).
- 10.1.4** Spin the centrifuge tubes containing plasma at room temperature in a clinical centrifuge at 2500 xG for 15 minutes.
- 10.1.5** Label 6 cryovials as instructed in **section 8**. Make certain each vial is labeled completely and identically.
- 10.1.6** Carefully remove 6 ml of plasma (without touching the pellet) and divide into 6, 1 ml labeled cryovials. Leave a small amount of plasma to avoid obtaining red blood cells.
- 10.1.7** 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.

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10.2 “Buffy Coat” (White Blood Cell) Processing

- 10.2.1** Follow procedures in **section 10.1** for collecting and processing plasma from EDTA tubes.
- 10.2.2** Label 4 cryovials as instructed in **section 8**.
- 10.2.3** After removing the plasma, carefully remove the white, “buffy coat” white blood cell layer, avoiding the red blood cell mass as much as possible.
- 10.2.4** Transfer the buffy coat layer (approximately 0.2 – 0.5 ml) from each EDTA tube into the labeled cryovials. Immediately freeze the cryovials of buffy coat on dry ice or in liquid nitrogen vapor. Do NOT freeze buffy coat cells by placing a warm tube in a -70 to -90 degree Celsius ultralow freezer. Once completely frozen, the cryovials containing the buffy coat cells may be stored at -70 to -90 degrees C until ready for shipment on dry ice.

11. Biospecimen Shipping

11.1 Overview

- 11.1.1** 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.

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11.1.2 Unless otherwise specified, all biospecimens should be shipped on the same day that they are collected (Monday – Thursday) so they are received by the Biorepository 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 Alliance Biorepository Program Manager: 1-314-747-4402 or alliance@email.wustl.edu for further instructions, at least 24 hours prior to anticipated collection.

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

11.1.4 **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 University School of Medicine

425 S. Euclid Ave

Room 5120

St. Louis, MO 63110-1005

Phone: (314) 454-7615

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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** 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
2.0	Removed study collection kits	PAA	06/01/2021
1.0	New	PAA	11/12/2020