

<b>ALLIANCE FOR CLINICAL TRIALS IN ONCOLOGY</b>	<b>CORRELATIVE SCIENCE PROCEDURE MANUAL</b>  Biospecimen Collection for A Randomized Phase II Trial of Adjuvant Nivolumab With or Without Cabozantinib in Patients with Resected Mucosal Melanoma  Short Title- A091903	Version No: 3.1	Effective Date: 03/01/2024
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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 all patients enrolled or registered on A091903. This document also describes the procedures that will be followed subsequent to the receipt of biospecimens by Dartmouth Hitchcock Medical Center or 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 A091903 biospecimen collection, processing, and submission, including staff at satellite institutions.

### 2. Scope

This document applies to all biospecimens collected specifically for A091903 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
H&E	Hematoxylin and Eosin

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#### 4. Contact Information

<b>Protocol-related questions may be directed as follows:</b>	
<b>Questions</b>	<b>Contact (via email)</b>
Questions regarding patient eligibility, treatment, and dose modification:	Study Chair: Alexander N. Shoushtari, MD <a href="mailto:shoushta@mskcc.org">shoushta@mskcc.org</a>  Nursing Contact: Lisa Kottschade, APRN, MSN, CNP <a href="mailto:kottschade.lisa@mayo.edu">kottschade.lisa@mayo.edu</a> Protocol Coordinator: Beth Smith <a href="mailto:beth10@bsd.uchicago.edu">beth10@bsd.uchicago.edu</a>  (where applicable) Data Manager: Brandon Bright <a href="mailto:bright.brandon@mayo.edu">bright.brandon@mayo.edu</a>
Questions related to data submission, RAVE or patient follow-up:	Data Manager: Brandon Bright <a href="mailto:bright.brandon@mayo.edu">bright.brandon@mayo.edu</a>
Questions regarding the protocol document and model informed consent:	Protocol Coordinator: Beth Smith <a href="mailto:beth10@bsd.uchicago.edu">beth10@bsd.uchicago.edu</a>
Questions related to IRB review	Alliance Regulatory Inbox <a href="mailto:regulatory@allianceNCTN.org">regulatory@allianceNCTN.org</a>
Questions regarding CTEP-AERS reporting:	Alliance Pharmacovigilance Inbox <a href="mailto:pharmacovigilance@alliancencn.org">pharmacovigilance@alliancencn.org</a>
Questions regarding specimens/specimen submissions:	Alliance Biorepository at WUSTL: <a href="mailto:alliance@email.wustl.edu">alliance@email.wustl.edu</a>
Questions regarding drug supply	PMB <a href="mailto:PMBAfterHours@mail.nih.gov">PMBAfterHours@mail.nih.gov</a>
Questions regarding drug administration	Pharmacy Contact: Heidi Finnes, PharmD <a href="mailto:gunderson.heidi13@mayo.edu">gunderson.heidi13@mayo.edu</a>

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- 4.1** 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](mailto: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](mailto:bioms@alliancenctn.org).
- 4.2** 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](mailto:alliance@email.wustl.edu).

## 5. Site Preparation

- 5.1** Please refer to A091903 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 Dartmouth Hitchcock Medical Center and 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](mailto:bioms@alliancenctn.org).
- 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** Identify a reliable source of dry ice for freezing and shipping biospecimens and a -70 to -90 degree Celsius freezer (“ultralow”) in which frozen biospecimens may be stored prior to shipment.

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**6. Collection Schema**

The following biospecimens are to be collected at each of the time points below. 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	Notes
<b>Mandatory for all patients registered to A091903</b>						
After Pre-registration	N	H&E stained slide <u>AND</u> 4-5 um unstained slides	1 H&E stained slide <u>AND</u> 4 x 4-5 um Unstained slides	Unstained Slides for PD-L1 Testing (9.2)	Ambient / Dartmouth	1
<b>For patients registered to A091903 biobanking, submit the following:</b>						
Prior to treatment	Y	Fixed tissue block- tumor  <u>OR</u> H&E stained slide <u>AND</u> 10 um unstained slides	1  <u>OR</u> 1 H&E stained slide <u>AND</u> 5 x 10 um Unstained slides	Fixed tissue blocks (9.3)  <u>OR</u> Fixed tissue slides (9.4)	Ambient / ABWUSTL	2, 3, 7
Prior to treatment	Y	Whole blood (EDTA)	2 x 10 ml	Whole blood- EDTA tubes (10.1)	Ambient / ABWUSTL	2, 4, 7
Prior to treatment	Y	Whole blood for plasma	3 x 1 ml aliquots	Frozen plasma (10.2)	Dry Ice / ABWUSTL	2, 5, 7
Prior to treatment	Y	Whole blood for buffy coat	1 aliquot	Buffy Coat (10.3)	Dry Ice / ABWUSTL	2, 5, 7
Prior to treatment	Y	Stool	1 tube	Stool (11.0)	Ambient / ABWUSTL	2, 7
Week 4	Y	Whole blood (EDTA)	2 x 10 ml	Whole blood- EDTA tubes (10.1)	Ambient / ABWUSTL	2, 4
Week 4	Y	Whole blood for plasma	3 x 1 ml aliquots	Frozen plasma (10.2)	Dry Ice / ABWUSTL	2, 5
Week 4	Y	Whole blood for buffy coat	1 aliquot	Buffy Coat (10.3)	Dry Ice / ABWUSTL	2, 5
Recurrence/ Progression	Y	Fixed tissue block- tumor  <u>OR</u> H&E stained slide <u>AND</u> 10 um unstained slides	1  <u>OR</u> 1 H&E stained slide <u>AND</u> 5 x 10 um Unstained slides	Fixed tissue blocks (9.3)  <u>OR</u> Fixed tissue slides (9.4)	Ambient / ABWUSTL	2, 3, 6
Recurrence/ Progression	Y	Whole blood (EDTA)	2 x 10 ml	Whole blood- EDTA tubes (10.1)	Ambient / ABWUSTL	2, 4

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Recurrence/ Progression	Y	Whole blood for plasma	3 x 1 ml aliquots	Frozen plasma (10.2)	Dry Ice / ABWUSTL	2, 5
Recurrence/ Progression	Y	Whole blood for buffy coat	1 aliquot	Buffy Coat (10.3)	Dry Ice / ABWUSTL	2, 5

**Notes:**

1. Real time PDL1 testing using archival tissue from biopsies or surgical specimens is required for all patients pre-registered to A091903. Tissue must be submitted within 28 days of pre-registration. Please see section 9.2 for additional details.
2. All participating institutions must ask patients for their consent to participate in the banking of their specimens for future correlative studies, although patient participation is optional.
3. A representative, archived tumor tissue block from either the original primary tumor diagnostic biopsy or surgical resection specimen should be submitted, if available. If entire tissue block cannot be submitted, 1 H&E stained slide **AND** five (10 um) unstained slides will be accepted as an alternative. If tissue is limited, please submit H&E and as many unstained slides as possible. **BLOCK SUBMISSION IS STRONGLY PREFERRED.**
4. Whole blood (EDTA) 2 x 10 ml for PBMC isolation and cryopreservation at the Biorepository.
5. Peripheral blood (EDTA) 1 x 10 ml to be processed for plasma (3 x 1 ml aliquots) and buffy coat, frozen on site and shipped on dry ice.
6. A representative, archived tissue block from a recurrent or progressive tumor should be submitted, if available. If entire tissue block cannot be submitted, 1 H&E stained slide **AND** five (10 um) unstained slides will be accepted as an alternative. If tissue is limited, please submit H&E and as many unstained slides as possible. **BLOCK SUBMISSION IS STRONGLY PREFERRED.**
7. Optional biobanking prior to treatment occurs once. It can be done either after pre-registration or within 28 days of full registration.

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## 7. Biospecimen Collection Kits

### 7.1 Dartmouth Hitchcock Medical Center

- 7.1.1 There is no kit supplied for submission of slides to Dartmouth Hitchcock Medical Center for PD-L1 testing.
- 7.1.2 Slides should be packaged to avoid breakage using a padded envelope or, preferably, a small Styrofoam container.
- 7.1.3 During warm weather months, paraffin 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.
- 7.1.4 Slides may be shipped for priority overnight delivery according to institutional policies and using the preferred vendor. Sites are responsible for shipping costs.

### 7.2 ABWUSTL

- 7.2.1 To facilitate the proper collection and shipping of all biospecimens submitted to ABWUSTL, biospecimen collection kits and materials will be provided. The cost of the kit and shipping of 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.
  - 7.2.1.1 **NOTE:** Kits will be sent via FedEx at no additional cost to the participating institutions. **Kits will not be sent via rush delivery service unless the participating institution provides their own FedEx® account number or alternate billing number for express service.** The study will not cover the cost for rush delivery of kits.

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- 7.2.2** 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.
- 7.2.3** Distributed kits will have a minimum shelf life of 90 days; unless precluded by the stability of a particular component.
- 7.2.4** Kit contents and specific instructions for use of the kit are provided in the kit box. 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.
- 7.2.5** 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.
- 7.2.6** 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.
- 7.2.7** 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.
- 7.2.8** 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.



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**7.2.9** 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 incoming kits are tracked, and sites that have requested many more kits than they have returned will be charged for non-returned kits.

**7.2.10** 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 (i.e. OMNIgene GUT stool collection kits) and probably are not available at the institution.

**7.2.11** 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.

**7.2.12** Because paraffin 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:

**7.2.12.1** Blocks and slides should be packaged to avoid breakage using a padded envelope or, preferably, a small Styrofoam container.

**7.2.12.2** 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.

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

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**7.3** Please see **Section 12 – Biospecimen Shipping** for specific instructions on shipping to Dartmouth Hitchcock Medical Center and to ABWUJSTL.

## 8. Biospecimen Labeling and Tracking

**8.1** All research biospecimens (vacutainer tubes, cryovials, and tissue bags) **MUST** be labeled with the Alliance patient ID 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 slides are being submitted instead of the block, each tissue section slide should be labeled with the Alliance patient ID number, institutional surgical pathology number, the block identifier, and the serial section number (if applicable). Provide **a de-identified copy of the surgical pathology report**, labeled with the Alliance patient ID number, corresponding to the blocks or slides submitted. **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 Dartmouth Hitchcock Medical Center or 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@alliancenctn.org](mailto:bioms@alliancenctn.org).

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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, or tumor ‘debulking’) 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 Slides for PD-L1 Testing

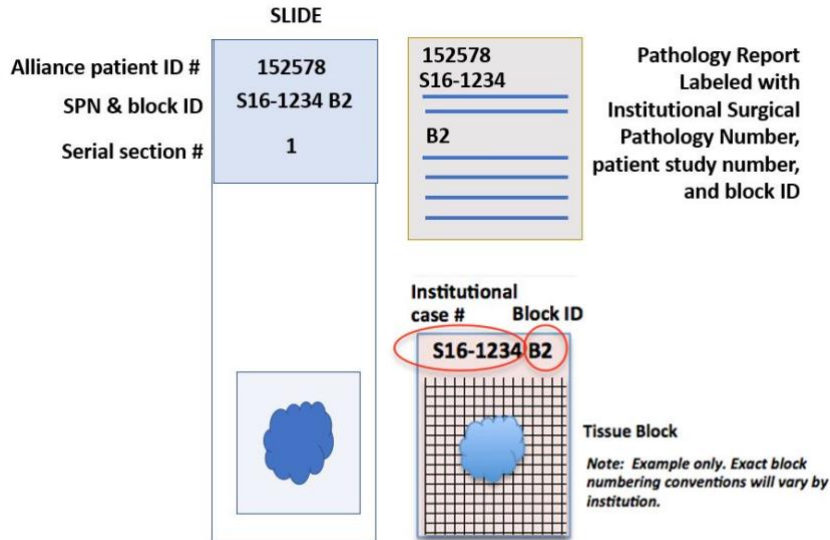
**9.2.1** Submission of fixed tissue slides from either the original primary tumor diagnostic biopsy or surgical resection is required for central PD-L1 testing. **One H&E stained slide and four (4) unstained slides should be submitted directly to Dartmouth Hitchcock Medical Center. The H&E stained slide should be from the same block from which the unstained slides were cut.** Tissue must be submitted directly to Dartmouth Hitchcock Medical Center within 28 days of pre-registration. Please follow the procedures below for submitting unstained tissue slides.

# of slides	Section thickness	Slide type	Purpose
4	4-5 micron	Positively Charged	Central PD-L1 testing

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- 9.2.2** Serial, tissue sections should be cut fresh (i.e. within 4 months of submission) from the appropriate formalin fixed, paraffin embedded tissue block.
- 9.2.3** Cut sections at 4-5 micron thickness as indicated onto positively charged slides.
- 9.2.4** Ensure that each slide is labeled with the Alliance patient ID number, the institutional surgical pathology number and block identifier, and the slide serial section number (1, 2, 3, 4).
- 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.

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- 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** The A091903 CLIA Laboratory PD-L1 Sample Submission Form must be submitted along with the slides to Dartmouth Hitchcock Medical Center. Failure to submit this form with the slides may delay turnaround time for central testing. The A091903 CLIA Laboratory PD-L1 Sample Submission Form can be located in **Appendix 1** of this manual or on the A091903 protocol-specific page on the CTSU and Alliance websites. A digital copy of the form and the shipment tracking number should be sent at time of shipment to [amber.j.barrows@hitchcock.org](mailto:amber.j.barrows@hitchcock.org).
- 9.2.13** A de-identified copy of the surgical pathology report should also accompany the slides and PD-L1 sample submission form.

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**9.2.14** Turnaround time is 7 business days from receipt of all required specimens and documents (i.e. slides, query free A091903 CLIA Laboratory PD-L1 Sample Submission Form, de-identified pathology report which matches the slides submitted). Dartmouth will reach out to sites if <4 unstained slides and 1 H&E stained slide are received or if there are missing or discrepant documents. If tissue has a QA issue (i.e. <100 tumor cells), the case will be considered not evaluable.

**9.2.15** Sites will receive results via secure email to the email address provided on the A091903 CLIA Laboratory PD-L1 Sample Submission Form. It is very important that the email address be entered correctly on the PD-L1 submission form to prevent delay. The cutoff for stratification factor is  $\geq 1\%$ .

### **9.3 Diagnostic Pathology Fixed Tissue Blocks.**

**9.3.1** This protocol requests submission of one representative, diagnostic pathology, formalin fixed paraffin embedded block from the original primary tumor diagnostic biopsy or surgical resection specimen at time of registration. An additional representative, diagnostic pathology, formalin fixed paraffin embedded block is requested at time of recurrence / progression, if applicable.

**9.3.2** 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.

**9.3.3** 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 (see **section 9.4**). **BLOCK SUBMISSION IS STRONGLY PREFERRED.**

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**9.3.4 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 (77degrees F) that may melt paraffin and damage the tissue specimens.**

#### **9.4 Unstained Slides from Diagnostic Fixed Tissue Blocks**

**9.4.1** In cases where institutions are unable or unwilling to submit the requested tissue blocks, sets of 5 unstained slides may be submitted as an alternative. **An H&E stained slide should accompany unstained slides. The H&E stained slide should be from the same block from which the unstained slides were cut.** If fewer than 5 unstained slides can be submitted, please submit as many as possible (up to 5 slides). 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	10 micron	Positively Charged	DNA, RNA

**9.4.2** Serial tissue sections should be cut fresh at 10 micron onto positively charged slides. Please follow mounting and labeling instructions located in **sections 9.2.4—9.2.11.**

**9.4.3** A de-identified surgical pathology report should accompany slide submission to ABWUSTL.

**9.4.4 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 (77degrees F) that may melt paraffin and damage the tissue specimens.**

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## 10. Blood Collection Methods

### 10.1 Whole blood (EDTA- no processing)

**10.1.1** Collect 10 ml of whole blood by standard venous phlebotomy technique into each of the 2 EDTA tubes (20 ml total). 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. **Blood should be collected Monday—Thursday only. Due to required processing, the tubes MUST be received at the Biorepository within 24 hours of collection.** 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 Processing

**10.2.1** Collect 10 ml of whole blood by standard venous phlebotomy technique into the purple top (EDTA) tube. Invert tube 10 times.

**10.2.2** Within 30 minutes of collection, spin the vacutainer tube at room temperature in a clinical centrifuge at 2500 xG for 15 minutes.

**10.2.3** Carefully remove the plasma layer (~3 ml), without touching the white, buffy coat layer, and transfer to a new, 15 ml conical centrifuge tube. Keep the vacutainer tube containing the white, buffy coat layer for white blood cell isolation (**section 10.3**).

**10.2.4** Spin the centrifuge tube containing plasma at room temperature in a clinical centrifuge at 2500 xG for 15 minutes.

**10.2.5** Label 3 cryovials as instructed in **section 8**. Make certain each vial is labeled completely and identically.



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**10.2.6** Carefully remove plasma without touching the pellet and divide into 1 ml labeled cryovials. Each 10 ml EDTA tube should yield 3 x 1 ml aliquots of plasma.

**10.2.7** 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.

### **10.3 Buffy Coat (White Blood Cell) Processing**

**10.3.1** Follow procedures in **section 10.2** for collecting and processing plasma from EDTA tubes.

**10.3.2** Label 1 cryovial as instructed in **section 8**.

**10.3.3** After removing the plasma, carefully remove the white, buffy coat white blood cell layer from the EDTA tube, avoiding the red blood cell mass as much as possible.

**10.3.4** Transfer the buffy coat layer (approximately 0.2 – 0.5 ml) from the EDTA tube into the labeled cryovial. Immediately freeze the cryovial 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 cryovial containing the buffy coat cells may be stored at -70 to -90 degrees C until ready for shipment on dry ice.

## **11. Stool Collection**

**11.1** Instruct patients to collect stool sample using the DNA Genotek OMNIgene GUT kit provided by the Biorepository. Stool collection should follow guidelines in the study protocol.

**11.2** After stool is collected, collection tube should be stored at room temperature. The stool sample must be received at the Biorepository within 45 days of collection. Ensure that the stool specimen 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.**

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## 12. Biospecimen Shipping

### 12.1 Overview

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

**12.1.2** Frozen plasma and buffy coat aliquots should be placed in a biohazard bag inside of a Styrofoam cooler and covered with 3 to 4 lbs (2 kg) of commercially-prepared dry ice. Pellets or chunks are preferred. Make sure the box is filled with dry ice and the weight of the dry ice is noted on the dry ice label on the outside of the shipping container. It is the local sites' responsibility to obtain dry ice when shipping frozen specimens. Specimens should be shipped according to IATA guidelines. **Frozen aliquots should be shipped to the Biorepository within 30 days of collection. Batch shipment of frozen aliquots is allowed. If sending specimens from multiple patients within a single shipment, please ensure all specimens are properly labeled and logged in the BioMS system. Specimens from each individual patient should be placed into their own biohazard bag that is clearly labeled with the Alliance patient ID number. The accompanying BioMS manifest should be sealed within each individual bag.**

**12.1.3** Place the original, completed copy of the BioMS packing manifest in the shipment. Do not send specimens without a completed BioMS Packing Manifest or substitute "BioMS Downtime Form." Biospecimens cannot be accepted without this completed form. If sending tissue, include a copy of the de-identified surgical pathology report. Tissue being submitted to Dartmouth Hitchcock Medical Center should also be accompanied by the **A091903 CLIA Laboratory PD-L1 Sample Submission Form**.

**12.1.4** All biospecimens should be shipped within the time frame specified in **sections 9-11** above. 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](mailto:alliance@email.wustl.edu) for further instructions, at least 24 hours prior to anticipated collection.

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

**12.2 Shipping to Dartmouth Hitchcock Medical Center**

**12.2.1** Enclose slide mailer containing H&E and unstained tissue slides within a padded envelope or small Styrofoam cooler. Ship for PRIORITY OVERNIGHT DELIVERY according to IATA guidelines and standard institutional policies.

**Notice of shipment should be sent, along with the A091903 CLIA Laboratory PD-L1 Sample Submission Form and shipment tracking number to [amber.j.barrows@hitchcock.org](mailto:amber.j.barrows@hitchcock.org).**

Using the preferred vendor, ship to:

**Laboratory for Clinical Genomics and Advanced Technology (CGAT)**

**Department of Pathology and Laboratory Medicine**

**4<sup>th</sup> floor, WTRB**

**Attn: Amber Barrows / Dr. Tsongalis**

**Dartmouth Hitchcock Medical Center**

**1 Medical Center Drive**

**Lebanon, NH 03756**

**Phone: 603-650-6821**

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### 12.3 Shipping to ABWUSTL

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

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**

### 13. ABWUSTL Biospecimen Receipt and Quality Assurance Measures

**13.1** Upon receipt, all biospecimens will be accessioned into the TPC informatics system, OpenSpecimen.

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

**13.3** 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.

**13.4** 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.

**13.5** 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.

**13.6** Aliquoted biofluids will be stored under liquid nitrogen vapor.

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**13.7** All biospecimens will remain in storage until additional processing or review is requested in writing by the appropriate protocol PI.

#### 14. Document History

Version	Description and Justification of Change	Author	Effective Date
3.1	Added contact table. Changed name of prior to treatment timepoint. Changed stool acceptability time to 45 days. Updated footnote 1 in collection table.	KAL	03/01/2024
3.0	Updated Dartmouth Requisition Form	PAA	05/02/2022
2.1	Increased quantity of unstained slides submitted to Dartmouth to 4	PAA	04/13/2022
2.0	Changed assay lab from Hematogenix to Dartmouth Fixed minor grammatical errors and typos	PAA	03/02/2022
1.0	New	PAA	09/30/2021

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**Appendix 1- A091903 CLIA Laboratory PD-L1 Sample Submission Form**



## A091903 CLIA Laboratory PD-L1 Sample Submission Form

The submission of these samples for PD-L1 testing is required for all patients registered to this study.

**This form must be filled, printed out, and submitted along with the slides to the lab at Dartmouth Hitchcock Medical Center. The form must be completed by typing; DO NOT HANDWRITE**

Failure to submit this form with the specimen may delay turnaround time for PD-L1 testing

Patient Initials: \_\_\_\_\_ Alliance Patient ID: \_\_\_\_\_ Date of Birth (DD/MM/YYYY) \_\_\_\_\_

Site Name: \_\_\_\_\_ Site CTEP ID: \_\_\_\_\_

Collection Date: \_\_\_\_\_

Courier Tracking Number: \_\_\_\_\_ Percentage of tumor cell content (If available) \_\_\_\_\_

Diagnosis \_\_\_\_\_

Specimens being submitted:  1 H & E slide  4 Unstained slides, 4 - 6 microns.

Responsible CRA Name: \_\_\_\_\_

CRA E-mail Address: \_\_\_\_\_

Phone Number: \_\_\_\_\_ Emergency Contact Number\*: \_\_\_\_\_

Alternate CRA Name: \_\_\_\_\_

E-mail Address: \_\_\_\_\_

Phone Number: \_\_\_\_\_ Emergency Contact Number\* \_\_\_\_\_

*\*Please provide a pager or cell phone number for questions outside of regular business hours.*

Comments (unusual circumstances during collection/processing of samples):

Shipped by \_\_\_\_\_ Shipment Date \_\_\_\_\_

**Laboratory for Clinical Genomics and Advanced Technology (CGAT)  
Department of Pathology and Laboratory Medicine, 4th floor, WTRB  
Attn: Amber Barrows/ Dr. Tsongalis  
Dartmouth Hitchcock Medical Center  
1 Medical Center Drive Lebanon, NH 03756**