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 A151804. 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 A151804 biospecimen collection, processing, and submission; including staff at satellite institutions.

2. Scope

This document applies to all biospecimens collected specifically for A151804 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
IO	Immuno-Oncology
irAE	Immune-related Adverse Event

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 A151804 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 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**.**

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.

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.

Time Point	Kit (Y/N)	Biospecimen	Quantity	Collection / Processing Method	Shipping	Notes
Pre-registration Specimens						
Pre-therapy	Y	Whole blood for plasma	3 x 1 ml aliquots	Frozen plasma (10.1)	Dry Ice	1, 2, 11
Pre-therapy	Y	Whole blood for "buffy coat"	2 aliquots	"Buffy Coat" (10.2)	Dry Ice	1, 2, 11
Pre-therapy	Y	Whole blood (EDTA)	2 x 10 ml	Whole blood- EDTA tubes (10.3)	Ambient	1, 3, 11
Pre-therapy	Y	Stool	1 tube	Stool (11.0)	Ambient	1, 4, 11
1 month (± 10 days) after pre-registration	Y	Whole blood for plasma	3 x 1 ml aliquots	Frozen plasma (10.1)	Dry Ice	1, 2, 11
1 month (± 10 days) after pre-registration	Y	Whole blood for "buffy coat"	2 aliquots	"Buffy Coat" (10.2)	Dry Ice	1, 2, 11
1 month (± 10 days) after pre-registration	Y	Whole blood (EDTA)	2 x 10 ml	Whole blood- EDTA tubes (10.3)	Ambient	1, 3, 11
1 month (± 10 days) after pre-registration	Y	Stool	1 tube	Stool (11.0)	Ambient	1, 4, 11
Registration Specimens						
≤ 7 days after registration	Y	Fixed tissue block-original primary tumor	1	Fixed tissue blocks (9.2)	Ambient	5, 6, 11
≤ 7 days after registration	Y	Stained tissue slides-original primary tumor	Case dependent	Stained tissue slides (9.3)	Ambient	5, 6, 11
≤ 7 days after registration	N	Scanned slide images-original primary tumor	Case dependent	Scanned slide images (9.4)	N/A	5, 6, 11
≤ 7 days after registration	Y	Fresh tissue biopsy-irAE	See below (9.0)	Frozen tissue (9.5)	Dry Ice	5, 7, 11, 12
≤ 7 days after registration	Y	Fresh tissue biopsy-irAE	See below (9.0)	Formalin fixation (9.6)	Ambient	5, 8, 11, 12
≤ 7 days after registration	Y	Fixed tissue block-irAE	1	Fixed tissue blocks (9.2)	Ambient	5, 11, 12
≤ 7 days after registration	Y	Stained tissue slides-irAE	Case dependent	Stained tissue slides (9.3)	Ambient	5, 11, 12

≤ 7 days after registration	N	Scanned slide images-irAE	Case dependent	Scanned slide images (9.4)	N/A	5, 11, 12
≤ 24 hours after registration	Y	Whole blood for plasma	3 x 1 ml aliquots	Frozen plasma (10.1)	Dry Ice	2, 5, 11
≤ 24 hours after registration	Y	Whole blood for "buffy coat"	2 aliquots	"Buffy Coat" (10.2)	Dry Ice	2, 5, 11
≤ 24 hours after registration	Y	Whole blood (EDTA)	2 x 10 ml	Whole blood- EDTA tubes (10.3)	Ambient	3, 5, 11
≤ 24 hours after registration	Y	Stool	1 tube	Stool (11.0)	Ambient	5, 9, 11
Post-Registration Specimens						
1 month (± 10 days) after registration	Y	Whole blood for plasma	3 x 1 ml aliquots	Frozen plasma (10.1)	Dry Ice	2, 11
1 month (± 10 days) after registration	Y	Whole blood for "buffy coat"	2 aliquots	"Buffy Coat" (10.2)	Dry Ice	2, 11
1 month (± 10 days) after registration	Y	Whole blood (EDTA)	2 x 10 ml	Whole blood- EDTA tubes (10.3)	Ambient	3, 11
1 month (± 10 days) after registration	Y	Stool	1 tube	Stool (11.0)	Ambient	9, 11
At irAE recurrence (if applicable)	Y	Fresh tissue biopsy	See below (9.0)	Frozen tissue (9.5)	Dry Ice	7, 10, 11
At irAE recurrence (if applicable)	Y	Fresh tissue biopsy	See below (9.0)	Formalin fixation (9.6)	Ambient	8, 10, 11
At irAE recurrence (if applicable)	Y	Fixed tissue block	1	Fixed tissue blocks (9.2)	Ambient	10, 11, 12
At irAE recurrence (if applicable)	Y	Stained tissue slides	Case dependent	Stained tissue slides (9.3)	Ambient	10, 11, 12
At irAE recurrence (if applicable)	N	Scanned slide images	Case dependent	Scanned slide images (9.4)	N/A	10, 11, 12
At irAE recurrence (if applicable)	Y	Whole blood for plasma	3 x 1 ml aliquots	Frozen plasma (10.1)	Dry Ice	2, 10, 11
At irAE recurrence (if applicable)	Y	Whole blood for "buffy coat"	2 aliquots	"Buffy Coat" (10.2)	Dry Ice	2, 10, 11

At irAE recurrence (if applicable)	Y	Whole blood (EDTA)	2 x 10 ml	Whole blood- EDTA tubes (10.3)	Ambient	3, 10, 11
At irAE recurrence (if applicable)	Y	Stool	1 tube	Stool (11.0)	Ambient	9, 10, 11

Notes:

1. For patients pre-registering prior to treatment with IO therapy, pre-registration samples can be obtained any time after consent but prior to initiation of IO therapy, and again after initiation of IO therapy 1 month ± 10 days after pre-registration. No pre-registration samples will be collected for patients who are pre-registered and registered concurrently at the time of confirmation of irAE(s).
2. Peripheral blood (EDTA) 1 x 10 ml to be processed for plasma (3 x 1-1.5 ml aliquots) and "buffy coat," frozen on site and shipped on dry ice.
3. Whole blood (EDTA, 2 x 10 ml) for PBMC isolation and cryopreservation at the Biorepository.
4. One stool sample obtained at each time point after pre-registration.
5. Registration is to occur within 96 hours of confirmation of G3-4 irAE event (for all patients regardless of the timing of pre-registration).
6. A representative, archived tumor tissue block from the original primary tumor diagnostic biopsy or surgical resection should be submitted, if available. If tissue block cannot be supplied due to limited availability, stained slides including a hematoxylin and eosin (H&E) stained slide and any others produced for histopathologic diagnosis may be submitted to the Biorepository for digital slide scanning prior to being returned to the site. Alternatively, whole slide image scan files of the stained slides in .SVS file format may be uploaded digitally to the Biorepository. If the tissue cannot be submitted at the time of registration, it may be submitted at a later timepoint.
7. Additional or residual fresh tissue from any diagnostic biopsy of solid tissue or cytology preparation from a needle aspiration procedure (e.g., cell pellet of bronchoalveolar lavage or cerebrospinal fluid), performed to evaluate pathology related to a presumed irAE (e.g. myocarditis, colitis, hepatitis) which is subsequently snap frozen (priority 1) and will be used for research purposes. If the tissue is collected but unable to be submitted at the time of registration or irAE recurrence, it may be submitted at a later time point. Submission of fresh tissue samples is not mandatory but strongly encouraged.
8. Additional or residual fresh tissue of any diagnostic biopsy from solid tissue or cytology preparation from a needle aspiration procedure (e.g., unstained cytospin slides or cell pellet of bronchoalveolar lavage or cerebrospinal fluid), performed to evaluate pathology related to a presumed irAE (e.g. myocarditis, colitis, hepatitis), which is subsequently formalin fixed (priority 2) and will be used for research purposes only. If the tissue is collected but unable to be submitted at the time of registration or irAE recurrence, it may be submitted at a later timepoint. Submission of fresh tissue samples is not mandatory but strongly encouraged.
9. One stool sample obtained at each time point after registration. This is mandatory for patients who are pre-registered prior to initiation of IO therapy, but optional for patients who are concurrently pre-registered and registered at the time of confirmation of one or more irAEs.

10. Recurrence is defined as an irAE having resolved to grade 1 or 0, and subsequently recurring at a grade level that would have been eligible for initial study registration. The recurrence time point applies only to patients consented after the release of update #02.
11. Based upon prospective experiences with biospecimen collections and feedback from clinical sites, collection criteria will be modified to optimize the number and quality of biospecimens collected without interfering with standard of care practices.
12. If additional or residual fresh tissue of diagnostic biopsy related to a presumed irAE or irAE recurrence is not available for submission, a representative fixed tissue block is requested. If tissue block cannot be supplied due to limited availability, stained slides including a hematoxylin and eosin (H&E) stained slide and any others produced for histopathologic diagnosis may be submitted to the Biorepository for digital slide scanning prior to being returned to the site. Alternatively, whole slide image scan files of the stained slides in .SVS file format may be uploaded digitally to the Biorepository. If the tissue cannot be submitted at the time of registration or irAE recurrence, it may be submitted at a later timepoint.

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 Alliance Biorepository at Washington University in St. Louis via priority overnight shipping.

7.2 An initial biospecimen collection kit should be requested at time of trial activation so a kit is on-hand to collect biospecimens at the time a patient experiences an irAE.

7.3 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.

7.4 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.**

7.5 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.

7.6 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.

7.7 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.

7.8 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.

7.9 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.

7.10 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 (e.g. stool collection tubes) and probably are not available at the institution.

7.11 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.

7.12 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:

7.12.1 There is no independent "kit" for submission of paraffin blocks or slides.

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

7.12.3 During warm weather months, paraffin blocks and slides should be shipped in an insulated container that contains a refrigerant pack, to avoid heat > 25 degrees C (77 degrees F) that may melt paraffin and damage the tissue specimens.

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

7.13 Please see **Section 12 – Biospecimen Shipping** for specific instructions on shipping to ABWUSTL.

8. Biospecimen Labeling and Tracking

8.1 All research biospecimens (vacutainer tubes, cryovials, and 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 slides 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, and the serial section number (if applicable). Provide **a de-identified copy of the surgical pathology report**, labeled with the patient study 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 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.

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 Regardless of the specific procedure used to obtain tissue, each individual tissue segment to be processed (i.e. frozen or fixed) must be 0.5 cm or less in one or more dimensions, to allow for rapid freezing or fixation.

9.1.3 In cases where a biopsy is performed and individual tissue segments are limiting, frozen tissue is the preferred method of preservation followed by formalin fixation. At least 3 cores, preferably with a 16 gauge needle, are requested to the extent clinically feasible.

9.1.4 Record the time when the tissue sample is first procured from the patient (i.e. via needle core biopsy, laparoscopic biopsy, or surgical resection) and the time at which the individual tissue segment is either frozen or placed into fixative. This time difference is warm ischemia time (WIT). The WIT should be less than 30 minutes, but even if it is not, accurate record keeping of this time is essential.

9.1.5 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.

9.1.6 Fresh tissue biospecimens collected for this protocol are for research purposes only and cannot be used for clinical management. Ensure that all tissue biospecimens needed for routine diagnostic standard of care are collected and deemed adequate PRIOR to submitting tissue specimens for this research study. No clinical pathology diagnosis will be rendered on submitted tissue biospecimens. Research tissue biospecimens (unlike diagnostic surgical pathology blocks- see **section 9.2**) will not be returned to the institution.

9.2 Diagnostic Pathology Fixed Tissue Blocks.

9.2.1 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. Additional blocks are requested from irAE and irAE recurrence, if unable to submit fresh biopsy.

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 stained slides including a H&E stained slide and any others produced for histopathologic diagnosis. Alternatively, whole slide image scan files of the stained slides in .SVS format may be uploaded to the Biorepository. See **sections 9.3** and **9.4.**

9.3 Stained Tissue Slides

9.3.1 In the event that it is not possible to submit the requested tissue block, then site should submit a Hematoxylin and Eosin (H&E) stained slide and any others produced for histopathologic diagnosis to the Biorepository for scanning.

9.3.2 Any stained slide submitted for scanning must be labeled as indicated in **section 8**.

9.3.3 Submitted slides will be scanned at the Alliance Biorepository and slides will be returned to the site within 14 days.

9.3.4 Deidentified, whole slide image scans will be stored, distributed, and used by approved investigators for future correlative studies involving histopathology image analysis.

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

9.4 Scanned Slide Images

9.4.1 As an alternate to submitting stained tissue slides, sites may instead submit a whole slide image scan (WSI) at 40X magnification of an H/E stained slide and any others produced for histopathologic diagnosis, in .SVS file format.

9.4.2 Please contact the BioMS Helpdesk via email (bioms@alliancenctn.org) for further instruction on appropriate image labeling, file naming conventions, and how to submit digital pathology image files.

9.4.3 Scanned images should not contain PHI (patient name, date of birth).

9.4.4 Deidentified, whole slide image scans will be stored, distributed, and used by approved investigators for future correlative studies involving histopathology image analysis.

9.5 Fresh Tissue Biopsy-Frozen Tissue

9.5.1 Prior to procurement, prepare tissue for freezing by placing approximately six pounds of crushed dry ice into the bottom compartment of a Styrofoam cooler. Place a metal freezing plate on top of the dry ice and allow the surface of the plate to reach the approximate temperature of the dry ice.

9.5.1.1 An alternative method is to use the freezing plate found on a pathology cryostat.

9.5.1.2 An alternative method is to use a flat surface of a dry ice block.

9.5.1.3 An alternative method is to use a commercially available Cryocooler (OPS Diagnostics) which uses a metal platform and a liquid nitrogen saturated "pillow" to achieve freezing temperatures of -130 degrees C.

9.5.1.4 Do not freeze tissue by placing warm tissue in a -70 to -90 degree Celsius ultralow freezer.

9.5.1.5 Do not freeze tissue using a dry ice ethanol bath.

9.5.1.6 Do not freeze tissue by submersion in an isopentane cryobath.

9.5.2 Label one tissue cryomold for every tissue core that is to be frozen. Ensure that the cryomold(s) and tissue bag(s) are labeled with the participant study number as instructed in **section 8**.

9.5.3 Working quickly, gently place the tissue length-wise in the mold. Place the cryomold on the level cold plate or flat, level surface of dry ice. Allow the tissue to freeze for 3-5 minutes.

9.5.4 Once frozen, quickly wrap the mold with the tissue block in cooled foil and place the block in the corresponding labeled tissue bag. Maintain the tissue block buried in dry ice, in a -70 to -90 degree C freezer, or in liquid nitrogen vapor (not liquid phase) until ready for shipment. Tissue should be shipped to the Biorepository as soon as possible following procurement.

9.5.5 Repeat the above steps for each individual tissue core or biopsy specimen that is to be frozen.

9.6 Fresh Tissue Biopsy-Formalin Fixation

9.6.1 Label the formalin fixative vial with the participant study number, as instructed in **section 8**. Be certain to record the date and time that the tissue is placed into the formalin vial.

9.6.2 Place the fresh tissue core into the vial and secure the lid with parafilm. Ensure that the tissue is completely submerged into the formalin fixative.

9.6.3 Store and ship the formalin fixed tissue at ambient temperature. If possible, to avoid prolonged fixation, ship the tissue on the same day it is collected.

10. Blood Collection Methods

10.1 Plasma Processing

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

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

10.1.3 Carefully remove the plasma layer (~3—5 ml in volume), 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 10.2)**.

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

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

10.1.6 Carefully remove 3 ml of plasma (without touching the pellet) and divide into 3, 2 ml labeled cryovials. Each aliquot should be between 1—1.5 ml in volume.

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.

10.2 "Buffy Coat" (White Blood Cell) Processing

10.2.1 Follow procedures in **section 10.1** for collecting and processing plasma from EDTA tube.

10.2.2 Label 2 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 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.

10.3 Whole blood (EDTA- no processing)

10.3.1 Collect whole blood by standard venous phlebotomy technique into each of the EDTA tubes. Invert tubes 10 times.

10.3.2 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.**

11. Stool Collection

11.1 Instruct patients to collect stool sample following 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 10 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.**

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 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.

12.1.3 All biospecimens should be shipped within the timeframes indicated above in **sections 9, 10 and 11**. If collected biospecimens cannot be shipped within the specified timeframe (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.

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

12.1.5 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 at Washington University in St. Louis
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. 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 Frozen tissues and aliquoted biofluids will be stored under liquid nitrogen vapor.

13.7 Fixed tissue biospecimens will be processed and embedded into paraffin using TPC standard operating procedures.

13.8 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
3.0	Removed cryopreserved tissue Updated biospecimen collection schedule to match updated protocol Include provisions for shipping during warm weather months Include alternatives to fresh tissue biopsy for irAE tissue Removed stool collection instructions and point sites to protocol	PAA	12/07/2021
2.0	Updated PM email Provided instructions for reduced blood collection Provided instructions for stained slides and scanned slide images Updated biopsy submission guidelines	PAA	10/14/2020
1.0	New	PAA	10/23/2019