**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 A021806. 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 A021806 biospecimen collection, processing, and submission, including staff at satellite institutions.

## 2. Scope

This document applies to all biospecimens collected specifically for A021806 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 |
| SOC | Standard of Care |

## 4. Contact Information

| Protocol-related questions may be directed as follows: | |
|---|---|
| **Questions** | **Contact (via email)** |
| Questions regarding patient eligibility, treatment and dose modificiation | Study Chair: Cristina R. Ferrone, MD<br>cristina.ferrone@cshs.org<br>Nursing Contact: Barbara Kleiber, RN<br>Barbara.kleiber@osumc.edu<br>Protocol Coordinator: Jamie Crawley<br>jcrawley@bsd.uchicago.edu<br>Data Manager: Pamela Fain Pribyl<br>fainpribyl.pamela@mayo.edu |
| Questions related to data submission, RAVE, or patient follow-up | Data Manager: Pamela Fain Pribyl<br>fainpribyl.pamela@mayo.edu |
| Questions regarding the protocol document and model informed consent | Protocol Coordinator: Jamie Crawley<br>jcrawley@bsd.uchicago.edu |
| Questions related to IRB review | Alliance Regulatory Inbox<br>regulatory@allianceNCTN.org |
| Questions regarding CTEP-AERS reporting | Alliance Pharmacovigilance Inbox<br>pharmacovigilance@alliancenctn.org |
| Questions regarding specimens/specimen submission | Alliance Biorepository at Washington University (ABWUSTL)<br>alliance@email.wustl.edu |
| Questions regarding drug administration | Pharmacy Liasion: Maria Andrea Monckeberg, MS, RPh<br>mamonckeberg@lifespan.org |

**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. 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.

**4.2** 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.

## 5. Site Preparation

**5.1** Please refer to A021806 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.

**5.3** Prior to collection of stool specimens, 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. No kits are provided for collection of blood or tissue specimens.

**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. 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 |
|---|---|---|---|---|---|---|
| **Mandatory for all patients registered to A021806** | | | | | | |
| At surgery | N | Tumor tissue for central pathology review | 1 block **OR** 1 (5 um) unstained slide **AND** 15-20 (10 um) unstained slides | Tissue for central pathology review (9.2) | Ambient | 1 |
| **For patients registered to A021806 Biobanking** | | | | | | |
| Prior to initiation of therapy | N | Archival tumor tissue from diagnostic biopsy / FNA | 1 block **OR** 1 H&E stained slide **AND** 3 (10 um) unstained slides | Fixed tissue block (9.3); Tissue slides (9.4) | Ambient | 2, 3 |
| Prior to initiation of therapy | N | Fresh tissue from baseline biopsy (if SOC) | 1 H&E stained slide **AND** 1-3 cores embedded into paraffin block | Fresh tissue biopsy (9.5) | Ambient | 2, 3 |
| Prior to initiation of therapy | N | Whole blood for plasma | 12 x 1 ml aliquots | Frozen Plasma (10.1) | Dry Ice | 2, 4 |
| Prior to initiation of therapy | N | Whole blood for "buffy coat" | 3 aliquots | "Buffy coat" (10.2) | Dry Ice | 2, 4 |
| Prior to initiation of therapy | Y | Stool | 1 tube | Stool (11.0) | Ambient | 2 |
| | | | | | | |
| Prior to surgery | N | Whole blood for plasma | 12 x 1 ml aliquots | Frozen Plasma (10.1) | Dry Ice | 2, 4 |
| Prior to surgery | N | Whole blood for "buffy coat" | 3 aliquots | "Buffy coat" (10.2) | Dry Ice | 2, 4 |
| Prior to surgery- **ARM 1 ONLY** | Y | Stool | 1 tube | Stool (11.0) | Ambient | 2, 5 |
| | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| After 4 cycles of adjuvant therapy | N | Whole blood for plasma | 12 x 1 ml aliquots | Frozen Plasma (10.1) | Dry Ice | 2, 4 |
| After 4 cycles of adjuvant therapy | N | Whole blood for "buffy coat" | 3 aliquots | "Buffy coat" (10.2) | Dry Ice | 2, 4 |
| After 4 cycles of adjuvant therapy- **ARM 2 ONLY** | Y | Stool | 1 tube | Stool (11.0) | Ambient | 2, 6 |
| | | | | | | |
| End of treatment- **ARM 2 ONLY** | N | Whole blood for plasma | 12 x 1 ml aliquots | Frozen Plasma (10.1) | Dry Ice | 2, 4, 6 |
| End of treatment- **ARM 2 ONLY** | N | Whole blood for "buffy coat" | 3 aliquots | "Buffy coat" (10.2) | Dry Ice | 2, 4, 6 |
| End of treatment | Y | Stool | 1 tube | Stool (11.0) | Ambient | 2 |
| | | | | | | |
| At recurrence | N | Whole blood for plasma | 12 x 1 ml aliquots | Frozen Plasma (10.1) | Dry Ice | 2, 4 |
| At recurrence | N | Whole blood for "buffy coat" | 3 aliquots | "Buffy coat" (10.2) | Dry Ice | 2, 4 |
| At recurrence | N | Fresh tissue from recurrence biopsy (if SOC) | 1 H&E stained slide **AND** 1-3 cores embedded into paraffin block | Fresh tissue biopsy (9.5) | Ambient | 2, 3 |

**Notes:**

1. Retrospective histopathology review will be conducted using the paraffin-embedded pancreatic tissue from the surgical specimens. The submission of these samples for histopathology review is required for all patients registered to this study, including those who are found to be ineligible and those who do not receive protocol therapy. One fixed tissue block **OR** 1 (5 um) unstained slide **AND** 15-20 (10 um) unstained slides should be submitted. Block submission is highly preferred.
2. Collection is optional for patients but requires all sites offer to patients to consent. Please see protocol-specific consent documents.

3. Submission of an archival tumor tissue block **OR** 1 H&E stained slide and three (3) unstained slides at 10 um from diagnostic biopsy / FNA is requested. If tissue is limited, submit as many slides as possible up to 3. If a fresh biopsy is obtained as standard of care, 1 H&E and 1-3 tissue cores embedded into a paraffin block can be submitted as an alternative to the archival tumor tissue block.
4. Peripheral blood (EDTA) 3 x 10 ml to be processed for plasma (1 ml aliquots) and "buffy coat," frozen on site and shipped on dry ice.
5. Specimen should only be collected from patients on Arm 1.
6. Specimen should only be collected from patients on Arm 2.

## 7. Biospecimen Collection Kits

### 7.1 Stool Specimens

**7.1.1** To facilitate the proper collection and shipping of stool specimens, 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 stool back to the Alliance Biorepository at Washington University in St. Louis via **FedEx priority overnight shipping. Stool specimens must be received by the Biorepository within 45 days of collection. Please be aware that the kits only contain materials for stool collection. EDTA tubes and cryovials for blood and plasma collection are not included. Please use your local supply for the EDTA whole blood collection and processing.**

**7.1.2** Kits should be requested at least 10 working days in advance of the anticipated collection date. As many as 3 kits can be requested at one time. Since the collection materials (stool collection 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.

**7.1.3** 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.

**7.1.4** Distributed kits will have a minimum shelf life of 90 days; unless precluded by the stability of a particular component.

**7.1.5** Kit contents and specific instructions for use of the kit are provided in the kit box.

**7.1.6 Once a kit is received, please retain the outer two-way mailer box. The kit, containing stool specimens, is to be shipped back to the Biorepository in the same box in which it was received.**

**7.1.7** Well in advance of collecting stool, inspect the collection kit to ensure that all components are present and not expired, particularly if the kit has been onsite for longer than 30 days.

**7.1.8** 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).

### 7.2 Tissue Specimens

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

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

**7.2.3** 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.

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

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

### 7.3 Blood Specimens

**7.3.1** There is no independent "kit" for submission of frozen plasma or buffy coat aliquots. Sites are responsible for acquiring materials for collection and shipping of these specimens to the Biorepository.

**7.3.2** 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, stool collection tubes, cryovials, 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 sections 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, section thickness (in um), and the serial section number. Provide **a de-identified copy of the surgical pathology report**, labeled with the Alliance patient ID 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.

**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.

**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** 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.

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

### 9.2 Diagnostic Pathology Fixed Tissue for Central Pathology Review

**9.2.1** Retrospective central pathology review requires submissision of one (1) diagnostic tumor tissue block. In cases where an institution is unwilling or unable to submit a tissue block for central pathology review, 1 (5 micron) unstained slide AND 15-20 (10 micron) unstained slides cut from such a block may be sent as an alternative. Block submission is highly preferred.

**9.2.2** Please follow the procedures below for submitting unstained slides.

| **# of slides** | **Section thickness** | **Slide type** | **Purpose** |
|---|---|---|---|
| 1 | 5 micron | Charged | H&E stained slide |
| 15-20 | 10 micron | Non-Charged | DNA, RNA |

**9.2.3** Serial tissue sections should be cut fresh from the appropriate formalin fixed, paraffin embedded tissue block.

**9.2.4** Cut sections at 5 or 10 micron thickness onto glass slides (charged or non-charged) as indicated above.

**9.2.5** Ensure that each slide is labeled with the Alliance patient ID number, the institutional surgical pathology number and block ID, section thickness (in um) and the slide serial section number (1, 2, 3, etc.).

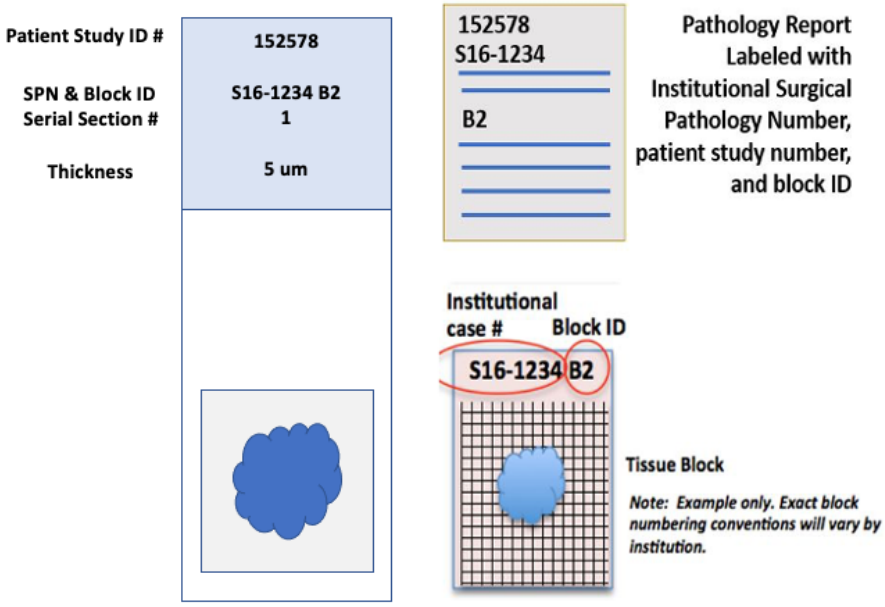
**9.2.6** Do not label slides with adhesive labels. Write or print information on the textured surface of the slide with indelible, solvent-resistant ink.

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

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

**9.2.9** 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.

**9.2.10** See figure below for proper mounting and labeling.




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

**9.2.12** 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.

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

### 9.3 Diagnostic Pathology Fixed Tissue Blocks.

**9.3.1** For patients who consent to A021806 biobanking for future research, a representative diagnostic block from the biopsy / FNA should be submitted, 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 tumor tissue block, the institution may instead submit an H&E stained slide and tissue sections, mounted and unstained to glass slides (**see section 9.4)**.

### 9.4 Slides from Diagnostic Fixed Tissue Blocks for Biobanking

**9.4.1** In cases where institutions are unable or unwilling to submit the requested tissue block, an H&E stained slide and a set of 3 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.

**9.4.2** All unstained slides will remain property of the Biorepository. However, H&E stained slides will be scanned and returned to the submitting institution upon request.

| # of slides | Section thickness | Slide type | Purpose |
|---|---|---|---|
| 3 | 10 micron | Charged | Biobanking |

**9.4.3** Follow sectioning and labeling instructions outlined in **section 9.2** and in the table above.

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

**9.4.5** If archival tissue is not available and patient has undergone new biopsy as standard of care, the re-embedded core biopsy block should be submitted for biobanking for consented patients (**see section 9.5**).

### 9.5 Fresh Tissue Biopsy

**9.5.1** If archival tissue is not available and patient has undergone new biopsy as standard of care, the re-embedded core biopsy block containing 1-3 cores should be submitted for biobank for consented patients.

**9.5.2** One (1) H&E stained slide corresponding to the block should also be submitted.

## 10. Blood Collection Methods

### 10.1 Plasma Processing

**10.1.1** Collect whole blood by standard venous phlebotomy technique into each of 3, 10 ml purple top (EDTA) tubes (total draw volume 30 ml). Invert tubes 10 times.

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

**10.1.3** Carefully remove the plasma layer from each tube (~4 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 12 cryovials as instructed in **section 8**. Make certain each vial is labeled completely and identically.

**10.1.6** Carefully remove plasma (without touching the pellet) and divide into 12, 1 ml labeled cryovials.

**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 tubes.

**10.2.2** Label 3 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 tubes 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. 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.**

## 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 stool specimens.

**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. 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.

**12.1.4** **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.

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. 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** Frozen aliquoted biofluids will be stored under liquid nitrogen vapor.

**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 |
|---|---|---|---|
| 2.0 | Updated biospecimen collection to align with protocol amendment<br>Updated contact information<br>Removed stool collection instructions<br>Added provisions for shipping batch aliquots | PAA | 05/09/2023 |
| 1.1 | Updated hyperlinks | PAA | 06/26/2020 |
| 1.0 | New | PAA | 05/05/2020 |