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 A032001. 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 A032001 biospecimen collection, processing, and submission; including staff at satellite institutions.

2. Scope

This document applies to all biospecimens collected specifically for A032001 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

4. Contact Information

Protocol-related questions may be directed as follows:	
Questions	**Contact (via email)**
Questions regarding patient eligibility, treatment, and dose modification:	Study Chair: Shilpa Gupta, MD guptas5@ccf.org Nursing Contact: Archana Ajmera aajmera@health.ucsd.edu Protocol Coordinator: Shiva Baghaie, MPH sbaghaie@bsd.uchicago.edu Data Manager: Alaina Carlson carlson.alaina@mayo.edu
Questions related to data submission, RAVE or patient follow-up:	Data Manager: Alaina Carlson carlson.alaina@mayo.edu
Questions regarding the protocol document and model informed consent:	Protocol Coordinator: Shiva Baghaie, MPH sbaghaie@bsd.uchicago.edu
Questions related to IRB review	Alliance Regulatory Inbox regulatory@allianceNCTN.org
Questions regarding CTEP-AERS reporting:	Alliance Pharmacovigilance Inbox pharmacovigilance@alliancenctn.org
Questions regarding specimens/specimen submissions:	Alliance Biorepository at Washington University (ABWUSTL) alliance@email.wustl.edu
Questions regarding drug supply	Pharmaceutical Management Branch, CTEP/DCTD/NCI PMBAfterHours@mail.nih.gov
Questions regarding drug administration	Pharmacy Contact: Jerline Hsin jhsin@coh.org

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 A032001 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
For patients registered to A032001-ST1						
After consent, prior to start of study treatment	N	Fixed tissue block	1 block	Fixed tissue block (9.2)	Ambient	1, 4, 6
After consent, prior to start of study treatment	N	Unstained tissue slides	10 (5 um) slides AND 15 (10 um) slides	Fixed tissue slides (9.3)	Ambient	1, 4, 6
After consent, prior to start of study treatment	Y	Whole blood (EDTA)	3 x 10 ml	Whole blood for PBMC-EDTA tubes (10.1)	Ambient	1, 2
After consent, prior to start of study treatment	Y	Whole blood for plasma	6 x 1 ml aliquots	Frozen plasma for Cytokine Analysis (10.2)	Dry Ice	1, 5
C2D1	Y	Whole blood (EDTA)	3 x 10 ml	Whole blood for PBMC-EDTA tubes (10.1)	Ambient	1, 2
C2D1	Y	Whole blood for plasma	6 x 1 ml aliquots	Frozen plasma for Cytokine Analysis (10.2)	Dry Ice	1, 5
Progression / End of Treatment	Y	Whole blood (EDTA)	3 x 10 ml	Whole blood for PBMC-EDTA tubes (10.1)	Ambient	1, 2, 3
Progression / End of Treatment	Y	Whole blood for plasma	6 x 1 ml aliquots	Frozen plasma for Cytokine Analysis (10.2)	Dry Ice	1, 3, 5
For patients consented to A032001 Biobanking						
After consent, prior to start of study treatment	Y	Whole blood (Streck BCT)	2 x 10 ml	Plasma for cfDNA (10.3)	Ambient	1
After consent, prior to start of study treatment	Y	Urine	50 ml	Urine (11.0)	Ambient	1
After consent, prior to start of study treatment	Y	Stool	1 tube	Stool (12.0)	Ambient	1
C2D1	Y	Whole blood (Streck BCT)	2 x 10 ml	Plasma for cfDNA (10.3)	Ambient	1
C2D1	Y	Urine	50 ml	Urine (11.0)	Ambient	1

Progression / End of Treatment	Y	Whole blood (Streck BCT)	2 x 10 ml	Plasma for cfDNA (10.3)	Ambient	1, 3
Progression / End of Treatment	Y	Urine	50 ml	Urine (11.0)	Ambient	1, 3
Progression / End of Treatment	Y	Stool	1 tube	Stool (12.0)	Ambient	1, 3

Notes:

1. Collection is optional for patients but requires all sites offer to patients during consent. Please see protocol-specific consent documents.
2. Whole blood (EDTA) for PBMC isolation and cryopreservation at the Biorepository.
3. Progression samples may be collected and submitted up to 3 months after progression.
4. A representative, archived tumor tissue block should be submitted, if available. If entire tissue block cannot be submitted, 10 unstained slides (5 um) AND 15 unstained slides (10 um) will be accepted. If tissue is limited, please submit as many unstained slides as possible. Please refer to **section 9.3** for further details. **BLOCK SUBMISSION IS STRONGLY PREFERRED.**
5. Whole blood (2 x 10 ml EDTA) will be collected and spun on site. Frozen plasma aliquots will be shipped to the Biorepository. Please refer to **section 10.2** for further details.
6. If tissue is unable to be submitted prior to start of study treatment, it may be submitted up to 120 days after patient starts treatment. All other specimens must be collected and submitted prior to start of study treatment.

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 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.

7.2.1 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.

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

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.

7.8.1 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.

7.8.2 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.

7.9 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.

7.10 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:

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

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

7.10.3 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.

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

7.11 Please see **Section 13 – 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).

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 study number (A032001), patient study number, institutional surgical pathology number, the block identifier, section thickness (in um), 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 identifier 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) 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 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.

9.2 Diagnostic Pathology Fixed Tissue Blocks for ST1

9.2.1 For patients who consent to A032001-ST1, one representative diagnostic tumor tissue block is requested.

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 tissue blocks, the institution may instead submit a set of twenty-five (25) unstained slides from the requested block. **BLOCK SUBMISSION IS STRONGLY PREFERRED**. Please refer to **section 9.3** for more details.

9.3 Unstained Tissue Slides from Diagnostic Fixed Tissue Block for ST1

9.3.1 In cases where an institution is unwilling or unable to submit tissue blocks, a set of twenty-five (25**)** unstained slides cut from a single block and containing adequate tumor cellularity may be sent as an alternative. If fewer than 25 unstained slides can be submitted, please prioritize slides for IHC and submit as many as possible for DNA / RNA (up to 25 unstained slides total). Please follow the procedures below for submitting unstained slides. **If your pathology department is unwilling or unable to follow these tissue-sectioning instructions, please consider submission of tissue blocks, which can be cut at the Biorepository and returned to your institution at a later date.**

# of slides	Section thickness	Slide type	Purpose
10	5 micron	Charged	IHC
15	10 micron	Non-Charged	DNA, RNA

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

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

9.3.4 Ensure that each slide is labeled with the Alliance study number (A032001), patient study number, the institutional surgical pathology number and block identifer, section thickness (in um), and the slide serial section number (1, 2, 3, etc.).

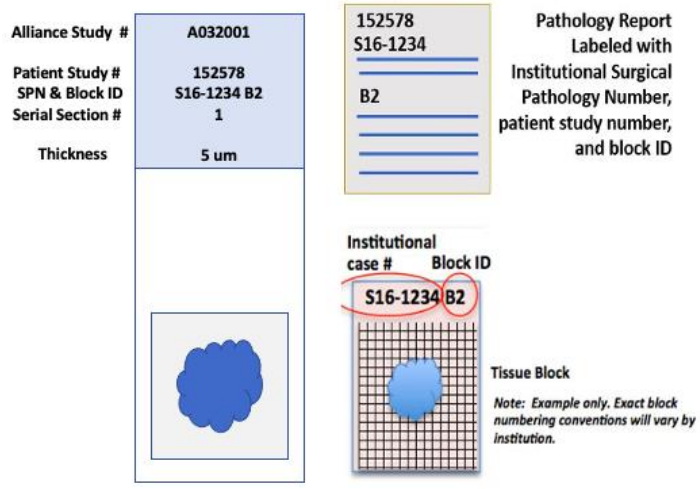
9.3.5 Do not label slides with adhesive labels. Write or print information on the textured surface of the slide with indelible, solvent-resistant ink.

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

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

9.3.8 When placing the sections onto the slides, ensure that the tissue is placed on the bottom third of the slide. Ensure that each serial section from the block is placed in the same orientation on each slide.

9.3.9 See figure below for proper mounting and labeling.



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

9.3.11 Use slide mailers or a slide box to ship slides. Slides should not be touching each other. Ensure that slides from only one patient are placed in one slide mailer.

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

10. Blood Collection Methods

10.1 Whole blood for PBMC (EDTA- no processing)

10.1.1 Collect 10 ml of whole blood by standard venous phlebotomy technique into each of the purple top (EDTA) tubes. A total of 30 ml of whole blood should be collected into the EDTA tubes (3 x 10 ml). Following collection, 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 for Cytokine Analysis (EDTA- plasma processing)

10.2.1 Collect 10 ml of whole blood by standard venous phlebotomy technique into each of the purple top (EDTA) tubes. A total of 20 ml of whole blood should be collected into the EDTA tubes (2 x 10 ml). Following collection, invert tubes 10 times.

10.2.2 The plasma should be spun and aliquoted as soon as possible. This is ideally within 30 minutes, but more practically, within 3-4 hours of collection in a busy clinic. More than 6 hours is not acceptable.

10.2.3 Spin the vacutainer tubes at room temperature in a clinical centrifuge at 2500 xG for 15 minutes.

10.2.4 Carefully remove the plasma layer (~3—5 ml in volume), without touching the white, buffy coat layer, and transfer to new 15 ml conical centrifuge tubes.

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

10.2.6 Label 6 cryovials as instructed in **section 8**. Make certain each vial is labeled completely and identically.

10.2.7 Carefully remove 6 ml of plasma (without touching the pellet) and divide into 6, 2 ml labeled cryovials. Each aliquot should be between 1—1.5 ml in volume.

10.2.8 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 Plasma Nucleic Acid (Streck) Tube Processing

10.3.1 Collect 10 ml of whole blood by standard venous phlebotomy technique into each of the Streck tubes. A total of 20 ml of whole blood should be collected into the Streck tubes (2 x 10 ml). Following collection, invert tubes 10 times.

10.3.2 Store Streck tubes with whole blood at room temperature. Do not freeze or refrigerate the tubes. The tubes may be stored for up to 72 hours at ambient temperature before shipment. Ensure that the Streck 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. Urine Collection

11.1 Instruct patients to collect approximately 50 ml of urine into a **clean, untreated** specimen cup. It is very important that an untreated specimen cup be used. Introduction of other reagents may interfere with stabilization activity of the Streck Urine Preserve.

11.2 **Within 2 hours of urine collection**, add the entire contents (5 ml) of the Streck Urine Preserve to the urine sample. Seal the specimen collection cup and gently invert the urine sample between 3 to 5 times to mix the urine and Urine Preserve.

11.3 Urine in Streck Urine Preserve may be held for up to 72 hours at room temperature prior to shipping. Do not freeze or refrigerate the urine. Ensure that the urine 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. Stool Collection

12.1 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.

12.2 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.**

13. Biospecimen Shipping

13.1 Overview

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

13.1.2 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.

13.1.3 All biospecimens should be shipped within the timeframes indicated above in **sections 9, 10, 11, and 12**. 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.

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

13.1.5 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

14. Biospecimen Receipt and Quality Assurance Measures

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

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

14.3 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.

14.4 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.

14.5 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.

14.6 Aliquoted biofluids will be stored under liquid nitrogen vapor.

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

15. Document History

Version	Description and Justification of Change	Author	Effective Date
2.0	Added contact table, note about tissue submission up to 120 days after pt starts tx, information about kit shipping and shelf life, warm weather shipping instructions for tissue, relaxed plasma processing time, changed omnigene receipt window from 10 to 45 days, removed reference to blank airbill being provided in kits	KAL	05/08/2023
1.3	Relaxed processing time for plasma from 30 mins to 2 hours	PAA	04/15/2022
1.2	Added standard Biorepository language stating submission of FFPE block is strongly preferred over slides	PAA	01/21/2022
1.1	Added name of stool collection kit	PAA	01/21/2022
1.0	New	PAA	01/19/2022