

ALLIANCE FOR CLINICAL TRIALS IN ONCOLOGY	CORRELATIVE SCIENCE PROCEDURE MANUAL Biospecimen Collection for Randomized double-blind phase III trial of vitamin D supplementation in patients with previously untreated metastatic colorectal cancer (SOLARIS) Short Title- A021703 (SOLARIS)	Version No: 4.2	Effective Date: 04/07/2023
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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 A021703. 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 A021703 biospecimen collection, processing, and submission, including staff at satellite institutions.

2. Scope

This document applies to all biospecimens collected specifically for A021703 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
IHC	Immunohistochemistry

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4. Contact Information

- 4.1** For questions and problems related to protocol administration, eligibility, patient registration, and data submission, relevant contact information is listed on protocol pages 1 and 2.
- 4.2** For information on using the BioMS system, please refer to the ‘Help’ links on the BioMS webpage to access the online user manual, FAQs, and training videos. To report technical problems, such as login issues or application errors, please contact: 1-855-55-BIOMS or bioms@alliancenctn.org. For assistance in using the application or questions or problems related to specific specimen logging, please contact: 1-855-55-BIOMS or bioms@alliancenctn.org.
- 4.3** For all other questions regarding biospecimen procurement and shipping procedures, please contact the Alliance Biorepository Program Manager: 1-314-747-4402 or alliance@email.wustl.edu.

5. Site Preparation

- 5.1** Please refer to A021703 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 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.

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6. Collection Schema

The following biospecimens are to be collected at each of the time points. 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	Notes
Mandatory for all patients registered to A021703						
Prior to initiation of treatment	N	Whole blood for plasma	2 x 1 ml aliquots	Frozen Plasma (10.1)	Dry Ice	1
Prior to initiation of treatment	N	Whole blood for "buffy coat"	1 aliquot	"Buffy Coat" (10.2)	Dry Ice	1
A021703 Biobanking						
Prior to treatment on C5D1	N	Whole blood for plasma	2 x 1 ml aliquots	Frozen Plasma (10.1)	Dry Ice	1
Prior to treatment on C5D1	N	Whole blood for "buffy coat"	1 aliquot	"Buffy Coat" (10.2)	Dry Ice	1
A021703 Biobanking						
Prior to treatment on C9D1	N	Whole blood for plasma	2 x 1 ml aliquots	Frozen Plasma (10.1)	Dry Ice	1
Prior to treatment on C9D1	N	Whole blood for "buffy coat"	1 aliquot	"Buffy Coat" (10.2)	Dry Ice	1
A021703 Biobanking						
At treatment discontinuation	N	Whole blood for plasma	2 x 1 ml aliquots	Frozen Plasma (10.1)	Dry Ice	1
At treatment discontinuation	N	Whole blood for "buffy coat"	1 aliquot	"Buffy Coat" (10.2)	Dry Ice	1
A021703 Biobanking						
Prior to initiation of treatment	N	Fixed tissue block	1	Fixed tissue block (9.2)	Ambient	2, 3

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Prior to initiation of treatment	N	Unstained Slides	18	Fixed tissue slides (9.3)	Ambient	2, 3
Prior to initiation of treatment	N	Whole blood for plasma	12 x 1 ml aliquots	Frozen Plasma (10.1)	Dry Ice	2
Prior to initiation of treatment	N	Whole blood for serum	3 x 1 ml aliquots	Frozen Serum (10.3)	Dry Ice	2
Prior to initiation of treatment	Y	Stool	2 tubes	Stool (11.1)	Ambient	2
Prior to treatment on C3D1	Y	Stool	2 tubes	Stool (11.1)	Ambient	2
Prior to treatment on C5D1	N	Whole blood for plasma	12 x 1 ml aliquots	Frozen Plasma (10.1)	Dry Ice	2
Prior to treatment on C5D1	N	Whole blood for serum	3 x 1 ml aliquots	Frozen Serum (10.3)	Dry Ice	2
Prior to treatment on C5D1	Y	Stool	2 tubes	Stool (11.1)	Ambient	2
Prior to treatment on C9D1	N	Whole blood for plasma	12 x 1 ml aliquots	Frozen Plasma (10.1)	Dry Ice	2
Prior to treatment on C9D1	N	Whole blood for serum	3 x 1 ml aliquots	Frozen Serum (10.3)	Dry Ice	2
At treatment discontinuation	N	Whole blood for plasma	12 x 1 ml aliquots	Frozen Plasma (10.1)	Dry Ice	2
At treatment discontinuation	N	Whole blood for serum	3 x 1 ml aliquots	Frozen Serum (10.3)	Dry Ice	2
At treatment discontinuation	Y	Stool	2 tubes	Stool (11.1)	Ambient	2

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Notes:

1. **Submission of frozen plasma for vitamin D level and buffy coat is required for all patients registered to A021703.**
2. Collection is optional for patients but requires all sites offer to patients to consent. Please see protocol-specific consent documents.
3. A paraffin tissue block with adequate tumor from primary or metastatic site **OR** 18 unstained slides (1 x 5 micron, 5 x 4 micron, 12 x 10 micron) from such a block should be submitted for patients opting in for A021703 biobanking. If fewer than 18 unstained slides can be submitted, please submit as many slides as possible in order of priority: 1 x 5 micron slide, 5 x 4 micron slides, 12 x 10 micron slides.

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 10 days of collection. Please be aware that the kits only contain materials for stool collection. EDTA tubes, no additive tubes, and cryovials for blood, plasma, and serum collection are not included. Please use your local supply for the whole blood collection and processing.**
- 7.1.2 Kits will be sent via FedEx® Ground 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.
- 7.1.3 Kits should be requested at least 10 working days in advance of the anticipated collection date. Due to limited supplies, distribution of stool kits will be limited to one kit per patient per time point. 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.

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7.1.4 Kit contents and specific instructions for use of the kit are provided in Appendix 1.

7.1.5 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.

7.1.6 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.

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

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 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, serum or buffy coat aliquots. Sites are responsible for acquiring materials for collection and shipping of these specimens to the Biorepository.

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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 (cryovials and stool tubes) **MUST** be labeled with the participant study number, patient initials (Last, First, Middle), the date and time (if applicable) of collection and specimen type (e.g. “plasma,” “buffy coat,” “serum”).
- 8.2** Surgical pathology tissue blocks should not be labeled in any manner. The institutional surgical pathology number (e.g. “S16-1234”) and the individual block identifier (e.g. “A3”) should be readable on the block. If tissue sections are being submitted instead of the block, each tissue section slide should be labeled with the patient study number, institutional surgical pathology number, the block identifier, the serial section number, and section thickness. Provide a **de-identified copy of the surgical pathology report**, labeled with the patient study number, corresponding to the blocks or slides submitted to ABWUSTL. Please ensure the institutional surgical pathology number (SPN) and block ID that match the SPN on the physical label on the tissue are maintained on the surgical pathology report. See section 9 for additional details on tissue submission.
- 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.

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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.

9.2 Fixed Tissue Block for A021703 Biobanking

9.2.1 For patients who consent to A021703 biobanking for future research, a representative diagnostic block from the primary or a metastatic site should be submitted, if applicable.

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.

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9.2.3 In the event than an institution will not release a tissue block, the institution may instead submit one (1), 5 micron unstained slide, five (5), 4 micron unstained slides **AND** twelve (12), 10 micron unstained slides.

9.2.4 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.

9.3 Unstained Slides from Diagnostic Fixed Tissue Blocks

9.3.1 In cases where an institution is unwilling or unable to submit a tissue block for biobanking for future research, a set of 18 unstained slides with adequate tumor may be sent as an alternative. If fewer than 18 unstained slides can be submitted, please submit as many slides as possible in order of priority: 1 x 5 micron slide, 5 x 4 micron slides, 12 x 10 micron slides. 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 a tissue block, which can be cut at the Biorepository and returned to your institution at a later date.

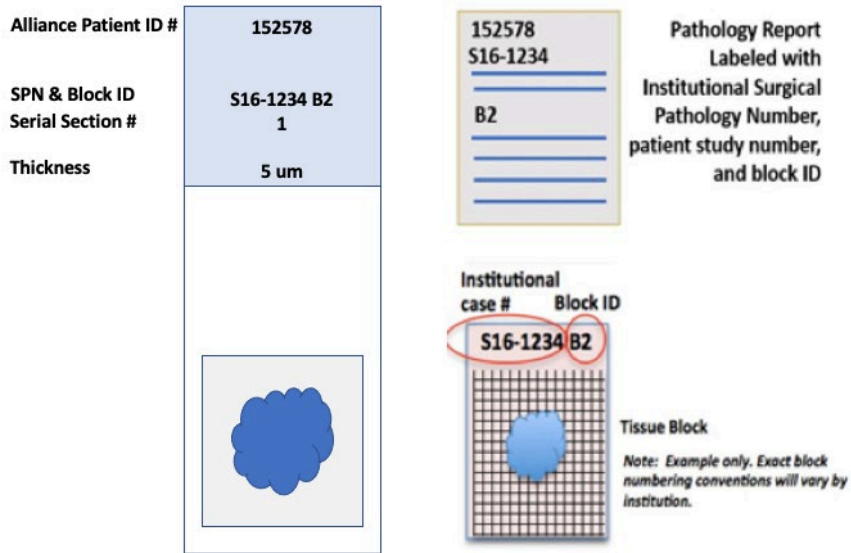
# of slides	Section thickness	Slide type	Purpose
1	5 micron	Charged	H&E staining for pathology review
5	4 micron	Charged	IHC
12	10 micron	Non-Charged	DNA, RNA

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

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- 9.3.3** Cut sections at 4, 5, 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 patient study number, the institutional surgical pathology number and block ID, slide serial section number (1, 2, 3, etc.), and section thickness (5 um, 4 um, 10 um).
- 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.

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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.**

9.3.13 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. Blood Collection Methods

10.1 Plasma Processing

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

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10.1.2 Within 2 hours 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 each), without touching the white, buffy coat layer, and transfer to new 15 ml conical centrifuge tubes. If required for collection, keep the vacutainer tube 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 4 cryovials per tube of blood collected (i.e. if 3 x 10 mL collected, 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 aliquot 1 ml into each of the 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. Frozen plasma should be shipped to the Biorepository within 30 days of collection. Batch shipment is allowed.

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 cryovial 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.

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10.2.4 Transfer the buffy coat layer (approximately 0.2 – 0.5 ml) from 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 Celsius until ready for shipment on dry ice. Buffy coat should be shipped to the Biorepository within 30 days of collection. Batch shipment is allowed.

10.3 Serum Processing

10.3.1 Collect whole blood by standard venous phlebotomy technique into the red top (plain glass with clot activator) tube. Do not collect whole blood into a “tiger top” / “SST” / “gel tube.” Invert tube 10 times

10.3.2 Allow blood to clot for 30 minutes.

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

10.3.4 Spin blood in vacutainer tube at 4 degrees in a clinical centrifuge using standard programming for serum separation. Usually this is 1200 xG (actual speed will depend upon the centrifuge) for 10 minutes.

10.3.5 Carefully remove 3 ml of serum (without touching the clot layer) and divide into 3, 1 ml labeled cryovials.

10.3.6 Freeze serum containing cryovials on dry ice or a -70 to -90 degree Celsius ultralow freezer. Store at -70 to -90 degrees until ready for shipment on dry ice. Serum should be shipped to the Biorepository within 30 days of collection. Batch shipment is allowed.

11. Stool Collection

11.1.1 Instruct patients to collect stool sample following guidelines in Appendix 1.

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11.1.2 After stool is collected, collection tubes should be stored at room temperature. **The stool samples must be received at the Biorepository within 10 days of collection.** Ensure that the stool specimens are shipped at ambient temperature to avoid freezing. **Stool questionnaires should be shipped with the stool sample. Please write study time point at the top of the stool questionnaire prior to return to the Biorepository.**

12. Biospecimen Shipping

12.1 Overview

12.1.1 Stool samples should be shipped according to guidelines in **Appendix 1. Stool samples should be shipped to the Biorepository within 10 days of collection. Stool questionnaires should be shipped to the Biorepository with the stool sample. Please write study time point at the top of the stool questionnaire prior to return to the Biorepository.**

12.1.2 Frozen plasma, serum 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.**

12.1.3 A completed copy of the BioMS packing manifest must accompany all shipments. 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 If sending tissue, include a copy of the de-identified surgical pathology report.

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12.1.5 Biospecimens should be shipped Monday—Thursday only. Do not ship on Friday, Saturday, Sunday or the day before a nationally recognized holiday.

12.2 Shipping to ABWUSTL

12.2.1 Ship container according to IATA guidelines and standard institutional policies via FedEx priority overnight shipping.

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.

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- 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** Stool specimens will be aliquoted and stored in a -70 to -90 degree Celsius ultralow freezer.
- 13.8** All biospecimens will remain in storage until additional processing or review is requested in writing by the appropriate protocol PI.

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14. Document History

Version	Description and Justification of Change	Author	Effective Date
4.2	Added information about kit expiration and shipping	KAL	04/07/2023
4.1	Updated effective date for posting.	PAA	01/15/2021
4.0	Updated hyperlinks. Added serum collection. Updated slide labeling requirements. Fixed minor grammatical errors and typos.	PAA	11/20/2020
3.1	Updated stool kit patient letter.	GLS	9/21/2020
3.0	Appendix 1 replaced in its entirety by new version to reflect change in stool kit tube color. Updated Program Management email address.	PAA	09/17/2020
2.0	Removed serum collection because serum is not a collection listed in the approved version of the protocol. Removed reference to FFQ questionnaire as sites will be automatically supplied with questionnaire when patient registers.	PAA	10/15/2019
1.0	New	PAA	09/11/2019

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		Replaces: 4.1	Page 19 of 27

Appendix 1- Stool Collection and Shipping Instructions

MICROBIOME SAMPLE COLLECTION

Thank you for your participation in this study! By providing stool samples, you are helping to answer questions about how to prevent, diagnose, and treat diseases like cancer.

You will be requested to submit a stool sample at these timepoints:

- BEFORE TREATMENT BEGINS (BASELINE)
- BEFORE CYCLE 3 DAY 1 TREATMENT IS RECEIVED
- BEFORE CYCLE 5 DAY 1 TREATMENT IS RECEIVED
- WHEN TREATMENT IS DISCONTINUED

The study team will provide you with a kit and collection instructions prior to each collection timepoint. As the collection instructions are generic, the following highlights what tubes your kit will include:












The kit WILL include the following tubes:

- Blue or Purple labeled ethanol tube
- Orange labeled anaerobic tube

The kit WILL NOT include the following tubes:

- Purple labeled OMNIgene-GUT tube
- White labeled oral tongue swab tube

Thus, instructions referencing these tubes can be ignored (pages 6-8)

 One mailer box  One air cushion	 One bio-specimen bag (with absorbent pad) for each tube	 Blue or Purple labeled ethanol tube	 Orange labeled anaerobic tube	 One stool questionnaire
 One barcode label for each tube and for the questionnaire	 Two spatulas per stool collection time point (one plus an extra if needed)	 Two toilet accessories per stool collection (one plus an extra if needed)	 Purple labeled OMNIgene-GUT tube.	 White labeled oral tongue swab tube

Please read the instructions carefully before starting collections and ensure all components are in the kit. If you are missing any items, please let the study team know as soon as possible.

After samples are collected, please place tubes and all components back into the kit box.

Bring the kit box to your next clinic visit.

If you have questions, please send an email or call your study coordinator.

THANK YOU FOR PARTICIPATING IN THIS STUDY!

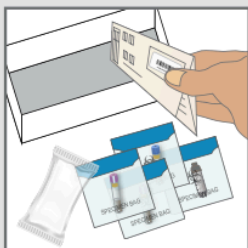
BIOM-Mass Microbiome Sample Collection

Thank you for your participation in this study! By providing samples, you are helping to research health, disease, and the human microbiome.

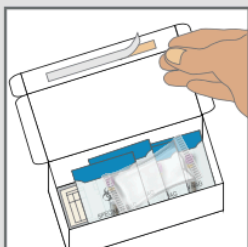
These instructions explain the collection and sample return process. The entire process is straightforward and hygienic. It is important to read all of the instructions prior to starting. Please collect and return your samples as soon as possible after your final collection. Check that your kit contains all of the materials shown below. If you are missing any items, please let the study team know as soon as possible.

Final Steps

UPON COMPLETION, PACKAGE AND RETURN YOUR SAMPLES FOR PROCESSING:



When you have completed collection for all samples in your study's collection plan, place any included paperwork in the bottom of the box. Next, place the sample bags inside the box, and then put the air cushion in the box on top of everything.



Once all items are inside the box, peel the adhesive liner off the box lid, close the box, and then press the adhesive and press the adhesive down firmly across its entire length to seal the box.

RETURN AS DIRECTED BY YOUR STUDY.

If this is in person, please return the box to your coordinator.

If this is by mail, please put the box in a mailbox or take it to your local post office. Postage is prepaid.

1

Kit Contents

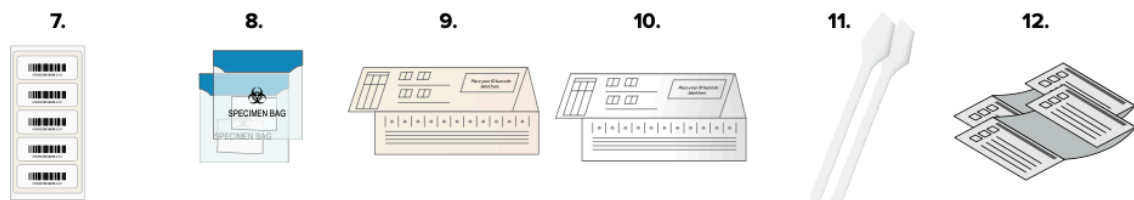
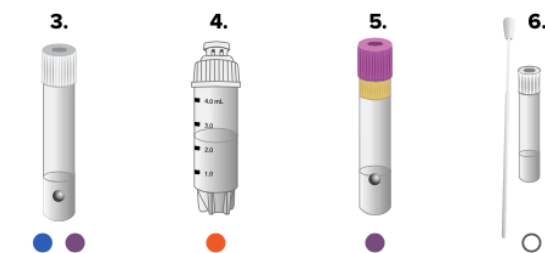
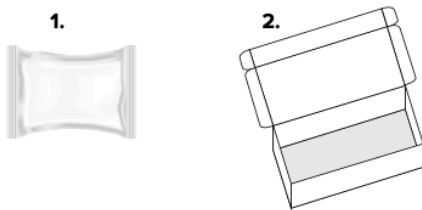
1. One air cushion.
2. One mailer box.

DEPENDING ON YOUR SAMPLE COLLECTION PLAN, YOUR KIT MAY CONTAIN ONE OR MORE:

3. **Blue** or **Purple** labeled ethanol tubes.
4. **Orange labeled anaerobic** stool collection tubes.
5. **Purple labeled OMNIgene-GUT** stool collection tubes.
6. **White** labeled oral tongue swab collection tubes.

ACCOMPANYING THESE COLLECTION TUBES, YOUR KIT MAY CONTAIN:

7. One **barcode label** for each tube and for each questionnaire.
8. One bio-specimen bag (with absorbent pad) for each tube.
9. One stool scantron questionnaire per stool time point.
10. One oral scantron questionnaire per tongue swab per collection.
11. Two spatulas per stool collection time point (one plus an extra if needed).
12. Two toilet accessories per stool collection (one plus an extra if needed).



STOOL COLLECTION INSTRUCTIONS

IMPORTANT:

When collecting multiple specimens from the same defecation using different tubes, do so from the same stool sample. Empty your bladder before beginning collection. The tubes are not designed to stand upright on their own. Placing the tube in a cup will allow you to not have to hold the tube in your hand at all times. Please be careful not to spill the preservative in the tubes.



STEP 1

Follow the instructions in the toilet accessory pack and attach the collection paper to the toilet seat by pressing firmly.

IMPORTANT: *Collect the samples quickly, as the toilet accessory will only hold for ~10 minutes once attached to the toilet seat.*

If your stool sample is liquid or you have diarrhea, wait until your next bowel movement to collect the sample. Additional toilet accessories have been provided if needed.

Do NOT urinate into the toilet accessory. The stool sample must be collected free of urine or toilet water. If the sample becomes contaminated with urine or falls into the toilet, do NOT collect the sample. Use additional toilet accessories if needed.

STEP 2

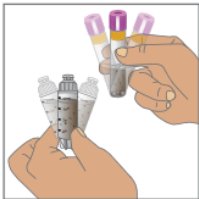
--> go to page **5** If your sample collection instructions include one or more blue ethanol tubes or orange anaerobic tubes, fill one of each now using the ETHANOL and ANAEROBIC stool tube instructions.

STEP 3

--> go to page **6** If your sample collection instructions include one or more purple OMNIgene-GUT tubes, fill one now using the OMNIgene-GUT stool tube instructions

STEP 4

Discard the spatula in the garbage, do NOT flush. Discard the toilet accessory collection paper in the toilet and flush as indicated in the instructions in the toilet accessory pack.

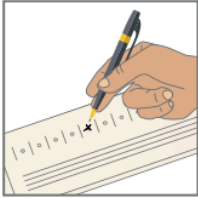


Shake each tightly sealed tube as hard and as fast as possible in a back and forth motion for at least 30 seconds each.



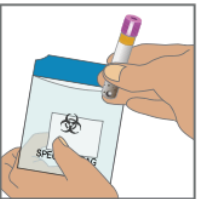
Locate the **barcode labels** and affix one to each tube.

STOOL COLLECTION INSTRUCTIONS



STEP 5

Fill out the stool sample questionnaire completely and affix a **barcode label** in the space provided.



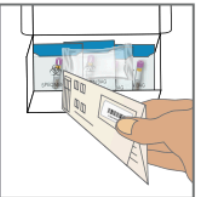
STEP 6

Insert each collected sample into its own biospecimen bag. Do NOT remove the absorbent pad from inside the bag.

Peel off the liner on the top of the bio-specimen bag and fold over to seal tightly.



Place all sealed bags back into the box.



Place the completed questionnaire into the box.

--- ➔ **back to page 1** Back to page 1 if your sample collection plan is complete, or continue with page 7 for additional sample types.

Thank you for participating in this study! If you have questions please send an email or call your study coordinator.

ETHANOL AND ANAEROBIC STOOL TUBE INSTRUCTIONS



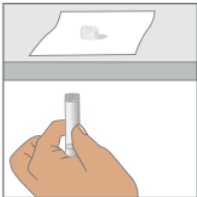
For each collection into a **Blue** or **Purple** labeled ethanol tube or an **Orange labeled anaerobic tube**:



STEP 1

Find the appropriate **ethanol** stool collection package with a **blue** or **purple label** or **anaerobic** collection package with an **orange label**, open it, and remove the tube.

You may discard the cellophane packaging of each tube after it has been opened.

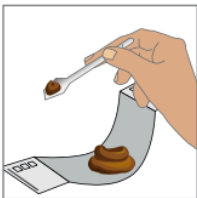


STEP 2

Unscrew the cap from the tube and place the cap aside on a clean surface. The tube is not designed to stand upright.

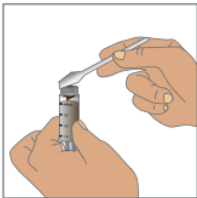
Please be careful not to spill the preservative in the tube.

Wash with water if liquid comes in contact with skin or eyes. Do NOT ingest.



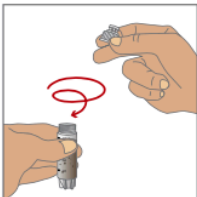
STEP 3

Use the spatula to collect a small scoop of stool from the paper accessory into the tube, approximately 1/2 the size of the spatula's end. Repeat with a second small scoop of stool. Do NOT fill the tube completely.



Scrape across the tube top to remove any excess stool.

The total amount of stool in the tube should be approximately the size of a jelly bean. It is not necessary to provide any more or less.



STEP 4

Screw the cap back on the tube tightly. Wipe the exterior of tube with toilet paper as needed.

Retain the spatula for other samples collected from this specimen.

---> **back to page 3** Back to page 3 if all stool samples have been collected

FOR THE STUDY COORDINATOR:

STEP 1:

- Ensure stool samples are labeled as per guidelines in A021703 CSM. Log stool specimens into BioMS and generate a packing manifest. Place manifest and stool questionnaire inside of box. **IT IS IMPORTANT THAT THE STUDY TIME POINT BE WRITTEN AT THE TOP OF THE STOOL QUESTIONNAIRE.**

STEP 2:

- Place the air cushion on top of the sample bags.
- Once all items are inside the box, peel the adhesive liner off the box lid and press the adhesive down firmly across its entire length to seal the box.

STEP 3:

- Return samples to the Alliance Biorepository. **STOOL QUESTIONNAIRE SHOULD BE SHIPPED TO THE BIOREPOSITORY WITH THE STOOL SAMPLE.**
- **Stool samples should be received at the Biorepository within 10 days of collection.**
- Ship container according to IATA guidelines and standard institutional policies via **FedEx priority overnight shipping**. **Stool samples must be received at the Biorepository within 10 days of collection.**

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**