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 A021502**.** This document also describes the procedures that will be followed subsequent to the receipt of biospecimens by the Alliance Biorepository at Mayo Clinic (ABMAYO), 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 A021502 biospecimen collection, processing, and submission; including staff at satellite institutions.

2. Scope

This document applies to all biospecimens collected specifically for A021502 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 and CTSU websites.**

3. Definitions/Abbreviations

Term	Definition
ABMAYO	Alliance Biorepository at Mayo Clinic
FFPE	Formalin fixed, paraffin embedded
Tx	Treatment

4. Contact Information

4.1 For questions and problems related to protocol administration, eligibility, patient registration, and data submission, please contact the relevant contact listed on protocol cover pages 1 & 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, or for assistance in using the application; or for questions or problems related to specific specimen logging please contact: 1-855-55-BIOMS or biomshelp@email.wustl.edu.

4.3 There are **THREE study kits for blood sample** collection/submission and **ONE study kit for stool sample** collection/submission; both types of kits can be ordered through the BioMS website https://bioms.wustl.edu/bioms/specimenKitRequest/list (see **section 7**) (Note: there is not a study kit for tissue sample collection/submission.) A small number of kits may be ordered prior to patient registration to ensure that sites are equipped for sample collection/submission.

4.4 For any other questions about biospecimen procurement and shipping procedures, please contact ABMAYO:

--Paraffin-embedded tissue samples: Amanda Sand
Phone: 507-284-3559
Email: Sand.Amanda@mayo.edu

--Non-paraffin-embedded (i.e. blood and stool) samples: Dr. Paola Ramos
Phone: 507-284-1156
Email: Ramos.Paola@mayo.edu

5. Site Preparation

5.1 Please refer to the A021502 protocol document for any specific requirements related to patient enrollment, registration, and regulatory compliance.

5.2 Please ensure that you have appropriate log in 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 ABMAYO. For kits needed urgently, please supply the site's FedEx account number for priority overnight delivery. For BioMS training, assistance in using the application or questions or problems related to specific specimen logging, please contact: 1-855-55-BIOMS or biomshelp@email.wustl.edu.

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 kits (unless there is urgent need; see **section 5.2**).

5.4 Please confirm that your institutional pathology department is willing to release 10 unstained charged slides, a normal tissue paraffin block, and a tumor tissue paraffin block **OR** will be willing to submit at least twenty-five unstained slides (fifteen 10 micron unstained, uncharged slides; ten 4-6 micron unstained, charged slides) from each block, and two cores (2mm) at each required time point designated in this document and in the trial protocol. Please do not bake or coverslip slides.

One H&E representative slide is also required to be submitted from the tumor tissue block. An institution whose pathology department is unable to comply with tumor block or slide submission cannot enroll patients to this study as tissue submission is required for eligibility.

5.5 Identify a reliable source of 4^{o}C refrigerators, dry ice, a -70^{o}C or lower freezer (if not, a -20^{o}C freezer can be used as alternative, see **section 10.2.5**) and cold pack (not frozen) for sample storage and shipment prior to shipment.

6. Collection Schema

Please refer to the specific protocol document (protocol **section 6.2**) for the precise biospecimen collection schedule. The following biospecimens are to be collected at each of the time points listed. Please refer to individual collection kit instructions, biospecimen collection and processing methods, and specific shipping procedures below.

Time	Kit (Y/N)	Biospecimen	Quantity	Collection / Methodology	Receiving Lab	Shipping	Notes
Mandatory for all patients registered to A021502							
Prior to treatment	N	Fixed tumor tissue	See section 9.1	Fixed tissue slides (9.1)	CellCarta (formerly Histogenex)	Ambient	1,2
Prior to treatment	N	Fixed tumor tissue	See section 9.2	Fixed tissue H&E slide and block (9.2)	ABMAYO FFPE	Ambient	1,3
Prior to treatment	N	Fixed **normal** tissue	See section 9.2	Fixed tissue block (9.2)	ABMAYO FFPE	Ambient	1,3
For patients registered to A021502-ST1 and/or A021502-PP1							
Prior to treatment	N	Fixed tumor tissue	See section 9.3	Fixed tissue slides/cores (9.3)	ABMAYO FFPE	Ambient	4, 5
Prior to treatment	Y (blood kit 1)	Platelet poor plasma & buffy coat from EDTA lavender top tubes. Processing at the site is required.	3 x 10 mL	Plasma nucleic acid & buffy coat (10.2)	ABMAYO BAP	Frozen/ dry ice	5,6
Prior to treatment	Y (blood kit 1)	Whole blood in ACD yellow top tubes. Do NOT process at the site	3 x 8.5 mL	Whole blood	Duke	Ambient	5,7
Prior to treatment	Y(fecal kit)	Stool	3 x 25 mL	Stool	ABMAYO BAP	Frozen/ dry ice	5
4 weeks (+/- 1 wk) after initiation of treatment	Y (blood kit 2)	Whole blood in ACD yellow top tubes. Do NOT process at the site	3 x 8.5 mL	Whole blood	Duke	Ambient	5,7
4.5 months (+/-1 wk) after initiation of treatment	Y (blood kit 1)	Whole blood in ACD yellow top tubes. Do NOT process at the site	3 x 8.5 mL	Whole blood	Duke	Ambient	5,7
4.5 months (+/-1 wk) after initiation of treatment	Y (blood kit 1)	Platelet poor plasma & buffy coat from EDTA lavender top tubes. Processing at the site is required.	3 x 10 mL	Plasma nucleic acid & buffy coat (10.2)	ABMAYO BAP	Frozen/ dry ice	5,6
4.5 months (+/-1 wk) after initiation of treatment	Y (fecal kit)	Stool	3 x 25 mL	Stool	ABMAYO BAP	Frozen/ dry ice	5
6 months (+/- 1 month) after end of therapy	Y (blood kit 1)	Platelet poor plasma & buffy coat from EDTA lavender top tubes. Processing at the site is required.	3 x 10 mL	Plasma nucleic acid & buffy coat (10.2)	ABMAYO BAP	Frozen/ dry ice	5,6

6 months (+/- 1 month) after end of therapy	Y (blood kit 1)	Whole blood in ACD yellow top tubes. Do NOT process at the site	3 x 8.5 mL	Whole blood	Duke	Ambient	5,7
6 months (+/- 1 month) after end of therapy	Y (fecal kit)	Stool	3 x 25 mL	Stool	ABMAYO BAP	Frozen/ dry ice	5
Time of recurrence	N	Fixed tumor tissue	See section 9.3	Fixed tissue block (9.3)	ABMAYO FFPE	Ambient	5, 8
Time of recurrence	Y (blood kit 3)	Platelet poor plasma & buffy coat from EDTA lavender top tubes. Processing at the site is required.	3 x 10 mL	Plasma nucleic acid & buffy coat (10.2)	ABMAYO BAP	Frozen/ dry ice	5

Notes:

1. Submission of these tissues are required for all patients registered to the parent study, A021502, please see section 9 for more details regarding each submission.
2. 10 unstained Superfrost® Plus Micro Slides must be submitted to the central laboratory for retrospective dMMR testing within 30 days of mounting the paraffin sections and within 30 days of registration. Please see **section 9.1** for alternatives to Superfrost Plus® Micro Slides. If tissue is limited, this mandatory submission is the **top** priority.
3. Please submit one H&E slide from the diagnostic block and one formalin-fixed, paraffin embedded (FFPE) tumor tissue block for retrospective pathology review and retrospective biomarker testing. If a block can't be submitted due to institutional policy, fifteen (15) unstained, uncharged slides at 10 microns, ten (10) unstained, charged slides at 4-6 microns, and two (2) cores (2mm) cut from the FFPE primary tumor are required. However, blocks are strongly preferred over slides/cores. If tissue is limited, this mandatory submission is the **second** priority.
4. If a tumor block was submitted above for mandatory retrospective pathology review (table row 2) (i.e. an alternative was not submitted), then no additional tissue submission for the prior to treatment time point is required for ST1. If a tumor block was not submitted above for the prior to treatment time point (i.e. an alternative was submitted), then please submit the following for patients consented for ST1: ten (10) unstained, charged slides at 4-6 microns, fifteen (15) unstained, uncharged slides at 10 microns, and two (2) cores (2mm). Biospecimen collection is optional and requires additional patient consent for A021502-ST1. Please see protocol-specific consent document. If tissue is limited, this optional submission is the **third** priority, and please submit as much tissue as possible up to the requested amount described above.
5. Biospecimen collection is optional and requires additional patient consent for A021502-ST1. Please see protocol – specific consent document. Patients who consent to A021502-ST1 are those who answer "Yes" to any of the following model consent questions #2, #3 or #4.
6. For patients consented to A021502-PP1 (model consent question #2), buffy coat will be used for PP1.
7. For patients randomized to Arm 1, the second whole blood draw should take place 4 weeks (+/- 1 week) after initiation of mFOLFOX6 + atezolizumab; for patients randomized to Arm 2, the second whole blood draw should take place 4 weeks (+/- 1 week) after initiation of mFOLFOX6.

8. For patients consented to A021502-ST1 who experience cancer recurrence, please submit tumor tissue block or alternatives as the following: ten (10) unstained, charged slides at 4-6 microns, fifteen (15) unstained, uncharged slides at 10 microns, and two (2) cores (2mm); see section 9.3 for submission details.

7. Biospecimen Collection Kits

7.1 To facilitate the proper collection and shipping of blood and stool 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 with no additional cost to the sites. Kits should be requested at least **10 working days** in advance of the anticipated collection date. Since many of the collection materials (vacutainer tubes) have expiration dates, do not request kits more than 60 days prior to their anticipated use. **NOTE: 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.**

7.2 All kits must be requested by using the BioMS system (see section 4.3 for URL of BioMS website).

7.3 This protocol uses **three kits** for blood specimen collection. All kits are designed for specific time points: blood kit 1 is designed for three time points: "prior to treatment", "4.5 months (+/- 1 week) after initiation of treatment," and "6 months (+/- 1 month) after the end of therapy". Blood kit 2 is designed for "4 weeks (+/- 1 week) after initiation of treatment" blood collection. Blood kit 3 is for "time of recurrence".

Prior to treatment (blood **kit 1**)	4 weeks (+/- 1 wk) after initiation of treatment (blood **kit 2**)	4.5 months (+/- 1 wk) after initiation of treatment (blood **kit 1**)	6 months (+/- 1 month) after end of therapy (blood **kit 1**)	Time of recurrence (blood **kit 3**)

Kit contents and specific instruction for use of the kit are provided in the kit box. The kit has a refrigerated shipper. Refrigerated cold packs will be placed on the bottom of the shipper and several layers of paper toweling should be placed on top of the cold pack. Refrigerated specimens will be placed on top of the paper toweling. Several more layers of paper toweling should be placed on top of the refrigerated specimens. Place the ambient specimens at the top of the return shipper.

7.4 This protocol also uses one kit (Fecal Kit) for all stool collections. Kit contents and specific instructions for use of the kit are provided in the kit box. The kit has a frozen shipper.

Place the "frozen specimen" transport bag, along with 3 to 4 lbs. (2 kg) of commercially-prepared dry ice, into the "frozen" Styrofoam® container. Pellets or chunks no more than 8 cm on a side are preferred. NOTE: It is local sites' responsibility to obtain dry ice when shipping frozen specimens via FedEx®. ABMAYO does not provide dry ice. Businesses that may have dry ice for purchase include ice cream shops, research labs, hospital and commercial blood banks, or chemical supply companies.

7.5 There is no kit for tissue collection/submission.

7.6 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.

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

7.8 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 (EDTA tubes) others (ACD tubes) may not be available at the institution.

Note that protocol requirements for blood collection **are based on blood volumes**, **not tube sizes**. If the protocol requires the collection of ~10 mL of whole blood, generally a 10 mL tube is provided in the kit for convenience. **Due to supply availability, it may be necessary for the Biorepository to substitute multiple, smaller collection tubes to facilitate collection of the required volume (i.e. 2 x 5 mL collection tubes in place of a 10 mL collection tube).**

7.9 Please see **section 12** – Biospecimen Shipping for specific instructions on packaging biospecimens into the shipping kit (if applicable) for shipment to the biorepository or laboratory.

8. Biospecimen Labeling and Tracking

8.1 All **blood and stool** biospecimens MUST be labeled with the Alliance study number, Alliance patient ID number, patient initials (Last, First, Middle), the date and time of collection, and specimen type (i.e. "PPP" for platelet poor plasma, "B" for buffy coat). Specimen labels must match BioMS records.

8.2 Surgical pathology tissue blocks should not be directly 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 section slides are being submitted instead of the block, each tissue section slide should be labeled with the surgical pathology number and the block identifier. **Please do NOT use sticky labels on slides**. Provide a **de-identified** copy of the surgical pathology report, labeled only with the participant study number, corresponding to the blocks or slides submitted. A copy of the de-identified pathology report is required for each and every tissue submission(s). Usually, this is generated by obscuring all PHI (names and dates) with white-out or a black magic marker, labeling each page of the report with the Alliance patient ID, and photocopying the report. 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 All unstained slides should be also labeled with thickness (e.g. 5 micron).

8.4 Label all containers and vials with indelible marker when they are at ambient temperature.

8.5 If using labels affixed to the collection tubes, wrap white scotch tape around the tube to ensure the labels will remain intact during shipping.

8.6 All biospecimens that are collected and sent to the ABMAYO, Duke Laboratory, or Central Laboratory must be logged and tracked in BioMS under A021502. Do not send samples to the Biorepository in which the patient has not been registered onto the study. Samples must include patient ID#. 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.

9. Tissue Collection Methods

9.1 Diagnostic Pathology Fixed Slides for Central dMMR Testing

9.1.1 This is a **mandatory submission** for all patients registered on A021502.

9.1.2 Please submit ten (10) Superfrost® Plus Micro Slides from the FFPE tumor tissue block to the central laboratory for retrospective central dMMR confirmation testing as consistent and accurate dMMR grading is important for this study. Superfrost® Plus Micro Slides are preferred, however, any ten (10) unstained, charged slides at 4-6 micron thickness are acceptable for submission. (Note: slides should not be paraffin dipped.) **The slides must be submitted to the central laboratory within 30 days of mounting the paraffin sections and within 30 days of registration.**

9.1.3 A study-specific requisition form and a de-identified surgical pathology report (see section 8.2) must be sent with all FFPE specimens to the central laboratory.

9.1.4 Slides for dMMR confirmation must be labeled with the following information:

- Alliance Study Number (A021502)
- Alliance Patient ID Number
- Specimen Surgical Pathology Number and Block Identifier either via your institution's standard method for labeling clinical slides, or using a permanent marker. Labeling with sticky labels is not acceptable.

Ship slides for dMMR confirmation to the central laboratory agreed upon by the Alliance and the drug company supporting this trial. The name and shipping address for the **central laboratory** are included in **section 11.4** or on the A021502 Requisition Form which can be found on the Alliance and CTSU websites. Results of the central retrospective dMMR confirmation testing will not be communicated back to sites or patients; results of the retrospective dMMR confirmation test will not be used for eligibility.

9.2 Diagnostic Pathology Fixed Tissue Blocks for Central Pathology Review and Biomarker Testing

9.2.1 This is a **mandatory submission** for all patients registered on A021502.

9.2.2 Please submit one H&E slide from the diagnostic tumor tissue block, one FFPE tumor tissue block, and one FFPE normal tissue block for retrospective pathology review and biomarker testing. It is required for all patients registered on A021502.

9.2.3 The Alliance has instituted special considerations for the small percentage of sites whose institutional policy prohibits long-term storage of blocks, and the smaller percentage of hospitals whose policies prohibit release of any block. If a block can't be submitted due to institutional policy, fifteen (15) unstained, uncharged slides at 10 microns, ten (10) unstained, charged slides at 4-6 microns, and two (2) cores (2mm) cut from the formalin-fixed, paraffin embedded (FFPE) primary tumor and/or normal tissue block(s) are required. However, blocks are strongly preferred over slides/cores. NOTE: if a block is submitted here for mandatory central pathology review and biomarker testing, then for patients who also consented to ST1, no additional tissue need to be submitted for ST1 for the prior to treatment submission (also see **section 9.3**).

9.2.4 A copy of the de-identified pathology report should be included in each and all tissue submission(s).

9.3 Diagnostic Pathology Fixed Tissue Block for A021502-ST1

9.3.1 This is an optional submission only for patients who consented for A021502-ST1.

9.3.2 Paraffin blocks of primary and, when available, metastatic tissue obtained from archival tumor specimens, should be submitted.

9.3.3 If a tumor block was submitted for the prior to treatment time point for mandatory retrospective biomarker testing (i.e. an alternative was not submitted), then no additional tissue submission for the "prior to treatment" time point is required. If a tumor or normal tissue block was not submitted for the prior to treatment time point (i.e. an alternative was submitted), then please also submit the following for the prior to treatment time point for patients consented to A021502-ST1 (model consent question #3): ten (10) unstained, charged slides at 4-6 microns, fifteen (15) unstained, uncharged slides at 10 microns, and two (2) cores (2mm). If a tumor block cannot be submitted for the time of recurrence, new polyps, or potential treatment toxicities time point(s), then please submit the following for patients consented to A021502-ST1 (model consent question #3): ten (10) unstained, charged slides at 4-6 microns, fifteen (15) unstained, uncharged slides at 10 microns, and two (2) cores (2mm), or as much as possible. However, blocks are strongly preferred over slides/cores.

9.3.4 A copy of the de-identified pathology report should be included in each and all tissue submissions.

10. Blood Collection Methods

10.1 Whole Blood in ACD Yellow Top Collection

10.1.1 Collect 3 x ~8.5 mL of peripheral venous whole blood into ACD (yellow top) vacutainer tubes (provided in the study kit). Invert tubes approximately 5-10 times to mix. Specimens should be kept in ambient temperature until shipping.

10.1.2 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 EDTA Platelet Poor Plasma and Buffy Coat Collection

10.2.1 Collect 3 x 10 mL of venous blood in EDTA lavender top (provided in the study kit) using standard venous phlebotomy. Both K2 and K3 EDTA tubes are acceptable.

10.2.2 The tubes should be inverted approximately 5-10 times to mix the EDTA. Store the EDTA tubes at refrigerated temperature (4 degrees Celsius) until centrifugation. Centrifugation of the EDTA blood samples should occur **within 2 hours** from blood draw.

10.2.3 Using a refrigerated centrifuge, spin samples at 2000 x g for 10 minutes. Using a new graduated pipette, aliquot the platelet rich plasma into a 15 mL conical tube with a cap and, using a new graduated pipette, aliquot the white blood cell layer (buffy coat) from each 10 mL EDTA tube from the first centrifugation into three (3) orange cap 2 mL cryovials (provided in the study kit) and label "B". Centrifugation of the EDTA blood samples should occur **within 2 hours** from blood draw. If a refrigerated centrifuge is unavailable on site, site should ensure that samples are on ice immediately before and after spinning.

10.2.4 Repeat the centrifugation of the plasma in the 15 mL conical tube at about 2000 x g for 10 minutes. Using a new graduated pipette, aliquot 1.0 mL plasma into nine (9) 2 mL white cap cryovials (provided in the study kit).

10.2.5 Immediately label with "PPP" (i.e. Platelet Poor Plasma) and freeze cryovials at –70°C or colder. If –70°C or colder freezer is not available, temporary storage on dry ice or at –20°C prior to shipment is acceptable for up to approximately 48 hours. Samples should be placed in a biohazard bag and shipped according to IATA guidelines within approximately 30 days of blood draw on dry ice by overnight express courier. Batch shipping is acceptable when feasible.

11. Stool Collection

11.1 Patient stool collection instructions can be found in the study protocol.

11.2 Stool collection will be performed using a Comprehensive Kit, developed at Mayo Clinic, that includes a 9x9x9" cardboard box with a Styrofoam container, two (2) nitrile gloves, a Ziploc bag with absorbent material containing 3 x 25 mL fecal collection vials with spoons for the stool sample, a disposable "specimen collection toilet adapter with white container" to be placed over the toilet bowl to collect the bowel movement, Wypall absorbent material, parafilm, and patient instruction sheet. Samples should be frozen on dry ice. Fecal samples should be stored in patient's home freezer (at -20 °C) until able to take to site for shipping on dry ice.

12. Biospecimen Shipping

12.1 Blood Specimen Shipping

12.1.1 Whole blood in ACD yellow top tubes should be shipped the same day that the blood is drawn. ACD tubes should be placed in a biohazard bag in the white shipping box provided with the study kit. Blood should be shipped according to IATA guidelines at ambient temperature. To avoid extreme heat during shipping, please include a room temperature (not frozen) cold pack to limit temperature variations during shipment. Shipping should be FedEx Priority Overnight or First Overnight. Send email to Substrate Services Core confirming shipment and provide the tracking number: surgerysubstrate@dm.duke.edu.

Ship **(using white shipping box contained in the study kit)** on **Monday through Thursday**, although Thursdays should be avoided when possible as delays can push shipment to the weekends. Do not ship on Fridays or Saturdays. Plan shipping such that delivery does not fall on a holiday.

12.1.2 EDTA platelet poor plasma and buffy coat should be placed in a biohazard bag and shipped according to IATA guidelines within approximately 30 days of blood draw **on dry ice**. Place the "frozen specimen" transport bag, along with 3 to 4 lbs. (2 kg) of commercially-prepared dry ice, into the "frozen" Styrofoam container. Pellets or chunks no more than 8 cm on a side are preferred. Make sure the box is filled with dry ice and the weight of the dry ice in noted on the dry ice label on the outside of the brown box. Batch shipping is acceptable when feasible. Specimens may be shipped Priority Overnight to the ABMAYO BAP freezer (see **section 12.5**) on Monday through Friday for next day delivery. The ABMAYO cannot receive specimens on Sundays or holidays. Do not send specimens on Saturday or the day before a holiday.

NOTE: It is local sites' responsibility to obtain dry ice when shipping frozen specimens via FedEx or other courier. ABMAYO does not provide dry ice. Businesses that may have dry ice for purchase include ice cream shops, research labs, hospital and commercial blood banks, or chemical supply companies.

12.1.3 Follow packaging instructions to ensure kit performance and shipment compliance with IATA Packaging Instructions.

12.2 Stool Specimen Shipping

Samples will be shipped frozen on dry ice. Samples should be placed in a biohazard bag and shipped according to IATA guidelines within approximately 30 days of collection. Place the "frozen specimen" transport bag, along with 3 to 4 lbs. (2 kg) of commercially-prepared dry ice, into the "frozen" Styrofoam container. Pellets or chunks no more than 8 cm on a side are preferred. Make sure the box is filled with dry ice and the weight of the dry ice in noted on the dry ice label on the outside of the brown box. Batch shipping is acceptable when feasible. Specimens may be shipped Priority Overnight to the ABMAYO BAP on Monday through Friday for next day delivery. The ABMAYO cannot receive specimens on Sundays or holidays. Do not send specimens on Saturday or the day before a holiday.

NOTE: It is local sites' responsibility to obtain dry ice when shipping frozen specimens via FedEx or other courier. ABMAYO does not provide dry ice. Businesses that may have dry ice for purchase include ice cream shops, research labs, hospital and commercial blood banks, or chemical supply companies.

12.3 Tissue Specimen Shipping

Because paraffin tissue 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:

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

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

12.3.3 During warm weather months, paraffin slides and blocks should be shipped in an insulated container that contains a refrigerant pack, to avoid heat > 25^{o}C (77^{o}F) that may melt paraffin and damage blocks and slides.

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

12.3.5 Slides for mandatory central dMMR testing must be submitted to the central laboratory within 30 days of mounting the paraffin section and within 30 days of registration. These slides must be accompanied by the completed requisition form and a copy of the de-identified surgical pathology report.

12.3.6 Tissues for mandatory retrospective pathology review and retrospective biomarker testing must be submitted to ABMAYO within 30 days of patient registration to A021502. Batch shipment is allowed.

12.4 BioMS Packing Manifest

Do not send specimens without a completed BioMS Packing Manifest or substitute. Biospecimens cannot be accepted without this completed form.

12.5 Shipping Addresses

There are **FOUR** different shipping destinations for the various specimens in this study.

Ship container(s) for PRIORITY OVERNIGHT DELIVERY according to institutional policies and using the preferred vendor. The following are the four destinations:

Slides for MANDATORY retrospective central dMMR testing should be sent to the following address:

CellCarta LLC
Attn: Sample Reception Team – P1931
1841 Centre Point Circle, Suite 100
Naperville, IL 60563
ustrials@histogenex.com
(630) 703-2098

Ship specimens to CellCarta (formerly HistoGeneX) on Monday through **Thursday** only. Do not ship specimens on Fridays, Saturdays, or the day before national holidays.

All other FFPE tissues (mandatory and/or optional ST1) should be sent to the following address:

Alliance Biorepository at Mayo Clinic FFPE Tissue
Attn: PC Office
RO-FF-03-24-CC/NW Clinic
200 First Street SW
Rochester, MN 55905

Ship specimens to ABMAYO on Monday through **Friday** only. Do not ship specimens on Saturdays or the day before national holidays.

ACD yellow top tubes from patients who consent to participate in A021502-ST1 should be sent to the following address in the white shipping box:

Attn: Substrate Services Core and Research Support (SSCRS)
Duke University Medical Center
203 Research Drive
MSRB I, Room 459
Durham, NC 27710

Ship specimens to the Duke Laboratory on Monday through **Thursday** only, however, Thursdays should be avoided when possible as delays can push shipment to the weekends. Do not ship specimens on Fridays, Saturdays, or the day before holidays.

All other blood specimens and stool specimens from patients who consent to participate in A021502-ST1 should be sent to the following address in the **brown** shipping box:

Alliance Biorepository at Mayo Clinic BAP Freezer
Stabile SL-16
150 Third Street SW
Rochester, MN 55902

Ship specimens to ABMAYO on Monday through **Friday** only. Do not ship specimens on Saturdays or the day before national holidays.

13. Biospecimen Receipt and Quality Assurance Measures

13.1 Tissues for mandatory retrospective central dMMR confirmation testing will be sent to the central laboratory, CellCarta (formerly HistoGeneX).

13.2 Blood specimens in ACD yellow top tubes will be sent to the laboratory at Duke University

13.3 Except the tissue specimens for mandatory retrospective central dMMR confirmation testing and the blood specimens in ACD yellow top tubes, all biospecimens will be shipped to and received by the ABMAYO, a CAP-accredited biorepository.

13.4 All biospecimens sent to the ABMAYO will be accessioned into the informatics system BioMS, at ABMAYO Research Laboratory Information Management System (RLIMS) which interfaces with BioMS.

13.5 All biospecimens received at the ABMAYO will be logged, associated, and tracked by the unique patient system generated number.

13.6 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 submitting site for reconciliation.

13.7 Upon receipt, any biospecimens received that are 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.

13.8 Tissue submitted to CellCarta (formerly HistoGeneX) will be kept under the custody of CellCarta (formerly HistoGeneX) and will be forwarded to the ABMAYO (in batches if needed) upon the completion of the study. All biospecimens submitted to ABMAYO will remain in storage until exhausted. In the event the patient withdraws consent, the specimens will be disposed/returned according to Alliance Policy and Procedure Chapter 11.

14. Document History

Version	Description and Justification of Change	Author	Effective Date
1.0	New (Master Template)	MAW	07/15/2018
2.0	Clarifications to Footnotes in Section 6.0	YW	02/01/2019
3.0	Revisions to Processing Instructions in Section 10.2	YW	03/01/2019
4.0	Removed patient facing stool collection instructions Updated ABMAYO contact info Updated timepoint titles in schema to align with protocol Updated stool collection quantity	PAA	01/27/2022
4.1	Updated site processing instructions to be more consistent with kit instructions	AAW	08/11/2022
4.2	Updated CellCarta shipping address	AAW	09/30/2022
4.3	Added info about kit shelf life and clarified pathology report requirements	KAL	04/07/2023
4.4	Updated ABMAYO contact info	KL	01/02/2024