

ALLIANCE FOR CLINICAL TRIALS IN ONCOLOGY	CORRELATIVE SCIENCE PROCEDURE MANUAL The Janus Rectal Cancer Trial: A Randomized Phase II Trial Testing the Efficacy of Triplet Versus Doublet Chemotherapy to Achieve Clinical Complete Response in Patients with Locally Advanced Rectal Cancer Short Title- A022104	Version No: 1.2	Effective Date: 05/24/2023
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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 A022104. 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 A022104 biospecimen collection, processing, and submission; including staff at satellite institutions.

2. Scope

This document applies to all biospecimens collected specifically for A022104 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
WW	Watch & Wait

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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: J. Joshua Smith, MD, PhD smithj5@mskcc.org Nursing Contact: Barb Kleiber, RN barbara.kleiber@osumc.edu Protocol Coordinator: Jamie Crawley jcrawley@bsd.uchicago.edu Data Manager: Kelsey Peterson peterson.kelsey1@mayo.edu
Questions related to data submission, RAVE or patient follow-up:	Data Manager: Kelsey Peterson peterson.kelsey1@mayo.edu
Questions regarding the protocol document and model informed consent:	Protocol Coordinator: Jamie Crawley jcrawley@bsd.uchicago.edu
Questions related to IRB review	Alliance Regulatory Inbox regulatory@allianceNCTN.org
Questions regarding CTEP-AERS reporting:	Alliance Pharmacovigilance Inbox pharmacovigilance@alliancencn.org
Questions regarding specimens/specimen submissions:	Alliance Biorepository alliance@email.wustl.edu
Questions regarding drug administration	Pharmacy Contact: Maria Andrea Monckeberg, MS, RPh, BCOP mamonckeberg@lifespan.org

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

5. Site Preparation

- 5.1** Please refer to A022104 protocol document for any specific requirements related to patient enrollment, registration, and regulatory compliance.
- 5.2** Please ensure that you have appropriate log on credentials and can successfully access the BiOMS application. The BiOMS application is used for logging the collection and shipment of biospecimens to the Alliance Biorepository at Washington University. For training and assistance in using the application or questions or problems related to specific specimen logging, please contact: 1-855-55-BIOMS or bioms@alliancenctn.org.
- 5.3** 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 biospecimen collection and processing methods and specific shipping procedures below.

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Time Point	Biospecimen	Quantity	Collection / Processing Method	Shipping	Notes
WW Patients					
After Registration / Prior to Initiation of Study Treatment	Fixed tissue block-diagnostic tumor biopsy	1 block	Fixed tissue block (9.2)	Ambient	1, 2
After Registration / Prior to Initiation of Study Treatment	H&E stained slide AND tissue scrolls-diagnostic tumor biopsy	1 H&E stained slide AND 10 x 10 micron scrolls	H&E stained slide AND Fixed tissue scrolls (9.3)	Ambient	1, 2
After Registration / Prior to Initiation of Study Treatment	Whole blood for plasma	9 x 1 ml aliquots	Frozen plasma (10.1)	Dry Ice	1, 5
After Registration / Prior to Initiation of Study Treatment	Whole blood for "buffy coat"	3 aliquots	"Buffy Coat" (10.2)	Dry Ice	1, 5
After Chemoradiation / Prior to first cycle of Consolidative Chemotherapy	Whole blood for plasma	9 x 1 ml aliquots	Frozen plasma (10.1)	Dry Ice	1, 5
At time of 1st restaging	Whole blood for plasma	9 x 1 ml aliquots	Frozen plasma (10.1)	Dry Ice	1, 5
4 months +/- 2 months after 1 st restaging	Whole blood for plasma	9 x 1 ml aliquots	Frozen plasma (10.1)	Dry Ice	1, 5
8 months +/- 2 months after 1 st restaging	Whole blood for plasma	9 x 1 ml aliquots	Frozen plasma (10.1)	Dry Ice	1, 5
12 months +/- 2 months after 1 st restaging	Whole blood for plasma	9 x 1 ml aliquots	Frozen plasma (10.1)	Dry Ice	1, 5

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At Progression	Fixed tissue block- recurrence / regrowth / metastases	1 block	Fixed tissue block (9.2)	Ambient	1, 4, 6
At Progression	H&E stained slide AND tissue scrolls- recurrence / regrowth/ metastases	1 H&E stained slide AND 10 x 10 micron scrolls	H&E stained slide AND Fixed tissue scrolls (9.3)	Ambient	1, 4, 6
At Progression	Whole blood for plasma	9 x 1 ml aliquots	Frozen plasma (10.1)	Dry Ice	1, 5, 6

Surgery Patients					
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After Registration / Prior to Initiation of Study Treatment	Fixed tissue block- diagnostic tumor biopsy	1 block	Fixed tissue block (9.2)	Ambient	1, 2
After Registration / Prior to Initiation of Study Treatment	H&E stained slide AND tissue scrolls- diagnostic tumor biopsy	1 H&E stained slide AND 10 x 10 micron scrolls	H&E stained slide AND Fixed tissue scrolls (9.3)	Ambient	1, 2
After Registration / Prior to Initiation of Study Treatment	Whole blood for plasma	9 x 1 ml aliquots	Frozen plasma (10.1)	Dry Ice	1, 5
After Registration / Prior to Initiation of Study Treatment	Whole blood for "buffy coat"	3 aliquots	"Buffy Coat" (10.2)	Dry Ice	1, 5

After Chemoradiation / Prior to first cycle of Consolidative Chemotherapy	Whole blood for plasma	9 x 1 ml aliquots	Frozen plasma (10.1)	Dry Ice	1, 5
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At time of 1st re- staging	Whole blood for plasma	9 x 1 ml aliquots	Frozen plasma (10.1)	Dry Ice	1, 5
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Surgery	Fixed tissue block- surgery specimen	1 block	Fixed tissue block (9.2)	Ambient	1, 3, 6
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Surgery	H&E stained slide AND tissue scrolls- surgery specimen	1 H&E stained slide AND 10 x 10 micron scrolls	H&E stained slide AND Fixed tissue scrolls (9.3)	Ambient	1, 3, 6
4 months +/- 2 months post surgery	Whole blood for plasma	9 x 1 ml aliquots	Frozen plasma (10.1)	Dry Ice	1, 5
8 months +/- 2 months post surgery	Whole blood for plasma	9 x 1 ml aliquots	Frozen plasma (10.1)	Dry Ice	1, 5
12 months +/- 2 months post surgery	Whole blood for plasma	9 x 1 ml aliquots	Frozen plasma (10.1)	Dry Ice	1, 5
At Progression	Fixed tissue block- recurrence / regrowth / metastases	1 block	Fixed tissue block (9.2)	Ambient	1, 4, 7
At Progression	H&E stained slide AND tissue scrolls- recurrence / regrowth/ metastases	1 H&E stained slide AND 10 x 10 micron scrolls	H&E stained slide AND Fixed tissue scrolls (9.3)	Ambient	1, 4, 7
At Progression	Whole blood for plasma	9 x 1 ml aliquots	Frozen plasma (10.1)	Dry Ice	1, 5, 7

Notes:

1. Collection is optional for patients but requires all sites offer to patients during consent. Please see protocol-specific consent documents.
2. A representative, archived tissue block from diagnostic tumor biopsy should be submitted, if available. If entire tissue block cannot be submitted, one H&E stained slide **AND** ten (10 um) serial tissue scrolls will be accepted as an alternative. If tissue is limited, please submit H&E and as many tissue scrolls as possible. **BLOCK SUBMISSION IS STRONGLY PREFERRED.**
3. A representative, archived tissue block from the surgical specimen should be submitted, for patients going through surgery after chemotherapy only. If entire tissue block cannot be submitted, one H&E stained slide **AND** ten (10 um) serial tissue scrolls will be accepted as an alternative. If tissue is limited, please submit H&E and as many tissue scrolls as possible. **BLOCK SUBMISSION IS STRONGLY PREFERRED.**

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4. A representative, archived tissue block from a recurrence / regrowth / metastasis should be submitted, if available. If entire tissue block cannot be submitted, one H&E stained slide **AND** ten (10 um) serial tissue scrolls will be accepted as an alternative. If tissue is limited, please submit H&E and as many tissue scrolls as possible. **BLOCK SUBMISSION IS STRONGLY PREFERRED.**
5. Peripheral blood (EDTA) 3 x 10 ml to be processed for plasma (9 x 1-1.5 ml aliquots) and “buffy coat,” frozen on site and shipped on dry ice. Buffy coat collection is only required at the After Registration / Prior to Initiation of Study Treatment time point.
6. For patients going through surgery after study chemotherapy only.
7. Progression here includes recurrence, regrowth, and/or metastases. The method for research tissue procurement (endoluminal biopsy with forceps through the scope as per standard practice (rectal cancer biopsies), needle core biopsy (for metastatic site sampling), and/ or sampling of surgically resected tumor (for patients with tumor recurrence or regrowth undergoing salvage surgery)) is dependent on the disease site, clinical scenario, and individual patient.

7. Biospecimen Collection Kits

7.1 Blood Specimens

7.1.1 There are no “kits” provided for submission of blood specimens for this study. Sites are responsible for acquiring materials for collection and shipping of these specimens to the Biorepository.

7.2 Tissue Specimens

7.2.1 There are no “kits” provided for submission of the paraffin block, H&E stained slides, or tissue scrolls for this study.

7.2.2 Tissue should be packaged to avoid breakage using a padded envelope or, preferably, a small Styrofoam container.

7.2.3 During warm weather months, fixed tissue 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 11 – Biospecimen Shipping for specific instructions on shipping to ABWUSTL.

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8. Biospecimen Labeling and Tracking

- 8.1** All research biospecimens (cryovials, microcentrifuge tubes, and tissue bags) **MUST** be labeled with the Alliance patient ID number, patient initials (Last, First, Middle), the date and time (if applicable) of collection and specimen type (i.e. plasma, “buffy coat”).
- 8.2** Surgical pathology tissue blocks should not be labeled in any manner. The institutional surgical pathology number (e.g. “S16-1234”) and the individual block identifier (e.g. “A3”) should be readable on the block. Provide a **de-identified copy of the surgical pathology report**, labeled with the Alliance patient ID number, corresponding to the blocks submitted. Please ensure the institutional surgical pathology number and block identifier are maintained on the surgical pathology report. The surgical pathology number (SPN) on the surgical pathology report must match the physical label on the tissue submitted to the Biorepository. 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 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>.

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NOTE: When logging tissue specimens in BioMS, the date of collection should be logged as the date of biopsy or surgery (i.e. the date the tissue was procured from the patient). This date should match the date printed on the corresponding de-identified surgical pathology report submitted with the tissue specimen.

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.
- 9.1.2** The method for research tissue procurement (endoluminal biopsy with forceps through the scope as per standard practice (rectal cancer biopsies), needle core biopsy (for metastatic site sample), and/ or sampling of surgically resected tumor (for patients with residual disease going for definitive surgery or for patients with tumor recurrence or regrowth undergoing salvage surgery)) is dependent upon the disease site, clinical scenario, and individual patient.
- 9.1.3** 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.
- 9.1.4** Retrieved fresh tissue can be formalin fixed and paraffin embedded (FFPE) as per institutional standard operating procedures (SOPs).
- 9.1.5** **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.**

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9.2 Diagnostic Pathology Fixed Tissue Blocks

9.2.1 For patients who consent to A022104, representative diagnostic tumor tissue blocks are requested from:

- a. a diagnostic tumor biopsy for WW patients and surgical patients
- b. the surgical specimen for patients going through surgery after study chemotherapy only
- c. a recurrence / regrowth / metastasis for WW patients and surgical patients with progression

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 endpoints have been met.

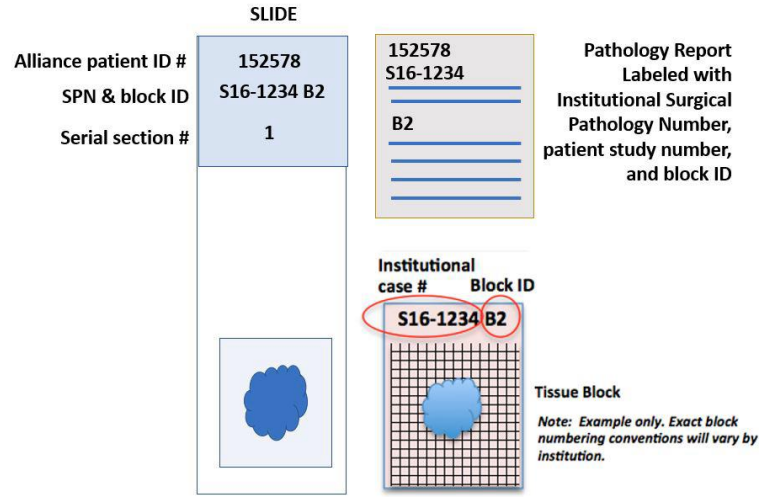
9.2.3 In the event that an institution will not release the requested tissue block(s), the institution may instead submit 1 H&E stained slide **AND** tissue scrolls as an alternative (see section 9.3). **BLOCK SUBMISSION IS STRONGLY PREFERRED.**

9.3 H&E Stained Slide and Fixed Tissue Scrolls

9.3.1 In cases where an institution is unwilling or unable to submit the requested tissue block(s) for biobanking, a single H&E stained slide for references and 10 x 10 micron scrolls serially cut from the same block may be submitted.

Cut and perform routine H&E stain on a single section from the tissue block. See figure below for proper mounting and labeling.

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9.3.2 Cut a “ribbon” (scroll) of 10 paraffin tissue sections at 10 microns. Place the ribbon of tissue directly into a single microcentrifuge tube or any other suitable container. Do not float the tissue ribbon or sections in a water bath. Label the tube of tissue following the guidelines in **section 8**.

10. Blood Collection Methods

10.1 Plasma 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. **If site supplies are limited and 10 ml EDTA tubes are not available, EDTA tubes of smaller volume may be substituted to achieve a total collection volume of 30 ml.**

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.

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- 10.1.3** Carefully remove the plasma layer from each vacutainer tube (~3—5 ml in volume per tube), without touching the white, buffy coat layer, and transfer to new 15 ml conical centrifuge tubes. Keep the vacutainer tubes containing the white, buffy coat layers for white blood cell (“buffy coat”) 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 9 (2 ml) cryovials as instructed in **section 8**. Make certain each vial is labeled completely and identically.
- 10.1.6** Carefully remove 9 ml of plasma (without touching the pellet) and divide into 9 (2 ml) labeled cryovials. Each aliquot should be between 1—1.5 ml in volume.
- 10.1.7** Freeze plasma containing cryovials on dry ice or a -70 to -90 degree Celsius ultralow freezer. Store at -70 to -90 degrees C until ready for shipment on dry ice. 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 tubes.
- 10.2.2** Label 3 (2 ml) cryovials as instructed in **section 8**.
- 10.2.3** After removing the plasma, carefully remove the white, “buffy coat” white blood cell layer, avoiding the red blood cell mass as much as possible.

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10.2.4 Transfer the buffy coat layer (approximately 0.2 – 0.5 ml) from each EDTA tube into the labeled cryovials. Immediately freeze the cryovials of buffy coat on dry ice or in liquid nitrogen vapor. Do NOT freeze buffy coat cells by placing a warm tube in a -70 to -90 degree Celsius ultralow freezer. Once completely frozen, the cryovials containing the buffy coat cells may be stored at -70 to -90 degrees C until ready for shipment on dry ice. Frozen buffy coat should be shipped to the Biorepository within 30 days of collection. Batch shipment is allowed.

11. Biospecimen Shipping

11.1 Overview

11.1.1 Frozen plasma and buffy coat aliquots should be placed in a biohazard bag inside of a Styrofoam cooler and covered with 3 to 4 lbs (2 kg) of commercially-prepared dry ice. Pellets or chunks are preferred. Make sure the box is filled with dry ice and the weight of the dry ice is noted on the dry ice label on the outside of the shipping container. It is the local sites' responsibility to obtain dry ice when shipping frozen specimens. Specimens should be shipped according to IATA guidelines. **Frozen aliquots should be shipped to the Biorepository within 30 days of collection. Batch shipment of frozen aliquots is allowed. If sending specimens from multiple patients within a single shipment, please ensure all specimens are properly labeled and logged in the BioMS system. Specimens from each individual patient should be placed in their own biohazard bag that is clearly labeled with the Alliance patient ID number. The accompanying BioMS manifest should be sealed within each bag.**

11.1.2 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.

11.1.3 If sending tissue, include a copy of the de-identified surgical pathology report.

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

11.2 Shipping to ABWUSTL

11.2.1 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**

12. Biospecimen Receipt and Quality Assurance Measures

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

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

12.3 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.

12.4 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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12.5 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.

12.6 Aliquoted biofluids will be stored under liquid nitrogen vapor.

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

13. Document History

Version	Description and Justification of Change	Author	Effective Date
1.2	Updated table footnotes to align with protocol Added clarification to section 9 for tissue submission Added contact information to section 4 Included instructions for sending aliquots in batch	PR, PAA, KAL	05/24/2023
1.1	Clarified that scrolls are being requested, rather than sections.	AAW	03/20/2023
1.0	New	PAA	10/17/2022