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 A031701. 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 A031701 biospecimen collection, processing, and submission, including staff at satellite institutions.

2. Scope

This document applies to all biospecimens collected specifically for A031701 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
MSKCC	Memorial Sloan Kettering Cancer Center
FFPE	Formalin fixed, paraffin embedded

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 A031701 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 and to the Breast and Imaging Center at Memorial Sloan Kettering Cancer Center. 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 whole blood specimens in Streck BCT tubes, a biospecimen collection kit must be at the collection site. Please see section 7 for requesting biospecimen collection kits. Please allow at least 10 working days to receive the collection kit.

5.4 Please confirm that your institutional pathology department is willing to submit a fixed tissue block **OR** twenty (20) 10 micron unstained slides and two (2) unstained 5 micron slides taken at the start and end of the 20 slides for 22 total unstained slides (do not bake or coverslip slides) at the required time point designated in this document and in the trial protocol. An institution whose pathology department is unwilling to comply with block or slide submission should not enroll patients to this study. In the rare case when there are fewer than 20 unstained sections available, please submit at least 10 unstained sections. However, 20 unstained sections are strongly preferred, and sites should submit 20 sections whenever possible to ensure enough tissue is available for testing.

5.5 Unstained slides or tissue block must be confirmed as being available within 7 days after registration. All efforts must be made to obtain and submit slides OR fixed tissue block to central laboratory for sequencing as soon as possible.

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.

Time Point	Kit (Y/ N)	Biospecimen	Quantity	Collection / Processing Method	Shipping	Recipient Lab	Notes
Mandatory for all patients registered to A031701							
Baseline (≤ 7 days after registration)	N	Fixed tissue block	1	Unstained slides or diagnostic block (9.2)	Ambient	MSKCC	1
Baseline (≤ 7 days after registration)	N	Unstained Tumor Tissue Slides	22	Unstained slides or diagnostic block (9.2)	Ambient	MSKCC	1
Baseline (≤ 7 days after registration	N	Whole blood (EDTA tube)	10 ml	Whole blood (10.1)	Ambient	MSKCC	1
A031701-ST1							
Baseline (≤ 7 days after registration)	Y	Whole blood (Streck BCT tube)	2 x 10 ml	Plasma for cfDNA (10.2)	Ambient	ABWUSTL	2
Baseline (≤ 7 days after registration)	N	Urine for ctDNA	20-50 ml	Urine (11.0)	Cold Pack	ABWUSTL	2, 3
Cycle 6, Day 1 (or at end of chemotherapy)	Y	Whole blood (Streck BCT tube)	2 x 10 ml	Plasma for cfDNA (10.2)	Ambient	ABWUSTL	2, 4
Cycle 6, Day 1 (or at end of chemotherapy)	N	Urine for ctDNA	20-50 ml	Urine (11.0)	Cold Pack	ABWUSTL	2, 3, 4
6 months after treatment	Y	Whole blood (Streck BCT tube)	2 x 10 ml	Plasma for cfDNA (10.2)	Ambient	ABWUSTL	2
6 months after treatment	N	Urine for ctDNA	20-50 ml	Urine (11.0)	Cold Pack	ABWUSTL	2, 3
After Cystectomy	N	Fixed tissue block	1	Fixed tissue block for ST1 (9.3)	Ambient	ABWUSTL	2, 5
After Cystectomy	N	Fixed tissue cores	3	Fixed tissue cores (9.4)	Ambient	ABWUSTL	2, 5

Notes:

1. **Submission of fixed tissue block OR unstained slides and whole blood for germline DNA (EDTA tube) is mandatory for all patients registered to A031701. Slides or tissue block must be confirmed as being available within 7 days after patient registration. All efforts must be made to obtain and submit slides OR fixed tissue block to central laboratory for sequencing AS SOON AS POSSIBLE. At least 20 unstained tissue sections cut at 10 microns, plus 2 unstained sections cut at 5 microns taken before and after the 20 sections, and provided on non-charged slides are requested. In rare cases when there are fewer than 20 unstained sections available to submit, please submit at least 10 unstained sections. However, 20 unstained sections are strongly preferred and sites should submit 20 sections whenever it is possible to ensure enough tissue is available for testing.**
2. Collection is optional for patients but requires all sites offer to patients to consent. Please see protocol-specific consent documents.
3. A minimum of 20 ml of urine should be collected. See additional details in **section 11.0.**
4. If collection cannot occur on cycle 6 day 1, collection may occur after the last dose of chemotherapy has been administered, at the discretion of the treating physician. If blood collection cannot occur on C6D1, urine collection can be delayed and obtained on the same day as the post-chemotherapy blood collection for ease of kit processing and shipping.
5. A paraffin tissue block from the radical cystectomy **OR** three (3) 2 mm cores from such block should be submitted for patients opting in for A031701 ST-1.

7. Biospecimen Collection Kits

7.1 Blood Specimens

7.1.1 To facilitate the proper collection and shipping of whole blood specimens in Streck BCT tubes, biospecimen collection kits and materials will be provided. The cost of the kit and shipping the kit to the site will be paid for. The institution is expected to pay for shipping the kit with the biospecimens back to the Alliance Biorepository at Washington University in St. Louis via priority overnight shipping. **Please be aware that the kits only contain Streck BCT tubes. EDTA tubes are not included. Please use your local supply for the EDTA whole blood collection.**

7.1.2 Kits should be requested at least 10 working days in advance of the anticipated collection date. As many as 3 kits can be requested at one time. Since the collection materials (vacutainer tubes) have expiration dates, do not request kits more than 90 days prior to their anticipated use. All kits must be requested by using the BioMS system.

7.1.3 Kit contents and specific instructions for use of the kit are provided in the kit box. Please return any used collection materials with the kit.

7.1.4 Once a kit is received, do not discard the outer cardboard overwrap. The kit, containing biospecimens, is to be shipped back in the same box.

7.1.5 Please return all components of the kit, regardless of whether they have been used or not. Kits and kit components are recycled when possible, minimizing the kit cost.

7.1.6 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.

7.1.7 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).

7.1.8 Please return all kits that have expired or missing components. Return the ENTIRE kit using the cheapest possible shipping method at your expense. DO NOT DISCARD kits that have missing or expired components. Recycling kits keeps the cost of kit materials to a minimum. Please note that all outgoing and incoming kits are tracked, and sites that have requested many more kits than they have returned will be charged for non-returned kits.

7.1.9 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 kit components are highly specialized (e.g. Streck BCT) and probably are not available at the institution.

7.1.10 Note that protocol requirements are based on blood volumes, not tube sizes. If the protocol requires the collection of 8 cc of whole blood, generally a 10 cc tube is provided in the kit for convenience. If desirable or necessary to collect 8 cc in 3 x 3 cc tubes (for example), that is permissible.

7.2 Urine Specimens

7.2.1 There is no independent "kit" for collection or submission of urine specimens. Sites are responsible for supplying materials to facilitate urine collection.

7.2.2 Urine should be collected in a standard urine collection cup and subsequently transferred to EDTA tubes.

7.2.3 EDTA tubes should be placed into zip-top biohazard bags containing absorbent materials sufficient to absorb contents in case of leakage.

7.2.4 Specimens should be packaged to avoid breakage in a small, Styrofoam container including a cold pack to maintain temperatures between 2—15 degrees Celsius while shipping.

7.3 Tissue Specimens

7.3.1 There is no independent "kit" for submission of paraffin blocks, cores, or slides.

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

7.3.3 During warm weather months, paraffin blocks, cores, 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.

7.4 Please see Section 12 – Biospecimen Shipping for specific instructions on shipping to ABWUSTL and to The Breast and Imaging Center at MSKCC.

8. Biospecimen Labeling and Tracking

8.1 All research biospecimens (vacutainer tubes, urine tubes, and tissue bags) MUST be labeled with the participant study number, patient initials (Last, First, Middle), the date and time (if applicable) of collection and specimen type.

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 or cores are being submitted instead of the block, each tissue section slide or tube should be labeled with the patient study number, institutional surgical pathology number, the block identifier, and the serial section number. A copy of **the non-de-identified surgical pathology report** must be submitted with the tissue to MSKCC for **MSK-IMPACT testing for DDR gene status**. **Patient name, date of birth, gender, and block accession number should NOT be redacted. Other potential PHIs should be obscured usually using a permanent marker. Please also label the pathology report with patient ID on each page.** See section 9.2 for additional details. Provide **a de-identified copy of the surgical pathology report**, labeled with the patient study number, corresponding to the blocks or cores submitted to **ABWUSTL**. Please ensure the institutional surgical pathology number and block ID are maintained on the surgical pathology report. See section 9.3 for additional details.

8.3 Label all containers and vials with an indelible, solvent-resistant marker when they are at ambient temperature.

8.4 Do not affix any labels to vials, slides or tubes. Label the collection containers directly with the marking pen.

8.5 All biospecimens that are collected and sent to the Alliance Biorepository or to the Breast and Imaging Center at MSKCC must be **logged and tracked in BioMS**. The BioMS system is a web-based application that tracks the collection and shipping of biospecimens. Once individual biospecimens are logged and 'shipped' in the BioMS system, a packing manifest will be created by the system. This manifest must be printed out and must accompany all biospecimen shipments. To become familiar with the BioMS system and for further information about training, access, and use, please contact the BioMS Help desk at: 1-855-55-BIOMS or bioms@alliancenctn.org.

8.6 In the event that BioMS cannot be accessed, please complete a BioMS Specimen Log and Shipping Manifest form which can be found here- **http://tinyurl.com/alliance-bioms-contingency**.

9. Tissue Collection

9.1 Overview.

9.1.1 Please refer to protocol-specific instructions for procedures related to actual tissue procurement from individual participants. The method for research tissue procurement (needle core biopsy, sampling of surgically resected tumor) is dependent upon the disease site and the individual patient.

9.1.2 When procuring tissue biospecimens by any method, when possible, avoid tissue that is grossly necrotic, hemorrhagic, fatty, or fibrous. If in doubt, briefly (1 min or less) place the tissue segment in a sterile specimen cup containing physiologic (normal) saline to rinse the tissue. Necrotic, hemorrhagic, and fatty tissue will generally dissolve or float on the surface while tumor and parenchymal tissue will remain intact and sink to the bottom of the cup.

9.2 Unstained Slides from Diagnostic Fixed Tissue Blocks OR Diagnostic Fixed Tissue Blocks

9.2.1 At least 20 unstained tissue sections, cut at 10 microns, plus 2 unstained sections cut at 5 microns taken before and after the 20 sections, and provided on **non-charged** slides are required for MSK-IMPACT testing for DDR gene status. **In rare cases when there are fewer than 20 unstained sections available to submit, please submit at least 10 unstained sections. However, 20 unstained sections are strongly preferred, and sites should submit 20 sections whenever it is possible to ensure enough tissue is available for testing.**

9.2.2 For sites unable or unwilling to comply with unstained slide submission, submission of ONE representative, diagnostic pathology, formalin fixed paraffin embedded (FFPE) tissue block is required.

9.2.1 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. Unstained slides submitted for this study will not be returned to the submitting institution.

9.2.2 Please follow the procedures below for submitting unstained tissue slides. For this mandatory submission, please download and complete the "**Central DDR Gene Status Results Form**" from the Alliance or CTSU websites. **The completed results form is REQUIRED to be submitted for central DDR gene assay.** Histopathology review will be performed at MSKCC to ensure the adequacy of tissue submitted for DDR gene assay. Sites should expect to receive the DDR gene results **within 6-8 weeks** after tissue slides are received by MSKCC's Molecular Diagnostics Service laboratory. The MSK-IMPACT assay will be performed in the CLIA-certified Molecular Diagnostics Service laboratory at MSKCC. A report including the somatic mutations of the sequenced tumor will be returned to the treating physician for further review with his/her patient. The clinical molecular profiling data, obtained in the context of A031701, will be entered into a HIPAA-compliant, de-identified, access-controlled database, known as the cBio portal. This data will be stored in the cBio portal for future, unspecified use by Alliance institutions. Although exceedingly rare, it is possible that in the course of calling somatic variants within a patient's tumor sample, an incidental germline mutation is identified in genes that are known to be associated with an increased risk of cancer or other diseases. These findings will be reviewed by the study chair and will be returned to the outside Principal Investigator and local policies on returning these findings to the patient should be followed.

# of slides	Section thickness	Slide type	Purpose
20	10 microns	Non-Charged	IMPACT testing for DDR gene status
2	5 microns	Non-Charged	IMPACT testing for DDR gene status

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

9.2.4 Cut sections at 10 micron or 5 micron thickness as indicated onto non-charged slides.

9.2.5 Ensure that each slide is labeled with the patient study number, the institutional surgical pathology number and block ID, the slide serial section number (1, 2, 3, etc.), and section thickness (5 um or 10 um).

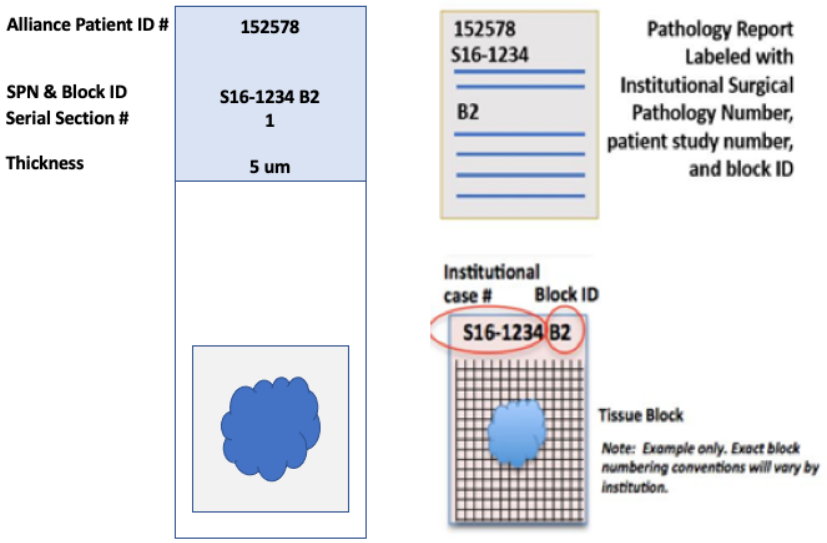
9.2.6 Do not label slides with adhesive labels. Write or print information on the textured surface of the slide with indelible, solvent-resistant ink.

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

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

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

9.2.10 See figure below for proper mounting and labeling.



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

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

9.2.13 Include a copy of the **pathology report (not de-identified). Please see section 8.2 for more instructions.**

9.3 Fixed Tissue Block for A031701-ST1

9.3.1 For patients who consented to sub-study A031701-ST1 and/or biobanking for future research, a representative diagnostic block from the radical cystectomy should be submitted, if applicable.

9.3.2 Include a copy of a de-identified pathology report, labeled with the patient study number with block submission.

9.4 Tissue Cores from Diagnostic Fixed Tissue Blocks

9.4.1 In cases where an institution is unwilling or unable to submit a tissue block for sub-study A031701-ST1, three (3), 2mm cores may be submitted as an alternative.

9.4.2 Place the tissue cores directly into a microcentrifuge tube or any other suitable container. Label the tube of tissue following the guidelines outlined in **section 9.2.**

10. Blood Collection Methods

10.1 Whole blood (EDTA Tube- no processing)

10.1.1 Collect 10 ml of blood into the EDTA tube using standard venous phlebotomy. Invert tube 10 times.

10.1.2 Store EDTA tube with whole blood at 4 degrees Celsius (i.e. refrigerated) until shipping. Do not freeze the tube. The tube may be stored for up to 72 hours at refrigerated temperature before shipment (i.e. if blood must be collected on Friday, it should be stored at 4 degrees Celsius over the weekend until Monday shipment). Ensure that the EDTA tube is shipped at ambient temperature to avoid freezing.

10.2 Plasma Nucleic Acid (Streck) Tube Processing

10.2.1 Collect 10 ml of blood into each of the Streck BCT tubes using standard venous phlebotomy. Invert tubes 10 times.

10.2.2 Store Streck tubes with whole blood at room temperature. Do not freeze or refrigerate the tubes. The tubes may be stored for up to 72 hours at ambient temperature before shipment. Ensure that the Streck tubes are shipped at ambient temperature to avoid freezing.

11. Urine Collection Methods

11.1 Instruct patients to collect 20-50 ml of first void urine into a standard urine collection cup.

11.2 Within 60 minutes of collection, distribute urine into 3 x 10 ml KEDTA vacutainer tubes.

11.3 Urine can be held for up to 72 hours at 4 degrees Celsius before shipping. Do not freeze the urine. Ensure that the urine is shipped on a cold pack to maintain temperature between 2-15 degrees Celsius during shipping.

12. Biospecimen Shipping

12.1 Overview

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

12.1.2 Place the original, completed copy of the BioMS packing manifest in the shipment. If sending tissue, include a copy of the DDR Gene Status Results Form (for MSKCC) and surgical pathology report (non-de-identified copy for MSKCC, de-identified copy for ABWUSTL, see **section 8.2**), labeled with the patient study number. Do not send specimens without a completed BioMS Packing Manifest or substitute "BioMS Downtime Form." Biospecimens cannot be accepted without this completed form.

12.1.3 **When shipping to MSKCC, notice of shipment should be sent to Seyram Doe-Tetteh (doetetts@mskcc.org) and Dr. Gopa Iyer (iyerg@mskcc.org). Notice of shipment should include FedEx tracking number and contact information for the individual sending the specimens.**

12.1.4 All biospecimens should be shipped within the timelines specified in **sections 9, 10, and 11.** Specimens should be shipped on Monday – Thursday only. If the event that patient or physician schedules require collection on a Friday, a Saturday, or on a holiday, please contact the Alliance Biorepository Program Manager: 1-314-747-4402 for further instructions, at least 24 hours prior to anticipated collection.

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

12.2 Shipping to The Breast and Imaging Center at MSKCC

12.2.1 Enclose whole blood (EDTA tube) and fixed tissue block **OR** slide mailer containing unstained tumor tissue slides within a padded envelope or small Styrofoam cooler. Ship for PRIORITY OVERNIGHT DELIVERY according to IATA guidelines and standard institutional policies.

Using the preferred vendor, ship to:

Seyram Doe-Tetteh
408 East 69th Street
New York, NY 10021
Phone: 646-888-3929

12.3 Shipping to ABWUSTL

12.3.1 Ship container for PRIORITY OVERNIGHT DELIVERY according to IATA guidelines and standard institutional policies and using the preferred vendor. A blank FedEx Air Bill is provided with the kit for convenience.

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.

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 DNA will be isolated from urine samples.

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

13.8 Fixed tissue biospecimens will be processed and embedded into paraffin using TPC standard operating procedures.

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

14. Document History

Version	Description and Justification of Change	Author	Effective Date
4.0	Updated shipping address for MSKCC Request for notice of shipment for MSKCC specimens	PAA	04/14/2021
3.0	Added urine collection Corrected minor typos and grammatical errors	PAA	03/10/2021
2.2	Updated BioMS email address	PAA	06/17/2019
2.1	Updated surgical pathology report requirements for MSKCC	YW, PAA	04/05/2019
2.0	Updated collection to include block Edited BioMS helpdesk contact info	YW, PAA	01/28/2019
1.5	Clarified study kit contents	YW, PAA	11/29/2018
1.4	Updated USS requirements Updated Cycle 6 timepoint to C6D1 Additional information provided on MSK-IMPACT assay	GI, YW, PAA	09/19/2018

1.3	Updated contact information for ABWUSTL Program Manager	PAA	06/13/2018
1.2	Updated requirements for surgical pathology reports	YW, PAA	06/08/2018
1.1	Clarification of mandatory collection and shipping procedures	YW, PAA	03/07/2018
1.0	New	PAA	12/08/2017