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 A032002. This document also describes the procedures that will be followed subsequent to the receipt of biospecimens by HistoGeneX or 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 A032002 biospecimen collection, processing, and submission; including staff at satellite institutions.

2. Scope

This document applies to all biospecimens collected specifically for A032002 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
H&E	Hematoxylin and Eosin

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 A032002 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 HistoGeneX and to the Alliance Biorepository at Washington University. For training and assistance in using the application or questions or problems related to specific specimen logging, please contact: 1-855-55-BIOMS or bioms@alliancenctn.org**.**

5.3 Prior to collection of biospecimens, a biospecimen collection kit must be at the collection site. Please see **section 7** for requesting biospecimen collection kits. Please allow at least 10 working days to receive the collection kit.

6. Collection Schema

The following biospecimens are to be collected at each of the time points below. Please refer to individual collection kit instructions, biospecimen collection and processing methods, and specific shipping procedures below.

Time Point	Kit (Y/N)	Biospecimen	Quantity	Collection / Processing Method	Shipping / Recipient	Notes
Mandatory for all patients registered to A032002						
<7 days after pre-registration	N	Unstained Slides	5	Unstained Slides for PD-L1 Testing (9.2)	Ambient / HistoGeneX	1
≤ 10 days before registration / prior to C1D1	N	Diagnostic H&E slides for histopathology review	Case dependent	H&E Slides for Histopathology Review (9.3)	Ambient / ABWUSTL	1
For patients registered to A032002 biobanking, submit the following:						
≤ 10 days before registration / prior to C1D1	Y	Fixed tissue block-tumor	1	Fixed tissue blocks (9.4)	Ambient / ABWUSTL	2, 4
≤ 10 days before registration / prior to C1D1	Y	H&E stained slide **AND** 5 um unstained slides **AND** 10 um unstained slides	1 H&E Stained Slide **And** 3 (5 um) Unstained Slides **AND** 20-25 (10 um) Unstained Slides	Fixed tissue slides (9.5)	Ambient / ABWUSTL	2, 4
≤ 10 days before registration / prior to C1D1	Y	Whole blood (Streck BCT)	2 x 10 ml	Plasma for cfDNA (10.1)	Ambient / ABWUSTL	2, 3
≤ 10 days before registration / prior to C1D1	Y	Urine	2 x 10 ml	Urine (11.0)	Cold Pack / ABWUSTL	2, 3

6 weeks	Y	Whole blood (Streck BCT)	2 x 10 ml	Plasma for cfDNA (10.1)	Ambient / ABWUSTL	2
6 weeks	Y	Urine	2 x 10 ml	Urine (11.0)	Cold Pack / ABWUSTL	2
3 months	Y	Whole blood (Streck BCT)	2 x 10 ml	Plasma for cfDNA (10.1)	Ambient / ABWUSTL	2
3 months	Y	Urine	2 x 10 ml	Urine (11.0)	Cold Pack / ABWUSTL	2
Disease Progression	Y	Whole blood (Streck BCT)	2 x 10 ml	Plasma for cfDNA (10.1)	Ambient / ABWUSTL	2, 5, 6
Disease Progression	Y	Urine	2 x 10 ml	Urine (11.0)	Cold Pack / ABWUSTL	2, 5

Notes:

1. Collection is mandatory for all patients registered to A032002. Please refer to **sections 9.2 and 9.3** for additional details.
2. All participating institutions must ask patients for their consent to participate in the banking of their specimens for future correlative studies, although patient participation is optional.
3. Should be collected before patient receives first dose of study drug, recommend on the same day of registration.
4. A representative, archived tumor tissue block from either the metastatic biopsy or surgical specimen should be submitted, if available. If entire tissue block cannot be submitted, 1 H&E stained slide **AND** three (5 um) **AND** 20-25 (10 um) unstained slides will be accepted as an alternative. If tissue is limited, please submit H&E and as many unstained slides as possible. **BLOCK SUBMISSION IS STRONGLY PREFERRED**.
5. Progression samples may be collected and submitted up to 1 month after progression.
6. Whole blood (Streck tubes) for ct-DNA specimen submission should continue to be collected if there is early discontinuation of protocol therapy until disease progression.

7. Biospecimen Collection Kits

7.1 HistoGeneX

7.1.1 There is no kit supplied for submission of slides to HistoGeneX for PD-L1 testing.

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

7.1.3 During warm weather months, paraffin 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.

7.1.4 Slides may be shipped for priority overnight delivery according to institutional policies and using the preferred vendor. Sites are responsible for shipping costs.

7.2 ABWUSTL

7.2.1 To facilitate the proper collection and shipping of all biospecimens submitted to ABWUSTL, biospecimen collection kits and materials will be provided. The cost of the kit and shipping of the kit to the site will be paid for. The institution is expected to pay for shipping the kit with the biospecimens back to the Alliance Biorepository at Washington University in St. Louis via priority overnight shipping.

7.2.2 Kits should be requested at least 10 working days in advance of the anticipated collection date. As many as 2 kits can be requested at one time. Since the collection materials (vacutainer tubes) have expiration dates, do not request kits more than 90 days prior to their anticipated use. All kits must be requested by using the BioMS system.

7.2.3 Kit contents and specific instructions for use of the kit are provided in the kit box. During warm weather months (i.e. June—August), a refrigerated pack (not frozen) should be included in the shipments containing blood specimens to maintain ambient temperature. When shipping blood during other months of the year, a room temperature pack should be included in the shipment. Regardless of time of year, urine should **always** be shipped with a refrigerated pack.

7.2.4 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.

7.2.5 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.

7.2.6 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.

7.2.7 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.

7.2.8 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 out-going and incoming kits are tracked, and sites that have requested many more kits than they have returned will be charged for non-returned kits.

7.2.9 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 while some kit components are generic (i.e. EDTA tubes), others are highly specialized (i.e. Streck BCT) and probably are not available at the institution.

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

7.2.11 Because paraffin blocks or slides cut from such blocks may be requisitioned and received from the surgical pathology department at a different time than the day of procurement for other biospecimens, paraffin blocks or cut slides may be sent independently of other biospecimens using the following guidelines:

7.2.11.1 There is no independent “kit” for submission of paraffin blocks or slides.

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

7.2.11.3 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.

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

7.3 Please see **Section 12 – Biospecimen Shipping** for specific instructions on shipping to HistoGeneX and to ABWUSTL.

8. Biospecimen Labeling and Tracking

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

8.2 Surgical pathology tissue blocks should not be labeled in any manner. The institutional surgical pathology number (e.g. “S16-1234”) and the individual block identifier (e.g. “A3”) should be readable on the block. If tissue slides are being submitted instead of the block, each tissue section slide should be labeled with the patient study number, institutional surgical pathology number, the block identifier, and the serial section number (if applicable). Provide **a de-identified copy of the surgical pathology report**, labeled with the patient study number, corresponding to the blocks or slides submitted. Please ensure the institutional surgical pathology number and block ID are maintained on the surgical pathology report. See **section 9** for additional details.

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

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

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

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

9. Tissue Collection

9.1 Overview.

9.1.1 Please refer to protocol-specific instructions for procedures related to actual tissue procurement from individual participants. The method for research tissue procurement (needle core biopsy, sampling of surgically resected tumor, or tumor 'debulking') 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 for PD-L1 Testing

9.2.1 Submission of unstained slides containing urothelial tissue from the metastatic biopsies or surgical specimens is required for central PD-L1 testing. Tissue must be submitted directly to HistoGeneX within 7 days of pre-registration. Please follow the procedures below for submitting unstained tissue slides.

# of slides	Section thickness	Slide type	Purpose
5	4-6 micron	Positively Charged	Central PD-L1 testing

9.2.2 Serial, tissue sections should be cut fresh (within 30 days of mounting; however, within 14 days preferred) from the appropriate formalin fixed, paraffin embedded tissue block.

9.2.3 Cut sections at 4-6 micron thickness as indicated onto positively charged slides.

9.2.4 Ensure that each slide is labeled with the patient study number, the institutional surgical pathology number and block identifier, and the slide serial section number (1, 2, 3, etc.).

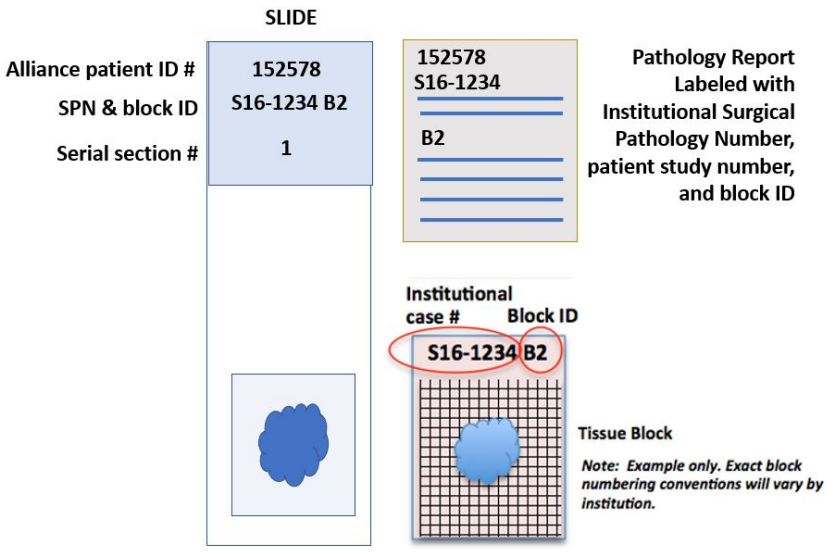
9.2.5 Do not label slides with adhesive labels. Write or print information on the textured surface of the slide with indelible, solvent-resistant ink. Labels must be xylene resistant.

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

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

9.2.8 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.

9.2.9 See figure below for proper mounting and labeling.



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

9.2.11 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.

9.2.12 The HistoGeneX Requisition Form must be submitted along with the slides to HistoGeneX. Failure to submit this form with the slides may delay turnaround time for central testing. The HistoGeneX Requisition Form can be located in Appendix 1 of this manual or on the A032002 protocol-specific page on the CTSU and Alliance websites. A digital copy of the form and the shipment tracking number should be sent at time of shipment to UStrials@histogenex.com.

9.2.13 A de-identified copy of the surgical pathology report should also accompany the slides and requisition form.

9.2.14 Turnaround time is 5 business days from receipt of all required specimens and documents (i.e. slides, query free HistoGeneX requisition form, de-identified pathology report which matches the slides submitted). HistoGeneX will reach out to sites if <5 unstained slides received or if there are missing or discrepant documents.

9.2.15 Sites will be provided a link to the HistoGeneX portal to access results. The cutoff for stratification factor is 5%.

9.3 H&E Stained Slides for Central Pathology Review

9.3.1 Submission of H&E stained slides from tumor tissue is required for retrospective central pathology review to confirm local diagnosis. Sites should submit H&E stained slides to the Biorepository.

9.3.2 Any stained slides submitted must be labeled as indicated in **section 9.2.9.**

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

9.4 Diagnostic Pathology Fixed Tissue Blocks.

9.4.1 This protocol requests submission of one representative, diagnostic pathology, formalin fixed paraffin embedded block containing urothelial tissue from the metastatic biopsy or surgical specimen.

9.4.2 Any clinical surgical pathology block that is submitted for research studies will not be exhausted or rendered unsuitable for future diagnostic use. Any clinical surgical pathology block that is submitted will be returned within ten working days of written request, when needed for clinical management or clinical trial enrollment for a specific patient. Otherwise, all blocks will be returned to the submitting institution when the trial and correlative science study end points have been met.

9.4.3 In the event that an institution will not release a tissue block, the institution may instead submit tissue sections, mounted and unstained to glass slides (see **section 9.5**). **BLOCK SUBMISSION IS STRONGLY PREFERRED.**

9.5 Unstained Slides from Diagnostic Fixed Tissue Blocks

9.5.1 In cases where institutions are unable or unwilling to submit the requested tissue block, a set of unstained slides may be submitted as an alternative. **An H&E stained slide should accompany unstained slides. The H&E stained slide should be from the same block where the unstained slides were cut from.** If fewer than the requested number of unstained slides can be submitted, please submit as many as possible. Please follow the procedures below for submitting unstained tissue 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
3	5 micron	Positively Charged	CyTOF
20-25	10 micron	Positively Charged	RNA, DNA

9.5.2 Serial tissue sections should be cut fresh at 5 or 10 micron onto positively charged slides. Please follow mounting and labeling instructions located in **sections 9.2.4—9.2.11.**

9.5.3 A de-identified surgical pathology report should accompany slide submission to ABWUSTL.

10. Blood Collection Methods

10.1 Plasma Nucleic Acid (Streck) Tube Processing

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

10.1.2 Store Streck BCT tubes with whole blood at room temperature until shipping. Do not freeze the tubes. **Whole blood collected in Streck BCT tubes can be stored for up to 72 hours at room temperature before shipping.** Ensure that the Streck BCT tubes are shipped at ambient temperature to avoid freezing. **During warm weather months (i.e. June—August), a refrigerated pack (not frozen) should be included in the shipment to maintain ambient temperature. When shipping during other months of the year, a room temperature pack should be included in the shipment.**

11. Urine Collection

11.1 Instruct patients to collect approximately 50 ml of urine into a clean specimen cup.

11.2 Within 60 minutes of collection, distribute 20 ml of urine into 2 x 10 ml EDTA vacutainer tubes. Remaining urine may be discarded following standard institutional procedures.

11.3 Urine in EDTA preservative may be held for up to 72 hours at 4 degrees Celsius (i.e. refrigerated) prior to shipping. Urine tubes should be shipped on a cold pack to maintain temperature between 2—15 degrees Celsius.

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. Do not send specimens without a completed BioMS Packing Manifest or substitute "BioMS Downtime Form." Biospecimens cannot be accepted without this completed form. If sending tissue, include a copy of the de-identified surgical pathology report. Tissue being submitted to HistoGeneX should also be accompanied by the HistoGeneX Requisition Form.

12.1.3 All biospecimens should be shipped within the time frame specified in **sections 9**-**11** above. If collected biospecimens cannot be shipped within the specified time frame (e.g. Friday – Saturday or Holiday collections), please contact the Alliance Biorepository Program Manager: 1-314-747-4402 or alliance@email.wustl.edu for further instructions, at least 24 hours prior to anticipated collection.

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

12.2 Shipping to HistoGeneX

12.2.1 Enclose slide mailer containing unstained tissue slides within a padded envelope or small Styrofoam cooler. Ship for PRIORITY OVERNIGHT DELIVERY according to IATA guidelines and standard institutional policies.

Notice of shipment should be sent, along with the HistoGeneX Requisition Form and shipment tracking number to UStrials@histogenex.com

Using the preferred vendor, ship to:

HistoGeneX LLC
Attn: Sample Reception Team- P0952
1331 W 75th Street, Suite 401
Naperville, IL, 60540
Phone: 630-473-6575

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 Aliquoted biofluids will be stored under liquid nitrogen vapor.

13.7 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
1.0	New	PAA	10/04/2021

Appendix 1- HistoGeneX Requisition Form

ALLIANCE A021502 REQUISITION FORM

(SPECIMEN SUBMISSION FORM FOR MMR ASSAY)

Instructions: After the patient has been registered, please complete this form electronically (i.e. typed, not handwritten) and submit this form with the required specimens to the HistoGeneX (HGX) Laboratory. **All fields are mandatory.**

Per Section 6.2 of the Protocol: Submit 10 Superfrost® Plus Micro Slides from the prior to treatment tumor tissue specimen. If Superfrost® Plus Micro Slides are not available, then any 10 unstained, charged slides at 4-6 micron thickness are acceptable. The specimens *must* be submitted to the central laboratory within 30 days of mounting the paraffin sections on slides (but within 14 days is preferred) for retrospective dMMR confirmation testing. Additionally, a de-identified surgical pathology report should be submitted along with the slides to the central laboratory.

Ship specimens to: **HistoGeneX LLC**
Attn: Sample Reception Team – P0952
1331 W 75th Street, Suite 401
Naperville, IL, 60540

Site Contact Information

Institution Name: _____

CTEP Institution Code (e.g. XY123): _____

Site Contact Name: (First) _____ (Last) _____

E-mail Address: _____

Phone Number: (___) ____ - _____ ext. _____

Specimen Information

Patient Initials: (First) _____ (Last) _____ Alliance Patient ID Number (e.g. 1234567): _____

Specimen Collection Date (MM/DD/YYYY): _____ Slide Section Mounting Date (MM/DD/YYYY): _____

Specimen Surgical Pathology Number/Block Number: _____

Tumor Type: ☒ Specimen is primary tumor, location: Colon

Collection Method: ☒ Excision/Resection

Fixation Method: ☒ Formalin Fixed, Paraffin Embedded (FFPE)

Slide Drying Method: ☐ Baked ☐ Air Dried ☐ Unknown

Specimen Type(s): ☐ Superfrost® Plus Micro Slides ☐ Unstained, Charged Slides (any)

Slide Quantity: ___ /10 Slide Quantity: ___ /10

De-identified Pathology Report Attached: ☐ Yes ☐ No, reason: _____

BioMS Shipment ID # (e.g. 12345678): _____

Questions? Email UStrials@histogenex.com or call HGX at (630) 473-6575.

Version Date: 12/01/2017