Title:	Tumor Frozen Needle Biopsy Preparation for the γH2AX IFA				Page 1 of 17
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National Clinical Target Validation Laboratory (NCTVL)

Applied Developmental Directorate

SAIC-Frederick, Inc.

NCI-Frederick Cancer Research Facility

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Change History

Revision	Approval Date	Description	Originator	Approval
--	10/22/08	New document	WHY	JJ
A	2/01/10	Format SOP, add Appendices 1 and 2, remove references to biopsy collection procedures, remove references to calibrator/control slide preparation, and define biopsy sectioning procedure by slide and use	YAE	JJ
B	12/29/10	Update SOP following in-house assay runs of patient samples.	WHY	JJ

Please check for revision status of the SOP at

http://dctd.cancer.gov/ResearchResources/ResearchResources-biomarkers.htm

and be sure to use the current version.

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SOP340507:
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- Collect and freeze tumor needle biopsies for use in biomarker assays

Ship to NCTVL or Certified Assay Site

SOP340522:
Tumor Frozen Needle Biopsy Preparation for the γH2AX IFA

- NBF fix and paraffin embed tumor needle biopsies and positive control sample
- Section biopsies for use in γH2AX IFA
- Stain 2 slides by H&E for standard histology analysis

Calibrator/Control Slides (DCTD-Provided Critical Reagent)

Ship to NCTVL or Certified Assay Site

SOP340523:
γH2AX IFA for Tumor Biopsy Slides

- Load biopsy slides into Bond-Max Processing Module; maximum of 3 patients, slides loaded so 1 patient per slide tray
- Bond-Max automated staining of slides with biotinylated-γH2AX monoclonal primary antibody as the detector and a streptavidin, Alexa Fluor 488 conjugate as the reporter
- Stain slides with DAPI and mount cover slips

Image within 18 to 72 h

SOP340533:
Image Capture of Tumor Biopsy Slides From γH2AX IFA

- Capture images of γH2AX-stained biopsy slides from a single patient (1 Bond-Max slide tray) using a Leica DM5000B microscope and Leica Plan Apo 20x/0.7 NA objective

SOP340534:
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- Quantitate captured images of γH2AX-stained biopsy slides using Image-Pro software and custom macros

1.0 PURPOSE

Standardize an immunohistochemical method for detecting and quantifying γH2AX staining in formalin-fixed, paraffin-embedded human tissue biopsies for pharmacodynamic studies of chemotherapeutic DNA-damaging agents.

2.0 SCOPE

This procedure applies to all personnel involved in processing clinical trial biopsy samples for the preparation of slides for quantitation of γH2AX using the γH2AX Immunofluorescence Assay (IFA) for Tumor Biopsy Slides (SOP340523). This SOP includes the procedures for specimen preparation by dehydration, fixation and paraffin-embedding for microtomy, and for slide preparation of sectioned tissues samples. The goal of the SOP and associated training is to ensure consistency of γH2AX measurement between operators and clinical sites.

3.0 ABBREVIATIONS

Cal	=	Calibrator
DAPI	=	4',6-Diamidino-2-Phenylindole
DCTD	=	Division of Cancer Treatment and Diagnosis
DI	=	Deionized
γH2AX	=	Histone H2AX Phosphorylated at Serine 139
H&E	=	Hemotoxylin and Eosin
ID	=	Identification/Identifier
IFA	=	Immunofluorescence Assay
LHTP	=	Laboratory of Human Toxicology & Pharmacology
NA	=	Numerical Aperture
NBF	=	Neutral Buffered Formalin
NCTVL	=	National Clinical Trial Validation Laboratory
QC	=	Quality Control
RT	=	Room Temperature
SOP	=	Standard Operating Procedure

4.0 INTRODUCTION

The γH2AX IFA is an immunohistochemistry-based staining assay developed to quantify the nuclear DNA damage marker, histone H2AX phosphorylated at serine 139 (γH2AX). The assay uses a biotinylated-γH2AX monoclonal antibody as the detector and an Alexa Fluor 488-streptavidin conjugate (Strp488) as the reporter for immunostaining.

5.0 ROLES AND RESPONSIBILITIES

Laboratory Director/Supervisor	The Laboratory Director/Supervisor, directs laboratory operations, supervises technical personnel and reporting of findings, and is responsible for the proper performance of all laboratory procedures. The Laboratory Director/Supervisor oversees the personnel who follow the SOPs in the laboratory and is responsible for ensuring the personnel are certified and have sufficient experience to handle clinical samples.
Certified Assay Operator	A Certified Assay Operator may be a Laboratory Technician/ Technologist, Research Associate, or Laboratory Scientist who has been certified through DCTD training on this SOP. The Certified Assay Operator works under the guidance of the Laboratory Director/Supervisor. This person performs laboratory procedures and examinations in accordance with the current SOP(s), as well as any other procedures conducted by a laboratory, including maintaining equipment and records and performing quality assurance activities related to performance.

*Depending on the laboratory, one person may have multiple roles.

5.1 It is the responsibility of the Laboratory Director/Supervisor to ensure that all personnel have documented DCTD training and qualification on this SOP prior to the actual handling and processing of samples from clinical trial patients. The Laboratory Director/Supervisor is responsible for ensuring the Certified Assay Operator running the SOP has sufficient experience to handle and analyze clinical samples.

5.2 The Certified Assay Operator for this SOP should be well versed and comfortable with tissue embedding and sectioning techniques.

5.3 The Certified Assay Operator responsible for conducting the assay is to follow this SOP and complete the required tasks and associated documentation. The Batch Record (Appendix 1) must be completed in ***real-time*** for each experimental run, with each page ***dated and initialed*****,** and placed with the clinical sample information.

5.4 All responsible personnel are to check the DCTD Biomarkers Web site (http://dctd.cancer.gov/ResearchResources/ResearchResources-biomarkers.htm) to verify that the most recent version of the SOP for the assay is being used.

6.0 MATERIALS, CRITICAL REAGENTS, AND EQUIPMENT REQUIRED

6.1 Fresh-frozen murine testes (DCTD-provided Critical Reagent; commercial sources are available but have not been validated for this SOP)

6.2 20-mL borosilicate glass scintillation vials (Fisher Scientific, Cat#: 03-337-15)
6.3 Scintillation vial caps with cone-shaped plastic liner (Fisher Scientific, Cat#: 03-337-7)
6.4 Transfer pipettes
6.5 Forceps
6.6 Tissue embedding cassettes and molds
6.7 Tissue/biopsy processing cassettes
6.8 Containers for graded ethanol and xylene washes of tissue embedding cassettes
6.9 Superfrost plus slides (Fisher Scientific, Cat#: 12-550-15)
6.10 Accu-Edge low-profile microtome blades (e.g., Sakura Finetek, Cat#: 4689 or Fisher Scientific, Cat#: NC9292148)
6.11 Slide box (e.g., Fisher Scientific, Cat#: 03-448-10)

6.12 Sterile-filtered, molecular biology grade deionized (DI) water (Invitrogen, Cat#: 10977-015)
6.13 Paraffin (e.g., Paraplast)
6.14 10% neutral buffered formalin (NBF; Thermo Scientific, Cat#: 5701)
6.15 Anhydrous ethanol, histology grade (Fisher Scientific, Cat#: A405-20 [Filtered using 0.22 µm pore size before use.])
6.16 Xylenes – histology grade
6.17 H&E staining solutions, histology grade (standard methods)

6.18 Tissue embedding station (should include paraffin dispenser with heated work block and a second cooling block). *Alternate*: 60°C incubator, 60°C heated work block, and cooling block (approx. -5°C)
6.19 Low-profile water bath, set to 48°C
6.20 Microtome (e.g., Leica RM2255 Automated Microtome, Leica Microsystems)

6.21 Dry ice
6.22 -80°C freezer
6.23 Liquid nitrogen storage system
6.24 37°C incubator
6.25 Tumor frozen needle biopsies processed following SOP340507

*If instruments and/or reagents differ from those specified above, the Laboratory performing the assay must prove their comparability or equivalence to those recommended using the manufacturer's specifications and experimental validation data.

7.0 OPERATING PROCEDURES

NOTE: The Batch Record (Appendix 1) is for biopsy samples from a single patient.

7.1 Clinical specimens for this assay will be frozen needle biopsies collected and stored according to SOP340507. After clinical biopsy collection, the specimens are snap-frozen and stored at -80°C. Biopsies can be stored at -80°C for up to 8 d after collection. After 8 d, the biopsies should be moved to liquid nitrogen storage.

Biopsy pairs (1st and 2nd biopsy) should always be stored together and processed as a pair. 1st and 2nd biopsy samples generally correspond to the pre- and post-dose biopsy time points.

7.2 Record the name and certification number of the Certified Assay Operator, the facility running the SOP, the Patient ID, the clinical trial site and the clinical protocol number in the Batch Record (Appendix 1).

7.3 Validated Critical Reagents for this SOP will be provided to clinical sites with Certified Assay Operators trained on the performance of this assay. For this SOP, the Critical Reagent is 6 vials containing ¼ of a fresh-frozen murine testis per vial. This is sufficient for analysis of paired tumor biopsy samples from 3 patients. Critical Reagents include a 2-fold surplus, so under optimal conditions, 6 sets of patient samples can be assayed.

7.4 Record the lot number and date of receipt of the Critical Reagent in the Batch Record (Appendix 1, Section 1). The reagent should be labeled with date of receipt and stored under the specified conditions for no longer than the recommended duration.

7.4.1 **Fresh-frozen murine testes:** Positive control sample. Store in -80°C for up to 1 mo.

7.5 For the 1st and 2nd biopsy samples, record the date of receipt, Sample ID, and other biopsy information as outlined in the Batch Record (Appendix 1, Section 2). **Note**: A 1st and 2nd biopsy from the same patient would have the same Patient ID but different Sample IDs.

7.5.1 For a single biopsy time point, multiple passes through the tumor may have been collected. A **single pass** of the **1st and 2nd biopsy** samples should be used for embedding. Additional passes should be stored in liquid nitrogen until needed. If data are acquired from the first pass, the remaining biopsy passes can be used per institutional guidelines.

7.6 For each patient, one paraffin tissue block will contain the patient's 1st biopsy sample and a positive control specimen (fresh-frozen murine testis). A second paraffin block will contain the patient's 2nd biopsy sample. Parallel processing of all 3 tissues should be done to ensure minimal sample handling and processing variability.

7.7 Protocol for Specimen Fixation

7.7.1 Remove a single pass of a 1st and 2nd biopsy sample for one patient, as well as the positive control Critical Reagent vial from -80°C/liquid nitrogen storage; immediately place on dry ice. Record biopsy information, for each biopsy to be embedded, in the Batch Record (Appendix 1, Section 3).

7.7.2 Biopsy Samples:

7.7.2.1 Warm microtubes containing frozen biopsies slightly by gently rolling between palms of hands for 10 sec. Fill the tubes with 10% NBF and let sit for at least 1 min at RT.

7.7.2.2 Label one 20-mL scintillation vial for each clinical sample with appropriate Sample ID. Using a transfer pipette, carefully transfer the NBF and clinical samples into the correctly labeled 20-mL scintillation vials. If necessary, flush the clinical sample microtubes with additional 10% NBF until the entire samples have been transferred to the scintillation tubes.

7.7.2.3 Top off the scintillation tube to 20-mL total volume with 10% NBF. Be sure each specimen is completely immersed in NBF in an individually labeled scintillation vial.

7.7.3 Positive Control Sample:

7.7.3.1 Label a 20-mL scintillation vial as "positive control" and fill with 10% NBF.

7.7.3.2 Using forceps slightly chilled on dry ice, transfer one fresh-frozen murine testes from the Critical Reagent vial to the 20-mL scintillation vial.

7.7.3.3 If needed, top off the scintillation tube to 20-mL total volume with 10% NBF. Be sure specimen is completely immersed in NBF. Return the Critical Reagent vial to -80°C/liquid nitrogen.

7.7.4 Allow the tissue to fix for 16 to 24 h at RT (optimal fixation time is 20 h). Do not let fixation proceed for longer than 96 h. Record the start and stop dates and times for fixation in the Batch Record (Appendix 1, Section 3).

7.8 Protocol for Paraffin-Embedding of Specimens

7.8.1 Prepare the tissue embedding station by pre-warming the paraffin and a heat block to 60°C and pre-cooling a cooling block to -5°C.

7.8.2 Prepare containers containing the graded-ethanol series (made with DI water and filtered) and xylenes as outlined in SOP Step 7.8.5.

7.8.3 For a single patient, pre-label 3 tissue processing cassettes and 2 embedding cassettes and molds.

7.8.3.1 For the 1st biopsy sample, label a processing cassette, embedding cassette, and mold with the **Patient ID** and **Sample ID**. Repeat for the 2nd biopsy sample. Each embedding cassette should be assigned a unique **Block Number** for tracking unsectioned samples. Record the Block Number for each cassette in the Batch Record (Appendix 1, Section 3).

7.8.3.2 The third processing cassette should be labeled as the **Positive Control** and will be embedded together with the 1st biopsy specimen.

7.8.4 Using a transfer pipette, remove the specimens from the NBF scintillation vials and place within the pre-labeled tissue processing cassettes. Place the cassettes into 70% ethanol and begin the paraffin-embedding sequence (SOP Step 7.8.5). Be sure to process a single patient's 1st and 2nd biopsy samples as well as one positive control tissue in parallel.

7.8.5 Paraffin-embedding sequence:

Step	Solution	Time	Temperature
1	70% Ethanol	30 min	RT
2	80% Ethanol	30 min	RT
3	80% Ethanol	30 min	RT
4	95% Ethanol	30 min	RT
5	95% Ethanol	30 min	RT
6	100% Ethanol	30 min	RT
7	100% Ethanol	30 min	RT
8	100% Ethanol	30 min	RT
9	100% Xylenes	30 min	RT
10	100% Xylenes	30 min	RT
11	Paraffin	45 min	60°C
12	Paraffin	45 min	60°C
13	Paraffin	45 min	60°C
14	Paraffin	30 min	60°C

7.8.6 Place a small amount of melted paraffin in the bottom of each pre-labeled embedding mold.

7.8.6.1 Using a transfer pipette, carefully transfer the positive control and 1st biopsy into the correctly labeled embedding mold and the 2nd biopsy into its mold. Use heated forceps to orient the biopsies within the molds to allow longitudinal sectioning of the biopsies.

7.8.6.2 The section orientation will match that in SOP Step 7.9.5. This embedding procedure and orientation ensures that the 1st and 2nd biopsy molds and sections are easily distinguishable.

7.8.7 Briefly transfer the mold onto a cooling block; the paraffin will partially solidify into a thin layer and hold the tissue pieces in position.

7.8.8 Immediately place the matching pre-labeled embedding cassette on top of each mold, then fill the combined mold and cassette with paraffin and return it to the cold plate to finish solidifying. Record the date the samples were embedded in the Batch Record (Appendix 1, Section 3).

7.8.9 Immediately proceed to microtomy. For temporary storage of blocks, store at 2-8°C away from volatile chemicals.

7.9 Protocol for Microtomy and Slide Preparation

7.9.1 Ensure a low-profile water bath is preheated to 48°C.

7.9.2 Select a paired set of clinical sample blocks (a positive control/1st biopsy block and a 2nd biopsy block) for a single patient.

7.9.2.1 A maximum of 26 slides will be made. This will include two slides for H&E staining, one set of 8 slides for initial γH2AX staining, and two backup sets of slides (8 slides each).

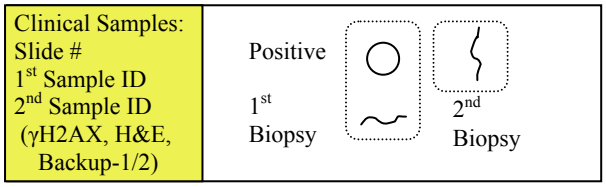
7.9.3 Pre-label 26 slides with the slide number (sequentially #1-26; may not use all slides), the Sample IDs for both the 1st and 2nd biopsies, and slide use as follows. Sections for each biopsy **MUST BE CONSECUTIVE CUTS, PLACED IN ORDER** on the slides**.** Section #1 (Slide #1) for each biopsy should be the first section from each block that has tissue pieces at least 2 mm^2.

Use and Slide/Section Number			
H&E	γH2AX	Backup-1	Backup-2
1	2	3	4
-	5	6	7
-	8	9	10
11	12	13	14
-	15	16	17
-	18	19	20
-	21	22	23
-	24	25	26

7.9.4 Section paraffin blocks in 5-micron sections. Each section placed on slides should have tissue pieces at least 2 mm^2. Record the date blocks are sectioned in the Batch Record (Appendix 1, Section 4).

7.9.5 Carefully float each section from each block on water in a 48°C water bath.

7.9.5.1 Collect paired specimen sections such that one section containing the positive control/1st biopsy specimens and one 2nd biopsy specimen section are placed onto each of the pre-labeled slides in the following **orientation**:



7.9.5.2 Sections #1 and #11 from each block are placed on the corresponding slides and will be used for H&E staining.

7.9.5.3 The 2nd section and every 3rd section thereafter (excluding #11) will be used for initial γH2AX staining (Slides #2, #5, #8, #12, etc.). These slides will be labeled "γH2AX."

7.9.5.4 Place the Backup-1 and Backup-2 sections (as designated in SOP Step 7.9.3) on their corresponding slides.

7.9.6 If any section is skipped or placed on a slide out of order, or if a slide is removed due to issues associated with placement of the paraffin section on the slide, make a notation of the deviation(s) for the slide(s) affected in the Batch Record (Appendix 1, Section 4).

7.9.7 Verify that all slides are labeled correctly and are generated with the appropriate tissues and orientation by placing a checkmark in the appropriate column of the Slide Preparation Table in the Batch Record (Appendix 1, Section 4).

7.9.8 Dry the slides overnight in a 37°C incubator.

7.10 H&E Slide Quality Control (QC)

7.10.1 Stain slides #1 and #11 with H&E according to standard methods. Both H&E-stained slides should be analyzed by qualified staff personnel and meet the following QC criteria:

- Morphology of each section should indicate acceptable nuclear and cellular definition,
- Sufficient tumor cellularity should be present in each section so that at least three 20x fields can be analyzed, and
- There should be sufficiently low necrotic areas in each section so that at least three 20x fields with 80% viable tissue can be analyzed.

7.10.1.1 If **both** H&E-stained slides **pass QC**, document this as ALL slides passed QC in the Batch Record (Appendix 1, Section 5) and proceed to SOP Step 7.11. Send Slide #1 to the clinic to be maintained with the patient records.

7.10.1.2 If **both** H&E-stained slides **fail QC**, all slides should be discarded. If a second pass of the biopsies was collected, repeat the SOP and embed the second pass, starting a new Batch Record. If none of the biopsy passes pass QC, the clinic should be informed that there was insufficient tumor tissue or cellularity in the biopsy to perform the biomarker assay.

7.10.1.3 If **either** H&E-stained slide **fails QC**, a bookend approach will be used to narrow down slides of sufficient quality for the γH2AX IFA. H&E stained slides #1 and #11 represent the starting bookends to determine tissue quality. Clearly document QC results for individual H&E stained slides in the Slide Preparation Table of the Batch Record (Appendix 1, Section 4).

- Beginning with the H&E bookend slide that failed QC, select the next sequential slide closer to the H&E bookend slide that passed QC. Stain this slide by H&E and determine QC pass/fail. If this slide fails, repeat this step until a slide passes QC.

  Example: If slide #1 passes but slide #11 fails, slide #10 would then be stained for H&E to determine if it passes QC. Continue sequentially until a slide passes QC; slides between #1 and the upper bookend would then be used for the γH2AX IFA.

- Document each slide that is stained by H&E in the Slide Preparation Table of the Batch Record (Appendix 1, Section 4) and the slide number of each bookend that passes QC (Appendix 1, Section 5).

- If H&E QC leads to a limited slide set being acceptable for γH2AX analysis or if the entire slide set fails, document reason in Appendix 1, Section 6. **Important**: A minimum of 3 slides need to be assayed to be confident in the image quantitation.

7.11 Place the slides labeled "γH2AX," "Backup-1," "Backup-2," and H&E-stained Slide #11 in a slide tray box, batched by slide label. The slides should be stored in a desiccator at 2-8°C away from volatile chemicals until use. If there is tissue remaining in the paraffin block, store with the Backup slides.

7.12 Slides labeled "γH2AX" should be processed following SOP340523 within 1 wk of sectioning. If the γH2AX slides do not pass QC, the Backup slides should be processed within 4 wk of sectioning. Once γH2AX data is acquired for a patient, any remaining Backup slides and the paraffin block can be used per institutional guidelines.

7.13 Review and finalize the Batch Record and document **ANY** and **ALL** deviations from this SOP in the Batch Record (Appendix 1, Section 6).

8.0 OPTIONAL: SHIP TO CERTIFIED ASSAY SITE FOR ANALYSIS

If the γH2AX Immunofluorescence Assay will be performed at a separate certified assay site or NCTVL, ship the slides as follows:

8.1 Send an e-mail to the certified assay site prior to shipping to advise recipient of scheduled shipping time (NCTVL, NCINCTVL@mail.NIH.gov). Be sure to request and receive a confirmation e-mail prior to shipping.

8.2 Generate a shipping list containing all the specimen records using the Shipping Manifest template as shown in Appendix 2. In the Batch Record, verify that all slides in the slide box are from a single patient, confirm that Slide #11 is H&E stained, and indicate if a paraffin block is included (Appendix 1, Section 7).

8.2.1 A Shipping Manifest may contain more than one patient's samples, but a single patient's slide box should contain only a single patient's slides and be clearly labeled.

8.3 Be sure to verify that the contents of the package match the Shipping Manifest.

8.4 Print and attach the shipping address onto the outside of the shipping container.

8.4.1 If shipping to NCTVL, use the following address:

Attn: Jay Ji, PhD
National Clinical Target Validation Laboratory (NCTVL)
DTP, DCTD, National Cancer Institute
37 Convent Drive
Building 37, Room 1048A
Bethesda, MD 20892
Phone: 301-443-2149

8.5 Record the shipping date, time, tracking number, and shipping information in the Batch Record (Appendix 1, Section 7).

8.6 Ship the specimens **with a copy of** the Shipping Manifest and copies of the completed Batch Records for all patient specimens. Retain copies of the completed Shipping Manifest and Batch Records in your records.

8.7 E-mail the certified assay site (NCTVL, NCINCTVL@mail.NIH.gov) a shipment notification. State "*Protocol Name* PD Specimen Shipment" in the subject line and reference the tracking number and shipping information in the e-mail.

APPENDIX 1: BATCH RECORD

A separate Batch Record should be started for **each** patient.

NOTE: Record times using **military time** (24-h designation); for example, specify 16:15 to indicate 4:15 PM.

Certified Assay Operator: _____

Certification Number: _____

Date: _____

Laboratory Director/Supervisor: _____

Date: _____

Facility Preparing Paraffin Blocks and Sections: _____

Patient ID: _____

Clinical Protocol Number: _____

Clinical Trial Site: _____

1. Critical Reagents

Critical Reagents validated for this SOP are provided to clinical sites with Certified Assay Operators upon request. Critical Reagents for this SOP are fresh-frozen murine testes.

Date Received	Reagent Name	Lot Number	No. Vials Used	No. Remaining
	Fresh-frozen murine testes		1	

2. Patient Samples Received From Clinical Site

	*Date of Receipt	*Sample ID	*Biopsy Date/Time	Diagnosis	No. of Passes Received
1st Biopsy:					
2nd Biopsy:					

3. Sample Information

*Date Embedded		Pass Number	Fixation Start (Date/Time)	Fixation Stop (Date/Time)	*Paraffin Block Number
	1st Biopsy				
	2nd Biopsy				
	Positive Control	N/A			N/A

*Required information

BATCH RECORD: INITIALS _____ DATE: _____

4. Slide Preparation

Date Blocks Sectioned: _____

Verify that each slide contains the following **CONSECUTIVE** sections in the appropriate orientation.
Note: Slides should contain tissue pieces at least 2 mm^2.

Slide No.	Positive Control & 1st Biopsy	2nd Biopsy	Notes or Deviations From SOP
1	☐	☐	H&E
2	☐	☐	
3	☐	☐	
4	☐	☐	
5	☐	☐	
6	☐	☐	
7	☐	☐	
8	☐	☐	
9	☐	☐	
10	☐	☐	
11	☐	☐	H&E
12	☐	☐	
13	☐	☐	
14	☐	☐	
15	☐	☐	
16	☐	☐	
17	☐	☐	
18	☐	☐	
19	☐	☐	
20	☐	☐	
21	☐	☐	
22	☐	☐	
23	☐	☐	
24	☐	☐	
25	☐	☐	
26	☐	☐	

Color Code: γH2AX | Backup-1 | Backup-2 | H&E

BATCH RECORD: INITIALS _____ DATE: _____

5. Pathology QC of H&E-Stained Slides*:

☐ **PASS**	Slide range that passed QC: ☐ All ☐ Bookend slide #____ to #____ **Important**: ≥ 3 slides need to be assayed to be confident in image quantitation.
☐ **FAIL**	All slides discarded

* If QC leads to a limited slide set being acceptable for γH2AX analysis or if the entire slide set fails, document reason in Section 6 below.

6. Notes, including any deviations from the SOP:

7. Shipping to Certified Assay Site

Verify a single patient's slides are in slide box: ☐ Yes ☐ No

Verify H&E stained slide included: ☐ Yes ☐ No

Paraffin block included: ☐ Yes ☐ No

Date and time samples shipped: _____

Tracking information: _____

Attach copy of Shipping Manifest

BATCH RECORD: INITIALS _____ DATE: _____

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APPENDIX 2: SAMPLE SHIPPING MANIFEST

Ship From: Contact Name: Tel: E-mail:	**Shipping Manifest**	**Ship To:** **Attn:** **Tel:** **E-mail:**

Shipping Date: **Carrier:**

In Package	Item No.	Patient ID	Sample ID	Clinical Protocol #	Item/Description
☒	*Example*	*06*	*AT12061306 and AT12043306*	*09-C-0000*	*Patient slide set, H&E slide, and paraffin block*
☐	1				
☐	2				
☐	3				
☐	4				
☐	5				
☐	6				
☐	7				
☐	8				
☐	9				
☐	10				