Title:	Tumor Frozen Needle Biopsy Preparation for the γH2AX IFA				Page 1 of 25
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National Clinical Target Validation Laboratory

Applied/Developmental Research Directorate, Leidos Biomedical Research, Inc.

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and be sure to use the current version.

Change History

Revision	Approval Date	Description	Originator	Approval
D	1/13/2015	Modified method for fixing and embedding tissue to ensure maximal tissue area will be available per section following microtomy. Clinical slide preparation and H&E slide quality control criteria updated. Added visual inspection of slides with operator-specified range of 18 or 35 slides for IFA analyses. Modified slide designations. Paraffin dipping of some backup slides added to preserve analytes.	KFG, YAE	KFG
C	9/22/2013	Update SOP flow diagram and relabel biopsy samples to specify location of pre- and post-dose biopsies. H&E QC criteria clarified and presence of neoplastic tissue added. Water bath temperature adjusted.	YAE	JJ
B	12/29/2010	Update SOP following in-house assay runs of patient samples.	WHY	JJ
A	2/01/2010	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
--	10/22/2008	New document	WHY	JJ

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OVERVIEW OF IMMUNOFLUORESCENCE ASSAY FOR BIOPSIES

SOP340507:

Tumor Frozen Needle Biopsy Specimen Collection and Handling

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

Ship to 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 slides by H&E for standard histology analysis

Calibrator/Control Slides

(PADIS/IQC-Provided Critical Reagent)

Ship to Certified Assay Site

SOP340523:

γH2AX IFA for Tumor Biopsy Slides

- Load biopsy slides into Bond-Max Processing Module
- 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

Ship to Certified Assay Site

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 fluorescent microscope and Plan Apo 20x objective with ≥ 0.7 NA.

SOP340534:

Image and Data Analysis of Tumor Biopsy Slides From γH2AX IFA

- Quantitate captured images of γH2AX-stained biopsy slides using Image-Pro software, custom macros, and a data analyses Excel.

1.0 PURPOSE

Standardize the method for fixing, embedding, and sectioning pre- and post-dose clinical biopsies along with control tissue for the immunohistochemical detection and quantification of histone H2AX phosphorylated at serine 139 (γH2AX) 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 fixation, dehydration 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	=	Hematoxylin 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 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.

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 Digital versions of the Slide Preparation Table in the Batch Record (Appendix 1, Sections 4) can be created for logging sample information as long as all column information exactly matches the tables in the Batch Record. A copy of the completed, digital sample tables must be printed and attached to the Batch Record in order to maintain a complete audit trail.

5.4 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.

5.5 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 AND EQUIPMENT REQUIRED

6.1 PADIS/IQC-Supplied Critical Reagents

6.1.1 Fresh-frozen murine testes halves (commercial sources are available but have not been validated for this SOP)

6.2 20-mL borosilicate glass scintillation vials (e.g., Fisher Scientific, Cat#: 03-337-15)
6.3 Scintillation vial caps with cone-shaped plastic liner (e.g., 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 Small petri dish (e.g., Falcon, Cat#: 351007, 60 x 15mm)
6.9 Metric ruler
6.10 Black paper
6.11 Digital camera
6.12 Laboratory utility wipe (e.g., Kimberly Clark, WYPALL-L10 Utility Wipes, Cat#: 05322). Wipe needs to be thick enough to prevent biopsy from curling during fixation process
6.13 Lens paper (e.g., Cat#: VWR Scientific, Cat#: 52846-001)
6.14 Containers for graded ethanol and xylene washes of tissue embedding cassettes
6.15 Superfrost plus slides (e.g., Fisher Scientific, Cat#: 12-550-15)
6.16 Accu-Edge low-profile microtome blades (e.g., Sakura Finetek, Cat#: 4689 or Fisher Scientific, Cat#: NC9292148)
6.17 Slide box (e.g., Fisher Scientific, Cat#: 03-448-10)
6.18 Dry ice
6.19 Sterile-filtered, molecular biology grade deionized (DI) water (e.g., Invitrogen, Cat#: 10977-015) or Milli-Q ultra-pure water
6.20 Paraffin (e.g., Paraplast)
6.21 10% neutral buffered formalin (NBF; e.g., Fisher Scientific, Cat#: 22-050-105)
6.22 Anhydrous ethanol, histology grade (e.g., Fisher Scientific, Cat#: A405-20 [Filtered using 0.22 µm pore size before use.]) ACS/USP Grade can be purchased and used without filtration (Pharmco-AAPER, Cat#: 111000200PL05)
6.23 Xylenes – histology grade
6.24 H&E staining solutions, histology grade (standard methods)
6.25 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)
6.26 Low-profile water bath, set to 50°C
6.27 Microtome (e.g., Leica RM2255 Automated Microtome, Leica Microsystems)
6.28 -80°C freezer
6.29 Liquid nitrogen storage system
6.30 37°C incubator
6.31 Frozen needle biopsies processed following SOP340507

7.0 OPERATING PROCEDURES

NOTE: A separate Batch Record (Appendix 1) should be started for each set of 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 (pre- and post-dose biopsy) should always be stored together and processed as a pair.

7.2 If samples were shipped from a separate site, save the clinical shipping manifest for the laboratories' record and attach a copy to this Batch Record.

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

7.4 Critical Reagent

7.4.1 Record the date of receipt, lot number, and expiration date for the Critical Reagent in the Batch Record (Appendix 1, Section 1).

7.4.2 Store the reagent as indicated below. Label reagent with the date of receipt and store under the specified conditions for no longer than the recommended duration.

- **Fresh-frozen murine testes:** Positive control sample, murine testes halves. Store at -80°C for up to 4 mo.

7.5 For the pre- and post-dose biopsy samples, record the date of receipt, Patient/Sample ID, and number of passes received in the Batch Record (Appendix 1, Section 2).

Note: A pre- and post-dose biopsy from the same patient would have the same Patient ID but different Sample IDs. The Patient/Sample ID should include the CTEP protocol number followed by a unique patient identifier and a sequential specimen ID (NCI tumor biopsies for PD sampling are series 500).

7.5.1 For a single biopsy time point, multiple passes through the tumor may have been collected. A **single pass** of the **pre- and post-dose 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.

7.5.2 For each patient, two paraffin tissue blocks will be prepared in parallel. One paraffin tissue block will contain the patient's pre-dose biopsy sample and a fresh-frozen murine testis specimen. A second paraffin block will contain the patient's post-dose biopsy sample. Parallel processing of all 3 tissues should be done to ensure minimal sample handling and processing variability.

7.6 Protocol for Specimen Fixation

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

7.6.2 Biopsy Samples:

7.6.2.1 Fill two (2) scintillation vials with 20 mL 10% NBF each and label one vial each for the pre- and post-dose biopsy with appropriate Sample ID.

7.6.2.2 One biopsy sample at a time, warm the microtube containing the frozen biopsy slightly by gently rolling between palms of hands for 10 sec. Using a transfer pipette, transfer 0.5-1 mL 10% NBF from the scintillation vial to the corresponding biopsy tube. Let sit for 2-5 min at RT.

7.6.2.3 Carefully pour the NBF and clinical sample into a small petri dish containing 7 mL NBF. If necessary, flush the microtube with 10% NBF from the scintillation vial until the entire sample is in the petri dish.

7.6.2.4 Optional: A digital photograph may be taken to document the tissue appearance at this point. Recommended photography steps:

- Place a black piece of paper under the petri dish and a metric ruler alongside the dish. A sample ID should also be visible in the photograph so that the correct identity of the sample is captured. The original sample vial label may be used or a pre-prepared label for the biopsy block cassette may also be used.
- Take a digital photograph of the sample at close range while ensuring the camera is focused. Most standard digital cameras have a mode for close-up photographs.
- Save the image to a secure location using the specimen ID in the file name.

7.6.2.5 Using forceps transfer the partially fixed tissue to a small piece of laboratory wipe orienting the tissue to prevent it from folding or curling during the fixation process. The laboratory wipe will be removed prior to embedding.

7.6.2.6 Transfer the tissue, adhered to the laboratory wipe, and 10% NBF into the correctly labeled 20-mL scintillation vial and discard the residual NBF from the petri dish.

7.6.2.7 Be sure each specimen is completely immersed in NBF in an individually labeled scintillation vial.

7.6.3 Testis Positive Control Sample:

7.6.3.1 Label a 20-mL scintillation vial as “positive control” and fill with 20 mL 10% NBF.

7.6.3.2 Warm the microtube containing the frozen testis half slightly by gently rolling between palms of hands for 10 sec. Using a transfer pipette, transfer 0.5-1 mL 10% NBF from the scintillation vial to the corresponding biopsy tube. Let sit for at least 1 min at RT.

7.6.3.3 Carefully pour the NBF and testis into the correctly labeled 20-mL scintillation vial. If necessary, flush the microtube with 10% NBF from the scintillation vial until the entire sample has been transferred to the vial.

7.6.3.4 Be sure specimen is completely immersed in NBF in an individually labeled scintillation vial.

7.6.4 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).

7.7 Protocol for Paraffin-Embedding of Specimens

7.7.1 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.

7.7.2 Prepare containers containing the graded-ethanol series (made with DI water and filtered) and xylenes as outlined in SOP Step 7.7.4.

7.7.3 For a single patient, pre-label 3 tissue processing cassettes.

7.7.3.1 For the pre-dose biopsy sample, label a processing cassette with the **Patient/Sample ID**; repeat for the post-dose biopsy sample. Each clinical biopsy processing cassette should be assigned a unique **Block Number** for tracking unsectioned samples.

7.7.3.2 Record the Block Number for each clinical cassette in the Batch Record (Appendix 1, Section 3). These processing cassettes will be placed on the embedding molds in SOP Step 7.7.6.

7.7.3.3 The third processing cassette should be labeled as the **Testis**; this tissue will be embedded together with the pre-dose biopsy specimen and therefore does not need a separate Block Number assigned.

7.7.4 Using clean forceps gently remove the specimens adhered to the laboratory wipe from the NBF scintillation vials and gently detach the tissue from the laboratory wipe.

7.7.4.1 Carefully orient the tissue for full-face presentation onto a small piece of lens paper pre-moistened with NBF.

7.7.4.2 Fold the lens paper over the biopsy to secure the tissue in the correct orientation and place within the pre-labeled tissue processing cassettes. The lens paper will help prevent the biopsy from curling up during the dehydration process; it will not be embedded with the tissue.

7.7.4.3 Place the cassettes into 70% ethanol and begin the paraffin-embedding sequence. Be sure to process a single patient's pre- and post-dose biopsy samples as well as the testis control tissue in parallel.

7.7.5 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

7.7.6 Place a small amount of melted paraffin in the bottom of an embedding mold.

7.7.6.1 Using a clean preheated forceps, carefully transfer the testis positive control and pre-dose biopsy from their processing cassettes into the embedding mold. For the pre-dose biopsy, carefully remove the tissue from the lens paper and use heated forceps to orient the biopsy within the mold to allow longitudinal sectioning of the biopsy.

7.7.6.2 The section orientation will match that in SOP Step 7.8.5. This embedding procedure and orientation ensures that the pre- and post-dose biopsy molds and sections are easily distinguishable.

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

7.7.6.4 Immediately place the correctly labeled clinical biopsy processing cassette on top of the 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).

7.7.7 Repeat Step 7.7.6 for the post-dose biopsy. The post-dose biopsy is embedded alone, without a testis specimen.

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

7.8 Protocol for Microtomy and Clinical Slide Preparation

7.8.1 Ensure a low-profile water bath filled with ddH_2O is preheated to 50°C.

7.8.2 Select a paired set of clinical sample blocks (a testis/pre-dose biopsy block and a post-dose biopsy block) for a single patient.

7.8.2.1 A maximum of 50 slides will be made.

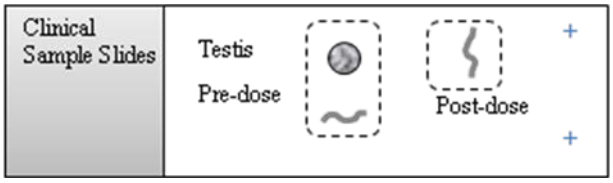
7.8.3 Pre-label 50 slides with sequential slide numbers (#1-50; may not use all slides), the Patient/Sample IDs for both the pre- and post-dose biopsies.

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.

7.8.4 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).

7.8.5 Carefully float each section from each block on water in a 50°C water bath.

7.8.5.1 Collect paired specimen sections such that one section of each of the two sections represented below (dashed lines) are placed onto each of the pre-labeled slides **in the following orientation**:



7.8.6 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).

7.8.7 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).

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

7.9 Visual Inspection of Slides, Determination of Slide Range for Analysis and Paraffin Dipping of Slides

7.9.1 The operator will visually inspect the entire set of up to 50 slides using a microscope. A range of 35 consecutive slides should be designated as optimal for H&E and subsequent IFA analysis.

- Selection of the 35-slide range designated for analysis is based on visual inspection of the slides and comparison of the relative area of the biopsy sections across the slide set.
- The designated range should have pre- and post-dose biopsy sections optimally at, or near, full-face longitudinal view and contain sufficient control tissue for the IFA analysis.

7.9.2 The 1st, 18th and 35th slide designated in the optimal 35-slide range will be used for H&E analysis. For example if cut slides 5 – 39 are designated as the slides that should proceed for further analysis, slides 5, 22 and 39 will be designated for H&E analysis. Selection of which slides in the 35-slide range are used for the primary IFA analysis and as backup slides are made after the H&E analysis of the slides is complete (SOP Section 7.10).

7.9.3 If < 35 slides were prepared during microtomy due to one of the tissues being exhausted the following guidelines should be followed:

7.9.3.1 A range of 18 consecutive slides should be designated from the available slide set as optimal for the H&E and subsequent IFA analysis following the guidelines in SOP Step 7.9.1.

7.9.3.2 The 1st and 18th slide in the optimal 18-slide range will be used for H&E analysis. For example. if a total of 24 slides are prepared during microtomy and slides 5 – 22 are designated as the slides that should proceed further for analysis, slides 5 and 22 will be designated for H&E analysis.

7.9.4 A minimum of 18 slides are needed to proceed with further analysis.

7.9.4.1 If < 18 slides were prepared during microtomy due to one of the tissues being exhausted, and a second pass of the biopsy is available for analysis, repeat the SOP and embed a second pass of both the pre- and post-dose biopsy, starting a new Batch Record.

7.9.4.2 If no second pass biopsy is available, or if a subsequent biopsy block also yields < 18 slides, no further analysis should be performed on the tissues. The tissues will be designated as "TQ" and not reportable due to insufficient or poor tissue quality on the Clinical Sample Data Report in SOP340534.

7.9.5 All slides except those designated for H&E analysis should be dipped in paraffin to prolong stability. The slides should be dipped in paraffin within 24 hours of the completion of microtomy.

7.9.5.1 Any additional slides prepared during microtomy that were determined to fall out of the optimal range (35 or 18) by visual inspection should be considered unanalyzable (UA).

7.10 H&E Slide Quality Control (QC)

7.10.1 The slides designated for H&E should be stained according to standard methods. The H&E-stained slides should be analyzed by a staff pathologist. Depending on the institution, a whole-slide digital scan of both H&E slides can be performed or the slides themselves can be provided to use for analyses.

7.10.2 H&E slides must meet the following QC criteria:

- Presence of neoplastic tissue
- Morphology of each section should indicate acceptable nuclear and cellular definition,
- Sufficient cellularity should be present in each section so that at least one (1) 20x field (~0.45 mm^2) can be analyzed, and
- There should be sufficiently low necrotic areas in each section so that at least one (1) 20x field with ≥ 80% viable tissue can be analyzed.

7.10.3 Pass/Fail QC Decisions and Designation of Slides for IFA Analysis

7.10.3.1 If the H&E-stained slides meet any of the following criteria the slides **Pass QC**:

- If the 1st, 18th and 35th slide were stained and each slide passes H&E Slide QC, the slide set **Passes QC**.
- If the 1st, 18th and 35th slide were stained and two consecutive slides (either the 1st and the 18th or the 18th and the 35th) pass H&E Slide QC and the third slide fails H&E Slide QC, the slide set **Passes QC**.
- If only the 1st and 18th slide were stained and each slide passes H&E slide QC, the slide set **Passes QC**.
- Proceed to SOP Step 7.11.

7.10.3.2 If the H&E-stained slides meet any of the following criteria the slides **Fail QC**:

- If the 1st, 18th and 35th slide were stained and no two consecutive slides pass QC, the slide set **Fails QC.**
- If only the 1st and 18th slide were stained and either or both slides fail H&E QC, the slide set **Fails QC.**

7.10.3.3 If the H&E-stained slides Fail QC and a second pass of the biopsies was collected, repeat the SOP and embed the second pass, starting a new Batch Record.

- If no second pass biopsy is available, or if a subsequent biopsy block also fails H&E slide QC, the tissues will be designated as "TQ" as not reportable due to insufficient or poor tissue quality on the Clinical Sample Data Report in SOP340534, Appendix 5.

7.10.4 If the H&E-stained slide set **Failed QC**, record the specific reason for QC failure (e.g., insufficient tumor tissue or cellularity in the biopsy) in the deviations section of the Batch Record (Appendix 1, Section 6) and proceed to SOP Step 7.12.

7.11 Slide Designations for IFA Analyses

7.11.1 Based on the optimal slide range and H&E Slide QC pass/fail outcome, assign slides to the following slide-use designations in the Batch Record (Appendix 1, Section 5).

7.11.1.1 Any additional slides prepared during microtomy that were determined to fall out of the optimal range (35 or 18) by visual inspection should be considered unanalyzable (UA).

7.11.2 If the optimal slide range was 35 slides and the 1st, 18th and 35th slide passed H&E slide QC, the slide designations are as follows:

Slide Designations: Slide numbers refer to **order** in the optimal 35-slide range based on visual inspection				
H&E	**IFA**	**Backup-1**	**Backup-2**	**Backup-3**
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
	27	28	29	30
	31	32	33	34
35				

7.11.3 If the optimal slide range was 35 slides and the 1st and 18th slide passed H&E slide QC (but slide 35 failed) OR if the optimal slide range was 18 and the 1st and 18th slide passed H&E slide QC, the slide designations are as follows:

Slide Designations: Slide numbers refer to **order** in the optimal 35-slide range based on visual inspection		
H&E	**IFA**	**Backup-1**
1	2	3
	4	5
	6	7
	8	9
	10	11
	12	13
	14	15
	16	17
18		

- If the optimal slide range was 35, slides 19 – 35 should be designated at UA.

7.11.4 If the optimal slide range was 35 slides and the 18th and 35th slide passed H&E QC (but slide 1 failed), the slide designations are as follows:

Slide Designations: Slide numbers refer to **order** in the optimal 35-slide range based on visual inspection		
H&E	**IFA**	**Backup-1**
18	19	20
	21	22
	23	24
	25	26
	27	28
	29	30
	31	32
	33	34
35		

- Slides 1 – 17 should be designated at UA.

7.12 If the 35- or 18-slide set failed H&E QC (SOP Step 7.10), all slides in the optimal slide range and any additional slides prepared during microtomy should be designated as UA.

7.13 Place the H&E and paraffin-dipped slides, grouped by label (i.e. H&E, IFA, Backup 1, Backup 2, Backup 3 or UA), in a slide box. The slides should be stored in a desiccator at 2°C to 8°C away from volatile chemicals until use. If there is tissue remaining in the paraffin block, store with the Backup slides.

7.14 Once IFA data are acquired for a patient, any remaining Backup slides and the paraffin block can be used per institutional guidelines.

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

7.16 The Laboratory Director/Supervisor should review the Batch Record and sample reports and sign the Batch Record affirming the data contained within the reports are correct (Appendix 1, Section 8).

8.0 OPTIONAL: SHIP TO CERTIFIED ASSAY SITE FOR ANALYSIS

If the IFA will be performed at a separate certified assay site, ship the slides as follows:

IMPORTANT: Include a copy of the Batch Record for all samples being shipped with the Shipping Manifest.

8.1 Send an e-mail to the certified assay site prior to shipping to advise recipient of scheduled shipping time. 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 and indicate if a paraffin block is included (Appendix 1, Section 7).

8.2.1 A Shipping Manifest may include 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 **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.

8.5 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 shipment notification. State "*Protocol Name* PD Specimen Shipment" in the subject line and reference the tracking number and shipping information in the e-mail.

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APPENDIX 1: BATCH RECORD

Pre-dose Specimen Label Here

Post-dose Specimen Label Here

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: _____

Facility Preparing Paraffin Blocks and Sections: _____

Patient ID: _____

Clinical Protocol Number: _____

1. Critical Reagents

Reagent Name	Date Received	Lot Number	Expiration Date
Fresh-frozen murine testis	/ /		/ /

2. Patient Samples Received From Clinical Site

	*Date Received	*Patient/Sample ID	No. of Passes Received
Pre-dose Biopsy:	/ /		
Post-dose Biopsy:	/ /		

3. Sample Information

	Pass Number	Fixation Start (Date/Time)	Fixation Stop (Date/Time)	Date Embedded	*Paraffin Block Number
Pre-dose Biopsy:		/ / :	/ / :	/ /	
Positive Control (testis):	N/A	/ / :	/ / :		
Post-dose Biopsy:		/ / :	/ / :	/ /	

*Required information

4. Slide Preparation and Visual Inspection

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.	Optimal 35 or 18 slide range based on Visual Inspection.	Notes or Deviations During Microtomy
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	☐	

(Slide Preparation and Visual Inspection, Continued)

Slide No.	Optimal 35 or 18 slide range based on Visual Inspection.	Notes or Deviations During Microtomy
26	☐	
27	☐	
28	☐	
29	☐	
30	☐	
31	☐	
32	☐	
33	☐	
34	☐	
35	☐	
36	☐	
37	☐	
38	☐	
39	☐	
40	☐	
41	☐	
42	☐	
43	☐	
44	☐	
45	☐	
46	☐	
47	☐	
48	☐	
49	☐	
50	☐	

BATCH RECORD: INITIALS _____ DATE: _____

5. H&E Slide QC and Slide-Use Designation

Order	Slide No. in Optimal Slide Range	H&E	IFA	Backup-1	Backup-2	Backup-3	UA
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			☐	☐	☐	☐	☐
27			☐	☐	☐	☐	☐
28			☐	☐	☐	☐	☐
29			☐	☐	☐	☐	☐
30			☐	☐	☐	☐	☐
31			☐	☐	☐	☐	☐
32			☐	☐	☐	☐	☐
33			☐	☐	☐	☐	☐
34			☐	☐	☐	☐	☐
35		☐					

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

8. Laboratory Director/Supervisor Review of Batch Record

Laboratory Director/Supervisor: _____ (PRINT)

_____ (SIGN)

Date: _____

BATCH RECORD: INITIALS _____ DATE: _____

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/Sample ID	Clinical Protocol/CTEP#	Item/Description
☒	*Example*	*1234-1025-500 and -501*	*12-C-0000/ 1234*	*Patient slide set, H&E slides, and paraffin block*
☐	1			
☐	2			
☐	3			
☐	4			
☐	5			
☐	6			
☐	7			
☐	8			
☐	9			
☐	10			