Title:	Tumor Frozen Needle Biopsy Preparation for Pharmacodynamic Immunofluorescence Assays Utilizing Murine Testis and/or Jejunum Control Tissues				Page 1 of 27
Doc. #:	SOP340550	Revision:	--	Effective Date:	3/26/2019

National Clinical Target Validation Laboratory

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Change History

Revision	Approval Date	Description	Originator	Approval
--	3/26/2019	New document	KFG	REP

TABLE OF CONTENTS

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

SOP340550: Tumor Frozen Needle Biopsy Preparation for Pharmacodynamic Immunofluorescence Assays Utilizing Murine Testis and/or Jejunum Control Tissues	• NBF fix and paraffin embed tumor needle biopsies and positive control sample • Section biopsies for use in IFA • Stain slides by H&E for standard histology analysis

1.0 PURPOSE

Standardize the method for fixing, embedding, and sectioning clinical biopsies along with murine testis and murine jejunum control tissues in preparation for multiplex immunofluorescence assays to support pharmacodynamic (PD) evaluations of DNA damage repair status and additional pharmacodynamic assays. The goal of the SOP and associated training is to ensure consistency of measurement between operators and clinical sites.

2.0 SCOPE

This procedure applies to all personnel involved in processing clinical trial biopsy samples for the preparation of slides for pharmacodynamic assays requiring murine testis and jejunum control tissues. This SOP includes the procedures for specimen preparation by fixation, dehydration and paraffin-embedding for microtomy, and for slide preparation of sectioned tissues samples.

3.0 ABBREVIATIONS

Cal	=	Calibrator
DAPI	=	4',6-Diamidino-2-Phenylindole
DCTD	=	Division of Cancer Treatment and Diagnosis
DI	=	Deionized
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

This protocol defines the procedure for fixing, embedding, and sectioning clinical biopsies along with the required murine testis and jejunum control tissues in preparation for multiplex immunofluorescence assays to support pharmacodynamic evaluations.

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 website (https://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 Critical Materials

6.1.1 Fresh-frozen murine testes: Fixation control tissue, murine testes halves. Testes serves as a positive or negative control tissue for several pharmacodynamic biomarkers.

6.1.2 Fresh-frozen murine jejunum: Fixation control tissue. Jejunum are collected from mice fed a chlorophyll free diet for two weeks prior to necropsy. Jejunum serves as a positive or negative control tissue for several pharmacodynamic biomarkers.

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 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
6.10 Lens paper (e.g., Cat#: VWR Scientific, Cat#: 52846-001)
6.11 Containers for graded ethanol and xylene washes of tissue embedding cassettes
6.12 Superfrost plus slides (e.g., Fisher Scientific, Cat#: 12-550-15)
6.13 Accu-Edge low-profile microtome blades (e.g., Sakura Finetek, Cat#: 4689 or Fisher Scientific, Cat#: NC9292148)
6.14 Slide box (e.g., Fisher Scientific, Cat#: 03-448-10)
6.15 Dry ice
6.16 Sterile-filtered, molecular biology grade deionized (DI) water (e.g., Invitrogen, Cat#: 10977-015) or Milli-Q ultra-pure water
6.17 Paraffin (e.g., Paraplast)
6.18 10% neutral buffered formalin (NBF; e.g., Fisher Scientific, Cat#: 22-050-105)
6.19 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)
6.20 Xylenes – histology grade
6.21 H&E staining solutions, histology grade (standard methods)
6.22 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)
6.23 Tissue processing station (capable of the tissue processing sequence detailed in Section 7.6, e.g., Sakura Tissue Tek VIP 6)
6.24 Low-profile water bath, set to 50°C
6.25 Microtome (e.g., Leica RM2255 Automated Microtome, Leica Microsystems)
6.26 -80°C freezer
6.27 Liquid nitrogen storage system
6.28 37°C incubator
6.29 Frozen needle biopsies collected and stored 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 procedure will be frozen needle biopsies collected and stored according to SOP340507. After clinical biopsy collection, the specimens are snap-frozen and stored at or below -80°C. Biopsies should be stored at -80°C or colder when possible (-140˚C, or liquid nitrogen). Biopsy sets (normally from two biopsy procedures, pre- and post-dose) should be stored together and processed as a pair when possible.

7.2 Record the name of the Certified Assay Operator, the facility running the SOP, the Patient/Sample ID, the clinical protocol number, the date the histology laboratory received the specimens, and the date the laboratory processed the specimens in the Batch Record (Appendix 1). The patient ID and the clinical protocol number should appear on each page of the batch record and on any accompanying document.

7.3 Critical Materials

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

Store the reagents as indicated below.

7.3.2 **Fresh-frozen murine testes**, murine testes halves, store at or below -80°C.

7.3.3 **Fresh-frozen murine jejunum**, store at or below -80˚C.

7.4 For the biopsy samples, record the date the biopsies were collected by the clinical site, when they were received, the Patient/Sample ID, and number of passes received in the Batch Record (Appendix 1, Section 2).

Note: Each 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).

7.4.1 For a single biopsy time point, multiple passes from the tumor may have been collected. Either a single pass or two passes of each biopsy sample should be used for embedding, as clarified in the histology request. Additional passes should be stored at -80°C or colder when possible (-140˚C, or liquid nitrogen).

7.4.2 For each patient, two paraffin tissue blocks will be prepared in parallel when possible. One paraffin tissue block will contain either one or two passes of the patient's earliest time-point biopsy sample (typically pre-dose) and both control tissues; murine testis tissue and murine jejunum tissue. If the earliest time point is not available, just the controls will be embedded in the first block. A second paraffin block will contain either one or two passes of the patient's second time-point biopsy sample. Parallel processing of all patient and control tissues should be done when possible to ensure minimal sample handling and processing variability.

7.4.2.1 If only the first time-point biopsy was collected, then only one paraffin block is prepared.

7.4.2.2 Further recommendations are provided in Section 8.0 for preparing a slide set from a single patient time-point.

7.4.2.3 For trials or individual patients with three biopsy timepoints see Section 9.0 for recommendations.

7.5 Protocol for Specimen Fixation

7.5.1 Remove either one or two passes of each biopsy sample time-point for one patient, as well as the testis and jejunum control specimens 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).

7.5.2 Biopsy Samples Fixation:

7.5.2.1 Label and fill an appropriate number of scintillation vials (one for each biopsy pass) with 20 mL of 10% Neutral Buffered Formalin (NBF). *Labels should have the assigned sample ID.*

7.5.2.2 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.

7.5.2.3 Carefully pour the NBF and clinical sample into a small petri dish or weigh boat 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 or weigh boat.

7.5.2.4 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.

7.5.2.5 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.

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

7.5.3 Testis and Jejunum Control Sample Fixation:

7.5.3.1 Label a 20-mL scintillation vial for each control tissue, the testis and the jejunum. Fill the vials with 20 mL 10% NBF.

7.5.3.2 One control tissue at a time, warm the microtube containing the tissue 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 sample tube. Let sit for at least **1 min** at RT.

7.5.3.3 Carefully pour the NBF and control tissue 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.

7.5.3.4 Ensure each tissue is completely immersed in NBF.

7.5.4 Allow all tissues 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).

7.6 Protocol for Tissue Processing and Paraffin-Embedding of Specimens

7.6.1 Prepare containers containing the graded-ethanol series (made with DI water and filtered) and xylenes as outlined in Step 7.6.2 below.

7.6.2 Tissue processing 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

7.6.3 For a single patient, pre-label tissue processing cassettes for each biopsy pass and control tissue.

7.6.3.1 For the first time-point biopsy sample(s), label a processing cassette with the **Patient/Sample ID for each pass**; repeat for all additional biopsy samples.

7.6.3.2 Two additional processing cassettes should be labeled as **Testis and Jejunum**.

7.6.4 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.

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

7.6.4.2 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.*

7.6.4.3 Place the cassettes into 70% ethanol and begin the tissue processing sequence. Process in parallel a single patient's biopsy samples and testis and jejunum control tissues.

7.6.5 Paraffin embedding

7.6.5.1 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.

7.6.5.2 Each clinical biopsy block should be assigned a unique **Block Number** for tracking unsectioned samples. Record the Block Number for each clinical biopsy time-point in the Batch Record (Appendix 1, Section 3).

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

7.6.5.4 Using clean preheated forceps, carefully transfer the testis control, the jejunum control and one or two passes of the first time-point biopsy from their individual processing cassettes into the final combined cassette within an embedding mold. For the biopsy samples, carefully remove the tissue from the lens paper and use heated forceps to orient the biopsy within the cassette to allow longitudinal sectioning of the biopsy.

7.6.5.5 The section orientations should match those shown in SOP Step 7.7.6. This embedding procedure and orientation ensure that the first and second time-point biopsy blocks and sections are easily distinguishable. For blocks in which two passes of a biopsy timepoint will be included, it is important to distinguish placement of each tissue and record which tissue will be in position "A" and "B" as noted in the block layout and the batch record.

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

7.6.5.7 Immediately fill the combined tissue cassette within the mold with paraffin. Return it to the cold plate to finish solidifying. Record the date the samples were embedded in the Batch Record (Appendix 1, Section 3).

7.6.6 Repeat Step 7.6.5 for the second time-point biopsy. **The second time-point biopsy is embedded without a control specimen.**

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

7.7 Protocol for Microtomy and Clinical Slide Preparation

7.7.1 Fill a low-profile water bath with ddH_2O and preheat to 50°C.

7.7.2 Select a paired set of clinical sample blocks (a control tissue/first time-point biopsy block and a second time-point biopsy block) for a single patient.

7.7.2.1 A maximum of 50 slides will be made when a single pass of each biopsy has been embedded.

7.7.2.2 A maximum of 25 slides will be made when two passes of each biopsy have been embedded.

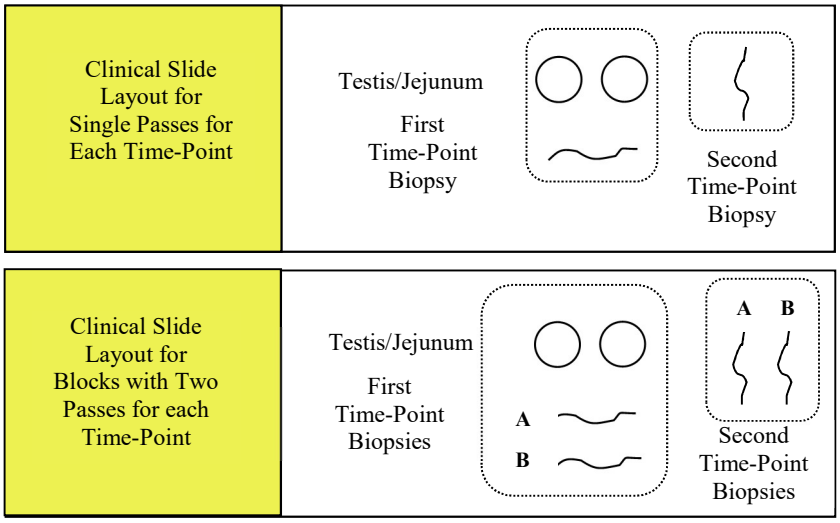
7.7.3 Pre-label slides with sequential slide numbers and the Patient/Sample IDs for both biopsy time points.

7.7.4 The block sections should be consecutively cut and placed in order on the slides. Section #1 on Slide #1 should be the first section from each block that has all tissue pieces with at least 2 mm^2.

7.7.5 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).

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

7.7.6.1 Arrange paired specimen sections so that one section of each of the two sections represented below (dashed lines) are placed onto each of the pre-labeled slides **in the orientation** shown below for both one pass and two pass clinical biopsy blocks.



7.7.7 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).

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

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

7.8.1 For slide sets from blocks with a single pass of each biopsy where a maximum of 50 slides were cut:

7.8.1.1 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 first and second time-point biopsy sections optimally at, or near, full-face longitudinal view and contain sufficient control tissue for the IFA analysis.

7.8.1.2 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. Only after H&E analysis is complete will it be decided which slides are designated and released for staining.

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

- If 35-slide range cannot be used, a range of 18 consecutive slides should be designated from the available slide set as optimal for the H&E and subsequent IFA analysis. 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.

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

- 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 first and second time-point biopsy, starting a new Batch Record.
- 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 SOP340545.

7.8.1.5 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. Record date of completion of paraffin dipping of the slides in the Batch Record (Appendix 1, Section 5).

- 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).

7.8.2 For slide sets from blocks with two passes of each biopsy where a maximum of 25 slides were cut:

7.8.2.1 The operator will visually inspect the entire set of up to 25 slides using a microscope. A range of 18 consecutive slides should be designated as optimal for H&E and subsequent IFA analysis.

- Selection of the 18-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 both first and second time-point biopsy sections optimally at, or near, full-face longitudinal view and contain sufficient control tissue for the IFA analysis.

7.8.2.2 The 1st and 18th slide in the optimal 18-slide range will be used for H&E analysis. For example, if a total of 25 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.

7.8.2.3 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. Record date of completion of paraffin dipping of the slides in the Batch Record (Appendix 1, Section 5).

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

7.8.3 Denote slides in the optimal 35 or 18 slide range and the slides H&E stained in the appropriate column of the Slide Preparation Table in the Batch Record (Appendix 1, Section 4).

7.9 H&E Slide Evaluation and Annotation

7.9.1 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. A whole-slide digital scan of the H&E slides should be utilized for the evaluation since this will allow for annotation of the viable tumor areas within the biopsy tissue sections.

7.9.2 Each biopsy tissue on the H&E slide should be evaluated for the presence of viable neoplastic tissue with acceptable nuclear and cellular definition. When possible, full annotation of a whole-slide digital scan of the H&E slides should be provided by a pathologist to demarcate all analyzable areas of viable tumor within the biopsy tissue sections.

7.9.3 Significant heterogeneity and low tumor content are frequent observations in post-diagnostic tumor biopsies. The root cause of lack of analyzable tumor cells has been attributed to several, often coexisting factors, including:

- Primarily or completely normal tissue
- Extensive mucin content
- Necrosis
- Fibrosis
- Too small
- Damaged post collection (i.e. crush artifact, freeze, artifact, autolysis)

7.9.4 The pathologist's evaluation of the H&E slide should include an estimation of the percentage of viable tumor within each biopsy tissue section. Additionally, the H&E evaluation should include an estimation of the percentage of the following factors that can interfere with analysis: Necrosis, Tumor Fibrosis, Mucin, Normal/Host Tissue, Artifact (Crush, Freeze, Air, Folds, Dry). A description of the tumor histology and cellularity pattern is also recommended.

7.9.5 With the final pathology report, each biopsy section is scored as follows:

7.9.5.1 >50% Viable Tumor Content

7.9.5.2 25-50% Viable Tumor Content

7.9.5.3 5-25% Viable Tumor Content

7.9.5.4 <5% Viable Tumor Content

7.9.6 Each biopsy is evaluated independently, and the goal is to identify a minimum of two consecutive H&E slides that are determined to be sufficient to proceed with analysis.

7.9.7 The following decisions and course of action are recommended based on the biopsy section score and the availability of additional biopsy passes:

Biopsy Score	Recommendations
>50%	Proceed to IFA Analysis
25-50%	Proceed to IFA Analysis
5-25%	Evaluate additional biopsy pass, if available. If there are no additional cores available or if this is the best score identified after evaluation of at least two cores, proceed with analysis.
<5%	Evaluate additional biopsy passes, if available. If there are no additional cores available or if this is the best score identified after evaluation of multiple cores discontinue analysis of the biopsy. The biopsy will be designated as "TQ" as not reportable due to insufficient or poor Tissue Quality on the Clinical Sample Data Report in SOP340545.

7.9.8 In some cases, only one biopsy of the pair will be determined to be analyzable and the SOP will be repeated when possible with an additional biopsy pass for the biopsy time point that fails to advance to analysis, following the guidelines in Section 8.0.

7.10 Slide Designations for IFA Analyses

7.10.1 Based on the optimal slide range and H&E Slide QC outcome, slides will be released for staining as appropriate from the optimal available slide range. Replicate slides selected for staining will be separated by a minimum of one slide and optimally three slides.

7.10.1.1 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).

7.10.2 If the optimal slide range was 35 slides and the 1st, 18th and 35th slide are sufficient to advance to analysis, the recommended 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				

7.10.3 If the optimal slide range was 35 slides and the 1st and 18th slide are sufficient for analysis (but slide 35 had a lower tumor content score, or was not sufficient to advance to analysis) OR if the optimal slide range was 18 and the 1st and 18th slide are sufficient to advance to analysis, the recommended 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 as UA.

7.10.4 If the optimal slide range was 35 slides and the 18th and 35th slide are sufficient for analysis (but slide 1 had a lower tumor content score, or was not sufficient to advance to analysis), the recommended 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 as UA.

7.11 For slide sets which contain two passes of each biopsy time-point and 18 slides designated as being in the optimal range based on visual inspection, follow 7.10.3 for slide use designations.

7.12 Place the H&E and paraffin-dipped slides in a slide box. The slides should be stored in a desiccator at RT away from volatile chemicals until use. If there is tissue remaining in the paraffin block, store with the Backup slides.

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

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

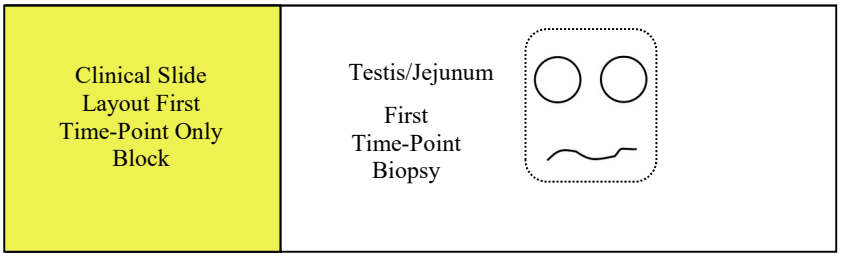
7.15 The Laboratory Director/Supervisor should review the Batch Record and sample reports and sign the Batch Record affirming the data contained within the reports are correct (Appendix 1, Section 7).

8.0 OPTIONAL: ALTERNATIVE PROCEDURE FOR PREPARING A SINGLE PATIENT BIOPSY TIME-POINT

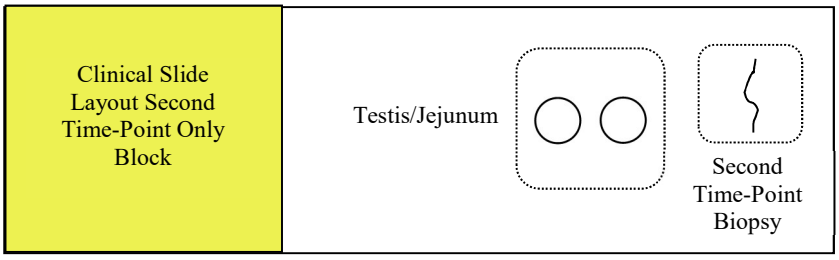
8.1 In some cases, it will be necessary to process a single patient biopsy rather than a pair. This section provides that alternative slide layout and guidelines to follow in that event.

8.2 Control tissues should be fixed, paraffin embedded and sectioned in parallel with the first time-point or second time-point biopsy. The alternative layouts for the first time-point or second time-point only clinical slides are provided below.

8.2.1 Pre-dose only clinical slide layout:



8.2.2 Post-dose only clinical slide layout



8.3 Clarify that only a first time-point or second time-point biopsy is being processed in the Batch Record (Appendix 1, Sections 2 & 3).

9.0 OPTIONAL: ALTERNATIVE PROCEDURE FOR PREPARING TISSUES INCLUDING THREE BIOPSY TIME-POINTS

9.1 For some patients and trials, three biopsy time-points will be received. This section provides the alternative slide layouts and guidelines to follow in that event.

9.2 Control tissues should be fixed, paraffin embedded and sectioned in parallel using the standard first and second time-point layouts for either one or two pass cases as detailed above in Section 7.7.6. The third time-point controls and patient biopsy tissues should be embedded and sectioned following the layout provided for post-dose only biopsy time-points layout shown above in Section 8.2.2.

9.3 Since two complete slide sets will be created, record information separately for the creation of the two slides sets on two batch records with clear denotation that the processing is for a patient including three biopsy time-points both within the patient specimen list as well as in the Notes and Deviations Section 6.0.

10.0 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.

10.1 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.

10.2 Generate a shipping list containing all the specimen records using the Shipping Manifest template as shown in (Appendix 2). Verify that all slides in the slide box are from a single patient and indicate if a paraffin block is included.

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

10.3 **Verify** that the contents of the package match the Shipping Manifest.

10.4 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.

10.5 E-mail the certified assay site shipment notification. State "*Protocol Name* PD Specimen Shipment" in the subject line and reference the tracking number, if applicable, 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.

1st Time-point Specimen A Label Here

1st Time-point Specimen B Label Here

2nd Time-point Specimen A Label Here

2nd Time-point Specimen B Label Here

Certified Assay Operator: _____

Facility Preparing Paraffin Blocks and Sections: _____

Patient ID: _____

Clinical Protocol Number: _____

Date Received by Histology Lab: _____

Date Histology Lab Processed Specimens: _____

1. Critical Materials

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

2. Patient Samples Received from Clinical Site

Biopsy Time Point/Pass #	Date Collected	*Date Received	*Patient/Sample ID
1st Time-point Biopsy:(pass 1; to be placed in "A" position)	/ /	/ /	
1st Time-point Biopsy:(pass 2; to be placed in "B" position)	/ /	/ /	
2nd Time-point Biopsy:(pass 1; to be placed in "A" position)	/ /	/ /	
2nd Time-point Biopsy:(pass 2; to be placed in "B" position)	/ /	/ /	

Patient ID:
Clinical Protocol No.:

3. Sample Information

	Time-point Designation	Fixation Start (Date/Time)	Fixation Stop (Date/Time)	Date Embedded	Paraffin Block Number
1st Time-point Biopsy: (pass 1; to be placed in "A" position)		/ / :	/ / :	/ /	
1st Time-point Biopsy: (pass 2; to be placed in "B" position)		/ / :	/ / :		
Control Tissue 1:	N/A	/ / :	/ / :		
Control Tissue 2:	N/A	/ / :	/ / :		
2nd Time-point Biopsy: (pass 1; to be placed in "A" position)		/ / :	/ / :	/ /	
2nd Time-point Biopsy: (pass 2; to be placed in "B" position)		/ / :	/ / :		

Patient ID:

Clinical Protocol No.:

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.	H&E	*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	☐	☐	

Patient ID:

Clinical Protocol No.:

(4. Slide Preparation and Visual Inspection, Continued)

Slide No.	Optimal 35 or 18 slide range based on Visual Inspection.	H&E	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	☐	☐	

5. Paraffin Dipping of Slides

Date of Completion of Paraffin Dipping of Slides: _____

BATCH RECORD: INITIALS _____ DATE: _____

Patient ID:
Clinical Protocol No.:

6. Notes, including any deviations from the SOP:

7. Laboratory Director/Supervisor Review of Batch Record

Laboratory Director/Supervisor: _____ (PRINT)

_____ (SIGN)

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/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			