Title:	Image and Data Analysis of Tumor Biopsy Slides from γH2AX Immunofluorescence Assay				Page 1 of 20
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National Clinical Target Validation Laboratory (NCTVL)

Applied Developmental Directorate

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Change History

Revision	Approval Date	Description	Originator	Approval
--	12/29/10	Image and data analysis section split into a separate SOP from SOP340533 Revision A. Update macro and Excel template information.	WHY	JJ
A	4/27/2011	Updates to macro scripts including version numbers, macro toolbar and capture menu. New macro scripts require the use of Image-Pro 7.0 or higher.	WHY	JJ

Please check for revision status at

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

and be sure to use the current version.

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OVERVIEW OF γH2AX 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 NCTVL or Certified Assay Site

SOP340522:
Tumor Frozen Needle Biopsy Preparation for the γH2AX IFA

- NBF fix and paraffin embed tumor frozen needle biopsies
- 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 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 and custom macros

1.0 PURPOSE

Standardize an immunohistochemical method for detecting and quantifying histone H2AX phosphorylated at serine 139 (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 the image capture and analysis of slides stained using the γH2AX Immunofluorescence Assay (IFA) for Tumor Biopsy Slides (SOP340523). This SOP outlines the recommended procedure for image capture and quantitation of γH2AX-stained, paraffin-embedded tumor biopsy sections. The goal of the SOP and associated training is to ensure consistency of γH2AX measurement between clinical sites.

3.0 ABBREVIATIONS

Cal	=	Calibrator
DAPI	=	4',6-Diamidino-2-Phenylindole
DCTD	=	Division of Cancer Treatment and Diagnosis
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 Target Validation Laboratory
%NAP	=	Percent Nuclear Area Positive for γH2AX
QC	=	Quality Control
SD	=	Standard Deviation
SOP	=	Standard Operating Procedure
Strp488	=	Alexa Fluor 488-Streptavidin Conjugate

4.0 INTRODUCTION

The γH2AX IFA is an immunohistochemistry-based staining assay developed to quantify the nuclear DNA damage marker, 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 within 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 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 image analysis and quality control 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 AND EQUIPMENT REQUIRED

6.1 Image Information Table from SOP340533 (Appendix 1, Section 2) for all images being analyzed

6.2 Image-Pro Pro 7.0 or higher (lower versions of Image-Pro are not supported and may not work with the macro)

6.3 Microsoft Excel 2003 or 2007; Windows XP (Windows 7 and Vista not supported)

6.4 The following file will be provided to DCTD Certified Assay Operators during the training course:

6.4.1 Macros for image analysis loaded in SOP340533

6.4.2 "SOP340534 gH2AX IFA Data Template.xlt" Microsoft Excel template for data analysis

*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

7.1 Image analysis should be completed for images from all sections of a single slide tray (up to 8 clinical slides from a single patient and 2 calibrator/control slides) captured in SOP340533.

7.1.1 Record the name and certification number of the Certified Assay Operator performing the image analysis, the facility running the SOP, the Patient ID, and the clinical protocol number in the Batch Record (Appendix 1).

7.1.2 Record the name of the Header Folder where the *.tif images captured in SOP340533 are stored in the Batch Record (Appendix 1, Section 1).

7.1.3 Use the Image Information Table from the Batch Record of SOP340533 for the capture order of the images as well as the reference Bond Slide ID number.

7.2 If image and data analysis are being performed on a different PC than image capture, be sure the Image-Pro software is installed and all DCTD-provided macros have been unzipped and loaded onto the PC.

7.2.1 Macro installation instructions can be found in SOP340533 (Appendix 3, Section 1A); the first time the macro is run on a computer the instruction in SOP340533 (Appendix 3, Section 1B) should be followed before proceeding.

7.2.2 If using software other than Image-Pro, the specifications for the macro scripts that are used for image capture and analysis are outlined in Appendix 3, Section 3 of SOP340533.

7.3 Protocol for Image Quantitation

7.3.1 Open the "SOP340534 gH2AX IFA Data Template.xlt" Excel template **(*.xlt)** workbook for data analysis and save as an Excel workbook (***.xls**) in the Header Folder.

7.3.1.1 The naming convention for the data analysis Excel workbook should match the Header Folder created in SOP340533 (e.g., ***CTEP1234_2010-10-24_1.xls).*** Record the name of the Excel workbook in the Batch Record (Appendix 1, Section 1).

7.3.2 Be sure the data analysis Excel workbook is open to "Sheet1" and all other Excel workbooks are closed as the macro may overwrite data in them.

7.3.3 If not already open, open the Image-Pro software and in the macro toolbar that pops up select **Analyze Images**. In the pop-up window that opens, browse and select the Header Folder where the images captured in SOP340533 are stored and click OK.

7.3.4 In the next window, leave everything at the default settings and click OK. The macro will create a new folder called "Masks" inside the Header Folder.

7.3.4.1 The macro-processed images for quantitation will be stored in the Masks Folder with the same file name as the *.tif files preceded by "***M_**."

7.3.4.2 Examples of an original captured image and a macro-processed image are shown in Appendix 2, Section 1A and 1B, respectively. A sorted macro-processed image is also created (file name preceded by "SL_") and stored in the Masks Folder (sample sorted image in Appendix 2, Section 1C).

7.3.5 In the **Open File Location** window, select the following files and then click **OK**.

1st field	Select the Header Folder as the location of images to be processed (location of *.tif images from microscope).
2nd field	Select the Masks Folder (within the Header Folder) as the location to store the processed images.
3rd field	Leave as the default: qIFA_gH2AX_v072007.ipm

7.3.6 In the next window, **Excel & Image Formatting**, the specified Row and Column numbers where the data will be pasted into "Sheet1" of the data analysis Excel workbook will be listed; leave these in the default setting. Change the field for **Image Format** to ***.tif** and click **OK**.

7.3.7 All *.tif images in the Header Folder will be processed; processing by the macro will take approximately 30 to 60 sec/image. Once the run is complete, a message saying, "**I made it!**" will appear in the **Output Window**. **Note:** Image processing speed can be increased by minimizing the Image-Pro window so that the program does not have to generate a digital image on the computer screen with each *.tif processed.

7.3.8 The data are exported to the open "Sheet1" of the data analysis Excel workbook.

7.3.8.1 Output data are grouped by imaged slide and then by tissue section. See a map of "Sheet1" in Appendix 3, Section 1.

7.3.8.2 Row 6 of "Sheet1" will contain a representative name for each section, the average percent nuclear area positive (%NAP) for γH2AX, and standard deviation (SD) for each set of images from one tissue section. The raw data for each tissue section are listed below the averaged data (Appendix 3, Section 2).

7.3.8.3 Visually inspect the data to ensure data for a single section are grouped underneath the appropriate heading. This is a quality assurance step to ensure the **Next→**, **Next Sample→,** or **Next Slide→** option was selected with each image captured in SOP340533.

8.0 DATA ANALYSIS AND ASSAY QUALITY CONTROL

8.1 The data saved on "Sheet1" are automatically sorted and organized into the second worksheet of the data analysis Excel workbook titled "Sorted Data Output" (Appendix 3, Section 3). The Excel sheet is organized based on the **recommended image capture order** in SOP340533.

8.2 Quality control (QC) **Pass/Fail** criteria are determined by Certified Assay Operators for the entire slide tray by first analyzing the calibrator/control slides in SOP Step 8.3 and then the individual clinical slides in SOP Step 8.4.

8.2.1 If the calibrator/control slides and at least half of a single patient's batched clinical slides pass QC, the clinical data for the slides can be reported.

8.2.2 Appendix 4 contains a flowchart that can be followed while determining whether γH2AX slide data pass QC criteria.

8.3 Calibrator/Control Control Slide QC

8.3.1 Using the sorted data on the "Sorted Data Output" worksheet, verify that the calibrator and control samples from the calibrator/control slides pass QC as follows:

8.3.1.1 Rows 15-24 of the "Sorted Data Output" worksheet correspond to images captured for the 2 calibrator/control slides (see Appendix 3, Section 3). For each calibrator and control level, there must be ≥ 3 analyzable images (captured images that lack necrotic regions, gaps, or folded tissue) in **aggregate** across both calibrator/control slides. Individually these images **do not** need to pass %NAP QC.

8.3.1.2 On the top of the "Sorted Data Output" worksheet, indicate if there are ≥ 3 analyzable fields with **Yes/No** for each calibrator/control level (cells B5-B9). If any level has < 3 analyzable fields, go to SOP Step 8.3.2.

8.3.1.3 If there were ≥ 3 analyzable fields for each calibrator and control level, then calculate the average %NAP for all analyzable images at each calibrator and control level from both slides and report these values at the top of the "Sorted Data Output" worksheet (cells D5-D9). **Note**: When calculating the average %NAP for the positive control, do not use values from the positive controls on the clinical slides.

8.3.1.4 At the top of the "Sorted Data Output" worksheet, indicate if the average %NAP for each calibrator and control level **passes or fails** QC (cells E5-E9) based on the following ranges:

Calibrator/Control	Acceptable Average %NAP Range*	Description
Positive Control	Intensely stained; > 10%	Mouse testes
Cal-High	7% – 15%	High positive
Cal-Mid	4% – 7%	Middle positive
Cal-Low	1% – 4%	Low positive
Negative Control	< Cal-Low	Mouse small intestine

* NAP ranges for calibrator/control slides with lot numbers **11001938, 11001939, and 11001940 only**. Check product insert with calibrator/control slides to verify %NAP ranges for the lot number being used.

8.3.2 If any of the calibrator/control levels **Fail QC** because either they have < 3 analyzable images or they have an average %NAP outside the indicated ranges, do the following:

8.3.2.1 Recapture images for the entire slide tray following SOP340533 as follows:

(1) Delete the Header Folder, and all files within it.

(2) Begin a new Batch Record for the new images and note in the original Batch Record that the calibrator/control slides **Failed QC** and samples were rerun. Keep Batch Records together.

(3) Begin with SOP340533 Step 7.3 and repeat image capture, then repeat image quantitation and data analysis using this SOP.

8.3.2.2 If **ALL** calibrator and control levels **now Pass QC**, proceed to SOP Step 8.4 with ONLY the new image data.

8.3.2.3 If the calibrator/control slides **Fail QC** a second time, then the entire slide tray fails QC.

- **Do not** analyze clinical slides and **do not** fill out a Sample Data Report**.**
- Indicate **Failed QC** in the new Batch Record and label the first page of the Batch Record and in cell G5 of the "Sorted Data Output" worksheet with "**Assay failed QC – do not use data**."
- The patient biopsy will need to be rerun using a Backup slide set and two new calibrator/control slides following SOP340523. Go to SOP Step 8.5.

8.3.3 If **ALL** calibrator and control levels pass QC, indicate **Pass QC** on the top of the "Sorted Data Output" worksheet in cell G5 and proceed to QC of clinical slides.

8.4 Clinical Slide QC

8.4.1 If the calibrator/control slides pass QC, use the data on the "Sorted Data Output" worksheet and determine if the clinical slides pass QC as follows.

8.4.1.1 Each clinical slide contains two biopsies (1st and 2nd), as well as a positive control section, images captured for clinical slides should be grouped by slide on the "Sorted Data Output" worksheet (Appendix 3, Section 3).

8.4.1.2 The positive control section on each clinical slide should be intensely stained for γH2AX and have > 10% average %NAP.

8.4.1.3 There must be ≥ 3 analyzable images for the positive control section and for each of the clinical biopsies on each clinical slide.

8.4.2 If either of these criteria is not met, then the individual clinical slide fails QC. Indicate whether each slide passes or fails QC criteria in **Column P** of the "Sorted Data Output" worksheet (see Appendix 3, Section 3).

8.4.2.1 If **at least half** of the slides from a single patient's batched slides pass QC, then proceed to SOP Step 8.5.

8.4.2.2 If **less than half** of the slides from a single patient's batched slides passes QC, **do not** fill out a Sample Data Report.

- Data from the failed slides should be discarded. A Backup set of slides for the patient and two new calibrator/control slides should be stained for γH2AX following SOP340523.
- Inquiries can be directed to the Laboratory Director/Supervisor to allow exceptions to failed QC. If, after expert review, a patient's slides that failed QC are permitted to pass, be sure to record this decision in the Batch Record as a deviation (Appendix 1, Section 3), complete a Sample Data Report, and note the deviation on the Report.

8.4.3 Once γH2AX data is acquired for a patient, any remaining "Backup" slides and embedded tissue can be used per institutional guidelines.

8.5 Record the final Header Folder name on the top of the "Sorted Data Output" worksheet (cell G9).

8.6 Print a copy of the "Sorted Data output" worksheet with QC information and attach to the Batch Record (Appendix 1, Section 2).

8.7 Review and finalize the Batch Record (Appendix 1) and obtain required signatures. Document ANY and ALL deviations from this SOP in the Batch Record (Appendix 1, Section 3).

8.8 Only prepare a Sample Data Report (next step) if all of the calibrator/control sections passed QC (SOP Step 8.3) and **at least half** of the slides from a single patient's batched slides **passed** QC (SOP Step 8.4).

8.9 Prepare a Sample Data Report for Each Patient

8.9.1 Use the data from the Image Information Table of SOP340533 and the data analysis Excel workbook to complete a Sample Data Report for each patient's batch of slides (Appendix 5).

8.9.2 For reporting of %NAP for calibrator and control samples on the Sample Data Report, use the average %NAP determined for each calibrator/control in the data analysis Excel "Sorted Data Output" worksheet (cells D5-D9).

8.9.3 For each clinical slide, report the **Specimen ID** and **Bond Slide ID Number** on the Sample Data Report. Indicate the average %NAP for all clinical slides that passed QC; can be found in **Column N** of the "Sorted Data Output" worksheet (see Appendix 3, Section 3). If a clinical slide failed QC, do not report the average %NAP for either biopsy on that slide. Instead, state **Failed QC** for that sample on the Sample Data Report.

8.9.4 The data on the "Sorted Data Output" worksheet are automatically sorted and graphed on the "Graph" worksheet in the Excel workbook (see Appendix 3, Section 4). Print 2 copies of the "Graph" worksheet and attach one to the Batch Record (Appendix 1, Section 2) and one to the Sample Data Report.

8.9.5 Once the Sample Data Report is completed by the Certified Assay Operator, the Laboratory Director/Supervisor needs to complete the bottom of the Report; their signature indicates they have reviewed and verified all data.

8.10 Send a copy of the final Sample Data Report and a copy of the graphed patient data on the "Graph" worksheet to the clinical site. Attach a copy of the completed Sample Data Report to the Batch Record (Appendix 1, Section 2).

8.11 Once all of the images have been processed, create a second folder within the Header Folder called "**TIFS**," and move all original *.tif images from the main Header Folder to this subfolder.

APPENDIX 1: BATCH RECORD

The Batch Record should contain information for one slide tray, and therefore a **single** patient's batched clinical slides.

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/Laboratory Running Image Analysis: _____

Patient ID: _____

Clinical Protocol Number: _____

1. File Names for Image Analysis

Clear and consistent labeling of folders and files is essential for easy data retrieval.

Name of the Header Folder: _____

Final name of "SOP340534 gH2AX IFA Data Template" Excel workbook: _____

Final storage location of Header Folder: _____

2. Perform data analysis and assay QC as outlined in SOP Step 8.0. Attach copies of the "Sorted Data Output" worksheet, "Graph" worksheet, and the Sample Data Report.

3. Notes, including any deviations from the SOP:

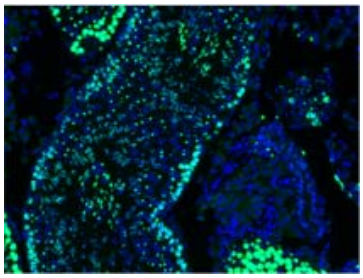
BATCH RECORD: INITIALS _____ DATE: _____

THIS PAGE LEFT BLANK ON PURPOSE

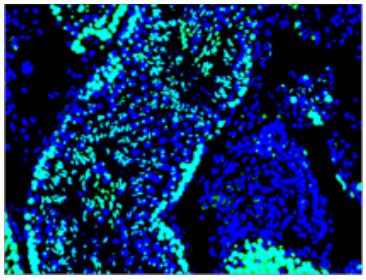
APPENDIX 2: IMAGE CAPTURE EXAMPLES

1. Captured fluorescent images:

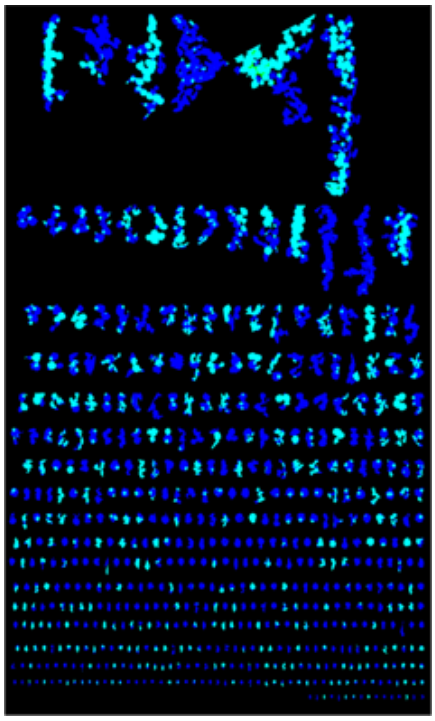
A. An example of the original *.tif image of a positive control section following image capture.



B. An example of a macro-processed image of a positive control section stored in the Masks folder. Image file name is preceded by "M_."



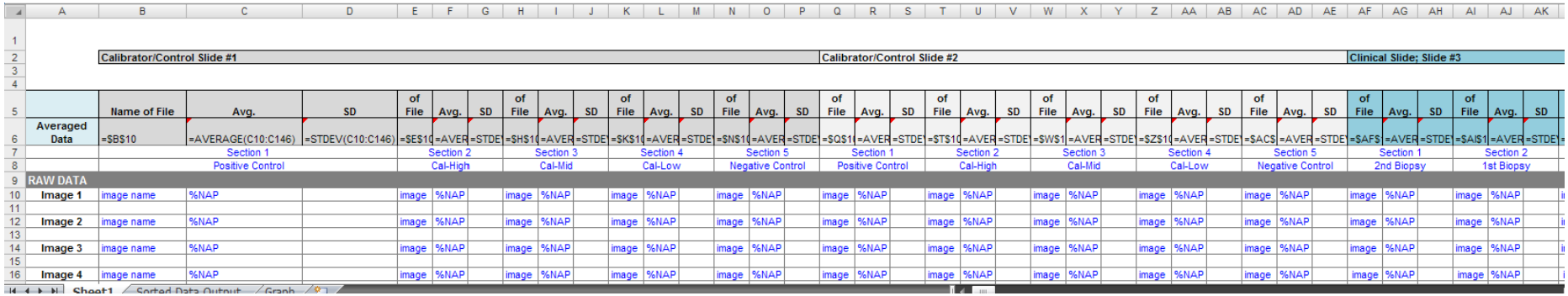
C. An example of a macro-processed image sorted by size and stored in the Masks folder. Image file name is preceded by "SL_."



APPENDIX 3: "SOP340534 gH2AX IFA DATA TEMPLATE" EXCEL WORKBOOK

1. Map of "Sheet1" Image Quantitation Output

Formulas are displayed in Row 6 to demonstrate how the information is generated from the imported image data in Row 10 and higher. Blue text in rows 7 and 8 indicates the location of each section captured following the recommended order in SOP340533. Blue text in rows 10-16 indicate the type of data that will be imported to "Sheet1" by the macro.

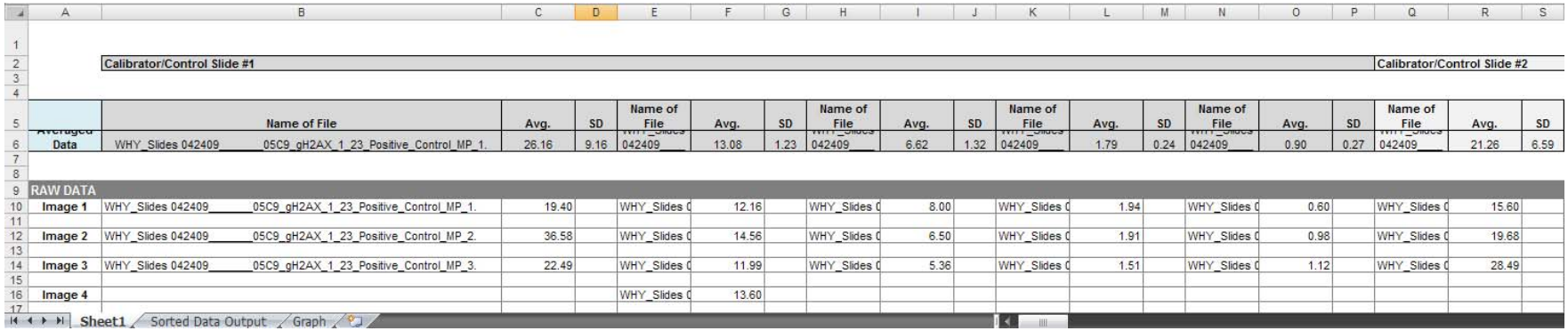


		Calibrator/Control Slide #1															Calibrator/Control Slide #2															Clinical Slide; Slide #3					
5		Name of File	Avg.	SD	of File	Avg.	SD	of File	Avg.	SD	of File	Avg.	SD	of File	Avg.	SD	of File	Avg.	SD	of File	Avg.	SD	of File	Avg.	SD	of File	Avg.	SD	of File	Avg.	SD	of File	Avg.	SD	of File	Avg.	SD
6	Averaged Data	=$B$10	=AVERAGE(C10:C146)	=STDEV(C10:C146)	=$E$1(	=AVER	=STDE'	=$H$1(	=AVER	=STDE'	=$K$1(	=AVER	=STDE'	=$N$1(	=AVER	=STDE'	=$Q$1(	=AVER	=STDE'	=$T$1(	=AVER	=STDE'	=$W$1	=AVER	=STDE'	=$Z$1(	=AVER	=STDE'	=AC	=AVER	=STDE'	=AF	=AVER	=STDE'	=$AI$1	=AVER	=STDE'
7		Section 1			Section 2			Section 3			Section 4			Section 5			Section 1			Section 2			Section 3			Section 4			Section 5			Section 1			Section 2		
8		Positive Control			Cal-High			Cal-Mid			Cal-Low			Negative Control			Positive Control			Cal-High			Cal-Mid			Cal-Low			Negative Control			2nd Biopsy			1st Biopsy		
9	RAW DATA																																				
10	Image 1	image name	%NAP		image	%NAP		image	%NAP		image	%NAP		image	%NAP		image	%NAP		image	%NAP		image	%NAP		image	%NAP		image	%NAP		image	%NAP		image	%NAP	
11																																					
12	Image 2	image name	%NAP		image	%NAP		image	%NAP		image	%NAP		image	%NAP		image	%NAP		image	%NAP		image	%NAP		image	%NAP		image	%NAP		image	%NAP		image	%NAP	
13																																					
14	Image 3	image name	%NAP		image	%NAP		image	%NAP		image	%NAP		image	%NAP		image	%NAP		image	%NAP		image	%NAP		image	%NAP		image	%NAP		image	%NAP		image	%NAP	
15																																					
16	Image 4	image name	%NAP		image	%NAP		image	%NAP		image	%NAP		image	%NAP		image	%NAP		image	%NAP		image	%NAP		image	%NAP		image	%NAP		image	%NAP		image	%NAP	

Sheet1 | Sorted Data Output | Graph

2. "Sheet1" With Sample Image Quantitation Output

Raw data collected during image acquisition and quantitation from each image of each section are saved into "Sheet1" of the data analysis Excel workbook. Data for 3 images of a positive control section are saved in cells B10 - C14 below. The data are automatically averaged and the SD determined in cells C6 and D6, respectively. *Additional slide data will be displayed in progressive columns to the right.*



		Calibrator/Control Slide #1												Calibrator/Control Slide #2									
5		Name of File	Avg.	SD	Name of File	Avg.	SD	Name of File	Avg.	SD	Name of File	Avg.	SD	Name of File	Avg.	SD	Name of File	Avg.	SD	Name of File	Avg.	SD
6	Averaged Data	WHY_Slides 042409_____05C9_gH2AX_1_23_Positive_Control_MP_1.	26.16	9.16	042409___	13.08	1.23	042409___	6.62	1.32	042409___	1.79	0.24	042409___	0.90	0.27	042409___	21.26	6.59			
9	RAW DATA																						
10	Image 1	WHY_Slides 042409_____05C9_gH2AX_1_23_Positive_Control_MP_1.	19.40		WHY_Slides 0	12.16		WHY_Slides 0	8.00		WHY_Slides 0	1.94		WHY_Slides 0	0.60		WHY_Slides 0	15.60				
12	Image 2	WHY_Slides 042409_____05C9_gH2AX_1_23_Positive_Control_MP_2.	36.58		WHY_Slides 0	14.56		WHY_Slides 0	6.50		WHY_Slides 0	1.91		WHY_Slides 0	0.98		WHY_Slides 0	19.68				
14	Image 3	WHY_Slides 042409_____05C9_gH2AX_1_23_Positive_Control_MP_3.	22.49		WHY_Slides 0	11.99		WHY_Slides 0	5.36		WHY_Slides 0	1.51		WHY_Slides 0	1.12		WHY_Slides 0	28.49				
16	Image 4				WHY_Slides 0	13.60																	

Sheet1 | Sorted Data Output | Graph

3. "Sorted Data Output" Worksheet With Sample Information

Data from "Sheet1" are auto-filled into the bottom half of the "Sorted Data Output sheet in all Rows >14 between Columns A and I.

In Columns J-O, the auto-filled data are sorted by slide type based on the tissue type selected (Sample, Calibrator, or Control) in the Capture Menu (SOP340533). The example below has 2 calibrator/control slides (blue bracket) and 4 clinical slides (purple bracket) captured in the **recommended image capture order** in SOP340533.

QC criteria are applied in SOP Step 8.0 and Pass/Fail is **filled in by the Certified Assay Operator** at the top of the worksheet for calibrator/control samples and in Column P for clinical samples. Conditional formatting in the Excel worksheet is applied so that Pass/Fail text (cells formatted green or red, respectively) are easily visible to the Assay Operator. If the calibrator/control slides fail QC, be sure to clearly label the worksheet with "**Assay failed QC – do not use data**" and analyze backup slides in a new experiment, with a separate Batch Record.

DATA ANALYSIS AND ASSAY QUALITY CONTROL Bond-Max Processing Date: 10/24/2010 Patient ID: 05C9 Slide Tray#: 1

Calibrator / Control QC to be completed by Certified Assay Operator

	≥3 analyzable fields: Yes/No	Avg. %NAP Ranges	Avg. %NAP	Calib/Control %NAP: Pass/Fail
Positive Control*	Yes	>10%	23.71	Pass
Cal-High	Yes	7% - 15%	13.82	Pass
Cal-Mid	Yes	4%-7%	6.80	Pass
Cal-Low	Yes	1%-4%	1.80	Pass
Negative Control	Yes	< Cal-Low	0.85	Pass

*Do not use the positive controls from the clinical sample slides for this calculation

Assay QC measures met? (Pass/Fail): Pass

Complete location and file name of the final stored Header Folder: C:\gH2AX qIFA\IpWin Source Files\Scripts\Projects\CTEP1234__2010-10-24__1

Data in Rows >14 between Columns A and I are auto-filled from the "Sheet1" worksheet

Clinical Sample QC to be completed by Certified Assay Operator

								All		Calibrator		Control		Sample		Clinical Samples	
Image 1	Image 2	Image 3	Image 4	Image 5	Image 6	Name of Sample	Avg. %NAP	SD:	Avg. %NAP	SD	Avg. %NAP	SD	Avg. %NAP	SD	%NAP Pass/Fail	Deviations
19.40	36.58	22.49	0.00	0.00	0.00	WHY_Slides 0424	26.16	9.16			26.16	9.16			N/A	
12.16	14.56	11.99	0.00	0.00	0.00	WHY_Slides 0424	13.08	1.23	13.08	1.23					N/A	
8.00	6.50	5.36	0.00	0.00	0.00	WHY_Slides 0424	6.62	1.32	6.62	1.32					N/A	
1.94	1.91	1.51	0.00	0.00	0.00	WHY_Slides 0424	1.79	0.24	1.79	0.24					N/A	
0.60	0.98	1.12	0.00	0.00	0.00	WHY_Slides 0424	0.90	0.27			0.90	0.27			N/A	
15.60	19.68	28.49	0.00	0.00	0.00	WHY_Slides 0424	21.26	6.59			21.26	6.59			N/A	
14.60	13.58	15.49	0.00	0.00	0.00	WHY_Slides 0424	14.56	0.96	14.56	0.96					N/A	
7.50	6.50	6.90	0.00	0.00	0.00	WHY_Slides 0424	6.97	0.50	6.97	0.50					N/A	
1.80	1.91	1.70	0.00	0.00	0.00	WHY_Slides 0424	1.80	0.11	1.80	0.11					N/A	
0.60	0.98	0.80	0.00	0.00	0.00	WHY_Slides 0424	0.79	0.19			0.79	0.19			N/A	
3.31	2.34	0.41	0.00	0.00	0.00	WHY_Slides 0424	2.02	1.47					2.02	1.47	Pass	
0.10	0.17	0.21	0.00	0.00	0.00	WHY_Slides 0424	0.16	0.06					0.16	0.06	Pass	
58.34	27.77	23.55	0.00	0.00	0.00	WHY_Slides 0424	36.55	18.99			36.55	18.99			Pass	
6.35	8.60	5.32	0.00	0.00	0.00	WHY_Slides 0424	6.76	1.68					6.76	1.68	Pass	
3.22	1.03	0.85	0.00	0.00	0.00	WHY_Slides 0424	1.70	1.32					1.70	1.32	Pass	
25.93	26.21	18.50	0.00	0.00	0.00	WHY_Slides 0424	23.55	4.37			23.55	4.37			Pass	
9.40	8.60	5.32	0.00	0.00	0.00	WHY_Slides 0424	7.77	2.16					7.77	2.16	Fail	pos control failed
3.22	5.20	0.85	0.00	0.00	0.00	WHY_Slides 0424	3.09	2.18					3.09	2.18	Fail	pos control failed
2.59	2.62	5.40	1.35	0.00	0.00	WHY_Slides 0424	2.99	1.71			2.99	1.71			Fail	Failed QC
6.35	3.60	5.32	0.00	0.00	0.00	WHY_Slides 0424	5.09	1.39					5.09	1.39	Pass	
3.22	2.50	0.85	0.00	0.00	0.00	WHY_Slides 0424	2.19	1.21					2.19	1.21	Pass	
25.93	26.21	20.60	0.00	0.00	0.00	WHY_Slides 0424	24.25	3.16			24.25	3.16			Pass	

Sheet1 | Sorted Data Output | Graph

4. Example of Plotted Patient Data From a Single Slide Tray on the "Graph" Worksheet

Data from the "Sorted Data Output" worksheet are automatically sorted and %NAP is plotted from both the calibrator/control slides (inset bar graph) and the clinical samples (line graph).

This graph represents the %NAP for progressive sections through a single patient's biopsy, and the shaded red box within the graph overlays any data points below 1% NAP, the minimum cut-off for reportable assay results.

Data on this sheet and in graph are auto-filled from "Sorted Data Output" worksheet

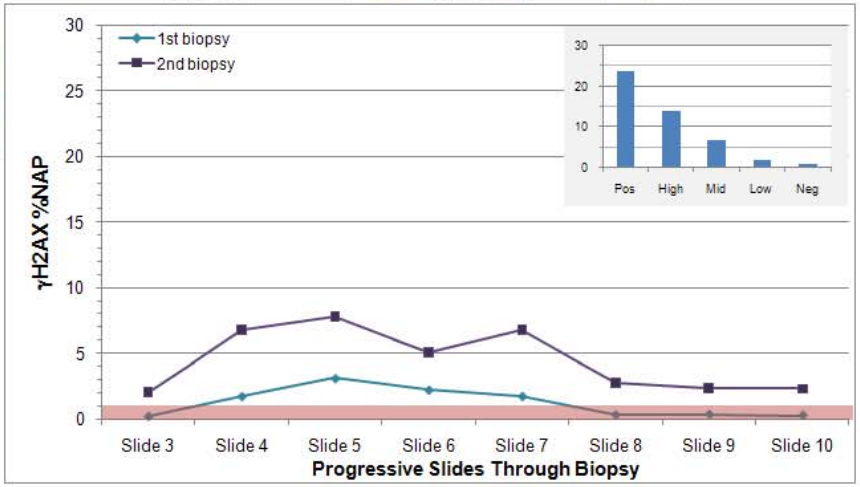
Name of Sample	Avg. %NAP	Slide recommended capture order
WHY_Slides	26.16	Slide 1; Pos
WHY_Slides	13.08	Slide 1; Cal-High
WHY_Slides	6.62	Slide 1; Cal-Mid
WHY_Slides	1.79	Slide 1; Cal-Low
WHY_Slides	0.90	Slide 1; Neg
WHY_Slides	21.26	Slide 2; Pos
WHY_Slides	14.56	Slide 2; Cal-High
WHY_Slides	6.97	Slide 2; Cal-Mid
WHY_Slides	1.80	Slide 2; Cal-Low
WHY_Slides	0.79	Slide 2; Neg
WHY_Slides	2.02	Slide 3; 2nd biopsy
WHY_Slides	0.16	Slide 3; 1st biopsy
WHY_Slides	6.76	Slide 4; 2nd biopsy
WHY_Slides	1.70	Slide 4; 1st biopsy
WHY_Slides	7.77	Slide 5; 2nd biopsy
WHY_Slides	3.09	Slide 5; 1st biopsy
WHY_Slides	5.09	Slide 6; 2nd biopsy
WHY_Slides	2.19	Slide 6; 1st biopsy
WHY_Slides	6.76	Slide 7; 2nd biopsy
WHY_Slides	1.70	Slide 7; 1st biopsy
WHY_Slides	2.71	Slide 8; 2nd biopsy
WHY_Slides	0.27	Slide 8; 1st biopsy
WHY_Slides	2.31	Slide 9; 2nd biopsy
WHY_Slides	0.32	Slide 9; 1st biopsy
WHY_Slides	2.28	Slide 10; 2nd biopsy
WHY_Slides	0.25	Slide 10; 1st biopsy

Calibrators/Controls

Plotted:	%NAP		
	Slide 1	Slide 2	Average
Pos	26.16	21.26	23.71
High	13.08	14.56	13.82
Mid	6.62	6.97	6.79
Low	1.79	1.80	1.79
Neg	0.90	0.79	0.85

Clinical Samples

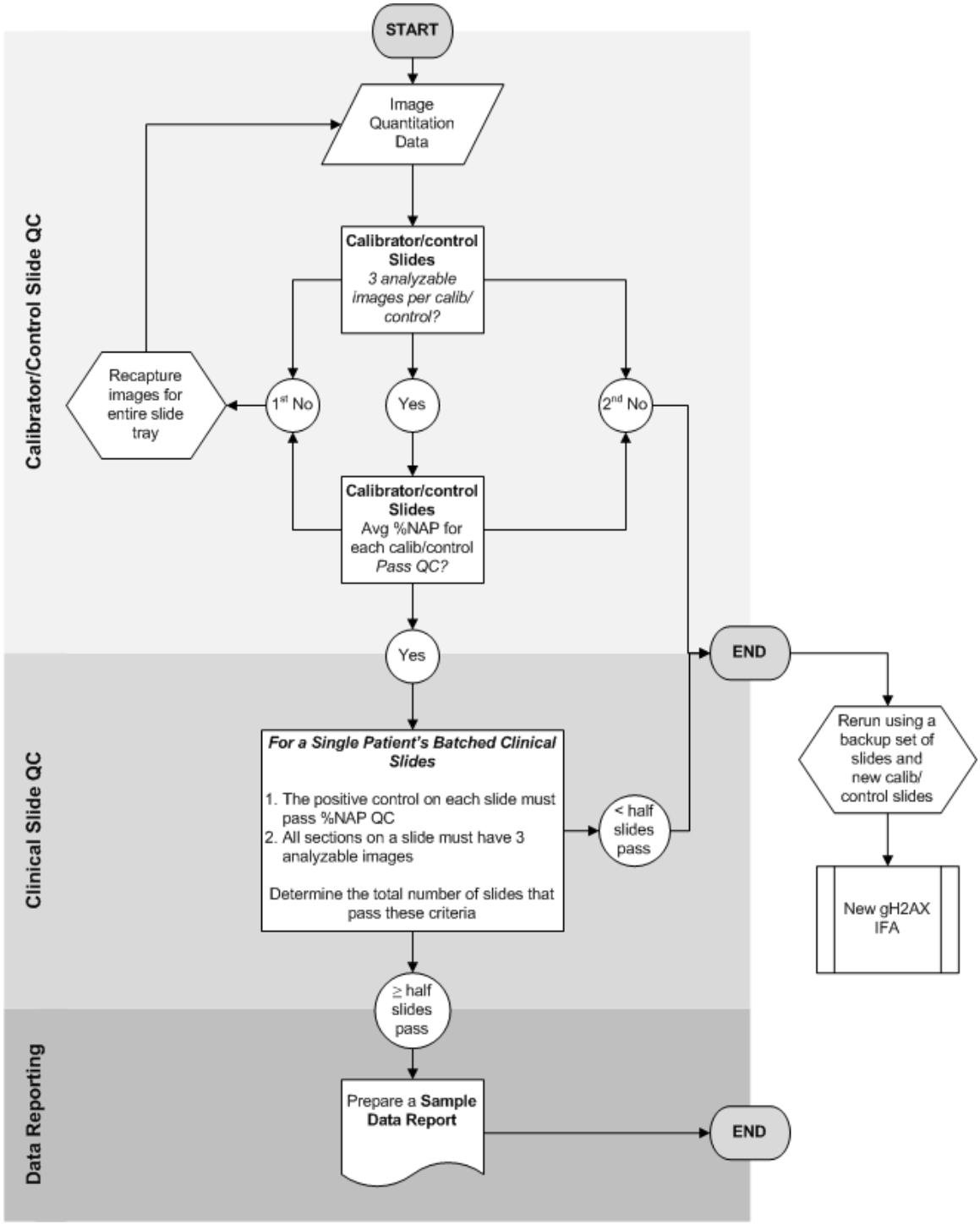
Plotted:	%NAP	
	1st biopsy	2nd biopsy
Slide 3	0.16	2.02
Slide 4	1.70	6.76
Slide 5	3.09	7.77
Slide 6	2.19	5.09
Slide 7	1.70	6.76
Slide 8	0.27	2.71
Slide 9	0.32	2.31
Slide 10	0.25	2.28



Sheet1 | Sorted Data Output | Graph

APPENDIX 4: QUALITY CONTROL FLOWCHART

General flowchart of QC pass/fail criteria outlined in SOP Step 8.0 for a single patient's batched slides from one Bond-Max slide tray.



APPENDIX 5: SAMPLE DATA REPORT

Sample Data Report - *To be completed for each slide tray from the Bond-Max System*
Attach a copy of the GRAPH of patient data from the Excel data analysis workbook

Certified Assay Operator (print): _____

Certification #: _____ Today's Date: _____

Patient Number: _____

Date Slides Processed in Bond-Max: _____ Slide Tray #: _____

Average %NAP		**Average %NAP**	
Cal-Low		Positive Control	
Cal-Mid		Negative Control	
Cal-High			

Assay Quality Control (QC) measures met? (Pass/Fail): _____

		Average %NAP	
	Bond Slide ID	1st Biopsy	2nd Biopsy
Specimen ID	**1**		
1st Biopsy	**2**		
	3		
	4		
2nd Biopsy	**5**		
	6		
	7		
	8		

Additional Information: *Images are available for review upon request.*

To be completed by Laboratory Director/Supervisor:

Complete the following table. Signature indicates assay results have been reviewed and verified.

Average %NAP		**Average %NAP**	
Cal-Low		Positive Control	
Cal-Mid		Negative Control	
Cal-High			

Assay Quality Control (QC) measures met? (Pass/Fail): _____

Director/Supervisor Signature: _____ Today's Date: _____