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Change History

Revision	Approval Date	Description	Originator	Approval
G	6/19/2019	Minor updates to collection and shipping procedures.	KFG	REP
F	2/11/2015	Updated contact shipping address and process for advance notification of shipments.	KFG	REP
E	7/3/2013	Updated tube-type to 1.5-mL conical bottom screw cap tubes to allow for broader use in DCTD assays and minimize the need to transfer biopsies during sample extraction steps. Decreased maximum time from biopsy collection to freezing to 2 minutes.	YAE	REP
D	1/8/2013	Update handling in surgical suite including details on halving of biopsy. Record total time elapsed from biopsy collection to freezing.	YAE, MM	JJ
C	12/29/2010	Update sample snap freeze to dry ice/ethanol bath or liquid nitrogen.	YAE	JJ
B	7/24/2009	Updated SOP format and prepared for publication to the DCTD Biomarkers Web site	YAE	JJ
A	10/13/2006	Revision with New Shipping Address	YZ	JJ
--	8/25/2006	New Document	YZ	JJ

Please check for revision status of the SOP at

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

and be sure to use the current version.

TABLE OF CONTENTS

1.0 PURPOSE

Standardize the method for collecting and handling frozen needle tumor biopsies to enable specimen use for measurement of pharmacodynamic (PD) markers following treatment with anticancer agents.

2.0 SCOPE

This procedure applies to all personnel involved in the collection and handling of frozen needle tumor biopsies for use in PD marker assays during clinical trials. The goal of this SOP and associated training is to ensure consistency in tumor needle biopsy collection and handling between clinical sites.

3.0 ABBREVIATIONS

DCTD	=	Division of Cancer Treatment and Diagnosis
ELISA	=	Enzyme-Linked ImmunoSorbent Assay
FNLCR	=	Frederick National Laboratory for Cancer Research
ID	=	Identification / Identifier
IQC	=	Internal Quality Control
LHTP	=	Laboratory of Human Toxicology and Pharmacology
NCTVL	=	National Clinical Target Validation Laboratory
PADIS	=	Pharmacodynamics Assay Development & Implementation Section
PD	=	Pharmacodynamic
SOP	=	Standard Operating Procedure

4.0 INTRODUCTION

Specimen handling, shipping, and storage procedures (pre-analytical variables) can have a significant impact on the reliability of biomarker measurements in the laboratory. Following detailed steps for sample collection and handling procedures and recording any deviations from this procedure allows retrospective identification of artifactual changes in biomarker readout and increases the reliability of the data and validity of the analytical results.

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. 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 and/or PK/PD Support Lab Personnel	A Certified Assay Operator and/or PK/PD Support Lab personnel may be a Laboratory Technician/Technologist, Research Associate, or Laboratory Scientist who has been certified through DCTD training on this SOP. 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 It is the responsibility of the Certified Assay Operator and/or PK/PD Support Lab personnel to confirm scheduled specimen collection time points, pre-print all labels and data collection sheets in advance, check documentation for accuracy, and verify that the required collection tubes, supplies, and equipment are available for successful collection and handling of biopsy samples.

5.3 The Certified Assay Operator and/or PK/PD Support Lab personnel responsible for conducting the specimen collection and handling procedures are 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 The responsible personnel are to check the DCTD Biomarkers Web site (http://dctd.cancer.gov/ResearchResources/ResearchResources-biomarkers.htm) to verify that the latest SOP version is being followed.

6.0 MATERIALS AND EQUIPMENT REQUIRED

6.1 Stopwatch, total time in minutes and seconds required
6.2 1.5-mL Sarstedt o-ring screw cap, conical bottomed tubes (Sarstedt, Cat#: 72.703.416)
6.3 Disposable, fine-tipped tweezers (e.g., VWR, Cat#: 83009-010). Tweezer tips need to easily fit to bottom of a 1.5-mL Sarstedt tube
6.4 Printable microcentrifuge tube labels or BSI labeling system
6.5 81-place freezer boxes (e.g., Fisher Scientific, Cat#: 12-565-182)
6.6 Thermoflask cooler or polystyrene foam container
6.7 Ice bucket
6.8 Liquid nitrogen or dry ice/ethanol bath
6.9 Wet ice
6.10 -80°C freezer (or lower)
6.11 Biohazard specimen bags
6.12 Insulating Styrofoam shipping container

7.0 OPERATING PROCEDURES

7.1 Record the name and certification number of the Certified Assay Operator and/or PK/PD Support Lab personnel performing the SOP, the facility/clinic collecting the specimens, the Patient/Sample ID, the primary diagnosis, and the clinical protocol number in the Batch Record (Appendix 1).

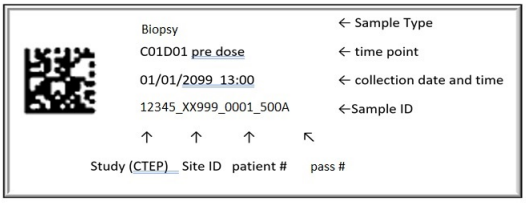
- The Batch Record for this SOP is sufficient for collection of a **single** set of biopsy samples collected from a single patient at a single timepoint. If collecting biopsy samples for more than one patient, prepare a separate Batch Record for each patient.

7.2 Labels

7.2.1 Prepare enough pre-printed specimen labels for each whole or halved biopsy sample to be collected and frozen as defined in the Pharmacodynamic/Correlative Study section of the Clinical Protocol; be sure to coordinate with the clinical center if they prepare the labels for sample collection. If two passes are collected from one tumor, the labels would be identical except that the specimen ID would be followed by a letter A/B to designate pass number. The specimen ID includes the CTEP protocol number followed by a unique patient identifier and a specimen series ID.

NCI tumor biopsy specimen IDs for PD sampling are series 500 with consecutive numbers identifying the collection time point as defined in the Clinical Protocol.

Sample pre-printed label for all frozen-tissue biopsy tubes:



7.2.2 Of the pre-printed labels prepared for each sample, one label will go on each 1.5-mL Sarstedt tube, one on the Batch Record (Appendix 1), and the last will be given to the research nurse to place into the patient record sheet. **Note:** be sure that no patient identifiable information is shown on the labels.

7.3 Tumor Needle Biopsy Collection and Handling

7.3.1 The research nurse is to notify the laboratory of scheduled PD sample collections, preferably giving at least 24 hours of notice. Arrive at the biopsy collection site early enough to allow sufficient time to set up laboratory supplies, collect relevant clinical information, and ensure rapid transport of specimens to the laboratory for placement at -80ºC (or lower) after collection.

7.3.2 Bring all necessary lab supplies including: disposable tweezers, a minimum of two 1.5-mL Sarstedt tubes (one for each whole biopsy core) pre-cooled on liquid nitrogen or dry ice/ethanol in an insulated bucket, and one pre-printed specimen label to give to the research nurse for the patient record.

Note: Pre-chill additional 1.5-mL Sarstedt tubes for specimen collection in case the interventional radiologist collects additional passes, or one of the other tubes is compromised prior to collection.

7.3.3 The total time elapsed between biopsy collection and placement into the pre-chilled tube is of **key importance** to biomarker analysis; biopsies should be frozen within **2 minutes** of collection. The interventional radiologist will eject the biopsy onto a sterile slide (for optimal analyte recovery the slide should be pre-chilled). Start a stopwatch (or note the time) at this point (Appendix 1, Section 1) and immediately walk the slide to the sample preparation table.

7.3.4 In the Batch Record (Appendix 1, Section 1), indicate if a full or halved biopsy, as defined in the Pharmacodynamic/Correlative Study section of the Clinical Protocol, is prepared.

7.3.4.1 For whole biopsies: Uncap an empty, pre-chilled 1.5-mL Sarstedt tube and, using disposable tweezers, pick up the freshly collected needle biopsy with the tweezers at one end, and touch the opposite end of the biopsy to the inner surface of the prechilled 1.5-mL Sarstedt tube. This should attach the tissue to the tube, allowing it to be dropped into the tube while releasing the tissue from the tweezers without sticking. Dispose of the tweezers in the appropriate biohazardous waste container(s).

7.3.4.2 For halved biopsies: Use 1-2 disposable tweezers and cut/shear the biopsy in half cross-wise while it is on the slide (do not pull or stretch the biopsy longitudinally). Use the tweezers to transfer the halved biopsies to sterile pre-chilled tubes as indicated above.

7.3.5 Immediately snap freeze the biopsy by placing the tube in liquid nitrogen or a dry ice/ethanol bath. **Note:** DO NOT let the tubes tip over in the dry ice/ethanol bath.

7.3.6 Calculate the total time elapsed from biopsy collection to biopsy freezing and record the total number of **minutes and seconds** elapsed in the Batch Record (Appendix 1, Section 1).

7.3.7 Note the specific location of each biopsy pass collected (*e.g.*, spleen, large left upper quadrant splenic mass) and a description of the appearance of the biopsy (*e.g.*, large whole core or small, fragmented core) in the Batch Record (Appendix 1, Section 1).

7.3.8 Note the biopsy timepoint (Appendix 1, Section 1).

7.4 If biopsy procedure details can be obtained from the interventional radiologist or research nurse, record them in the Batch Record (Appendix 1, Section 2). Some information may not be available until a later time from the clinical staff.

During **first-in-human** PD sample collection studies, information such as type of anesthesia and time-lag between biopsy needle withdrawal and sample freezing need to be tracked in order to determine the optimal sample collection procedure for the clinical community.

7.5 Return to the sample processing laboratory and transfer the frozen biopsy specimen(s) to -80°C (or lower) for storage until shipment to the PD processing laboratory. Record the date and time specimens are placed at -80ºC (or lower; Appendix 1, Section 3).

7.6 Review and finalize the Batch Record and document **ANY** and **ALL** deviations from this SOP in the Batch Record (Appendix 1, Section 5).

7.7 The Laboratory Director/Supervisor should review the Batch Record and sign to affirm the data contained within are correct (Appendix 1, Section 6).

8.0 SHIP TO FNLCR FOR ANALYSIS (OPTIONAL)

If shipping to a location other than FNLCR, use the following steps as a guide.

8.1 Sites are required to create a FedEx shipping label to accommodate the variable dry ice weight of the shipment package. Use only FedEx Priority Overnight Shipping. FNLCR PD Support will provide a FedEx account number to cover the cost of the shipment via NCI_PD_Support@mail.nih.gov.

8.1.1 By linking the FNLCR FedEx account number provided to the biopsy shipment, FNLCR PD Support can closely monitor the shipment and cover all shipping costs, and appropriate notification can be provided to pertinent staff of expected sample shipment arrival.

8.1.2 Please send all shipments via FedEx Priority Overnight shipping to the FNLCR PD Specimen Central Receiving address listed below:

Attention: Dan Danner
NCI-F/FNLCR
1073 Beasley Street, Building 1073
Fort Detrick
Frederick, MD 21701
Phone: (301) 846-5748

8.2 Once a tumor biopsy has been collected from a patient and placed at -80ºC (or lower), FNLCR PD Specimen Central Receiving should be notified that the specimens are ready for shipment. Preferably, if additional biopsies will be taken from the same patient (post-dose timepoint[s]), the biopsies are to be stored in a local freezer at -80ºC (or lower), and shipped together as a full set in one shipment.

8.3 Send an e-mail to FNLCR PD Specimen Central Receiving (NCI_PD_Support@mail.nih.gov) to advise that biopsy samples are being prepared for shipment. State "*Protocol Name* PD Specimens Ready for Shipment" in the subject line. Request a confirmation e-mail that personnel will be available on the expected delivery date and time. Personnel are generally available to receive frozen shipments Tuesday through Friday, exclusive of government holidays. If needed, FNLCR PD Central Receiving can be contacted directly at (240) 344-5697 (Rachel Andrews) or (301) 401-8070 (Amy Pantella).

8.4 Use the PD Sample Shipping Manifest template in Appendix 2 to generate a shipping list containing pertinent sample information.

8.5 Make a copy of the Shipping Manifest and specimen Batch Records so that one copy can be sent to FNLCR with the biopsy samples and one copy can be maintained at the collection site for internal records.

8.6 Day of Shipment

8.6.1 Just prior to shipment, place specimen tubes into a biohazard specimen bag then in an insulating Styrofoam shipping container. The insulating Styrofoam shipping containers are required to have dimensions of ***at least*** 14″×11″×9″ (length, width, height) with a ***minimum*** of 20 pounds of added dry ice. Sufficient dry ice is imperative to maintain the samples at -20°C for at least 72 hours. Expect 10 pounds of dry ice to sublimate per day during transit. Add additional dry ice to the required 20 pounds if shipping is anticipated to be longer than 24 hours.

8.6.2 All weekly processing specimens are recommended to ship out on Mondays through Thursdays via FedEx Priority Overnight (excluding any day before a federal holiday).

8.6.3 **Verify** that the contents of the package match the Shipping Manifest and sign and date the bottom of both copies of the Shipping Manifest. Place one copy of the Shipping Manifest inside the shipping box along with copies of the completed Batch Records for all specimens.

8.6.4 Seal the box and print and attach the shipping address onto the outside of the shipping container; be sure the container is labeled as containing biohazardous specimens.

8.6.5 Record the shipping date, time, tracking number, and shipping information in the Batch Record (Appendix 1, Section 4).

8.6.6 E-mail FNLCR PD Specimen Central Receiving (NCI_PD_Support@mail.nih.gov) a shipment notification. State "*Protocol Name* PD Specimen Shipment" in the subject line and reference the tracking number in the e-mail. Please notify FNLCR PD Specimen Central Receiving of any issues or protocol deviations as soon as possible and provide written notes on the Batch Record.

8.6.7 Once specimens arrive at the receiving laboratory, they should be immediately placed at -80ºC (or lower) pending delivery to the processing laboratory for protein extraction.

APPENDIX 1: BATCH RECORD

Place PD Specimen Label Here

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

Note: A pre-dose and post-dose sample from the same patient would have the same Patient ID, but different Specimen ID numbers.

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

Certified Assay Operator: _____

Certification Number: _____

☐ Check here if PK/PD Support Lab Personnel

Facility/Clinic Collecting Specimens: _____

Clinical Protocol Number: _____

Patient ID: _____

Primary Diagnosis: _____

1. Biopsy Collection

	1st Pass	2nd Pass	3rd Pass	4th Pass
Specimen ID				
Site of Biopsy (complete for all passes or note "same" for replicate cores)				
Description of Biopsy (*e.g.*, large intact core or small and fragmented core)				
Biopsy Timepoint (Cycle, Day, and Hours post dose, if post treatment)				
Biopsy size prepared for PD or histological analysis:	☐ Full ☐ Halved	☐ Full ☐ Halved	☐ Full ☐ Halved	☐ Full ☐ Halved
Required: Time elapsed from collection to placement in tube	min sec	min sec	min sec	min sec
Time biopsy collected (opt)	:	:	:	:
Time biopsy placed in tube (opt)	:	:	:	:

2. Biopsy Procedure Details

Specimen ID	
Time local anesthesia administered	:
Dose of local anesthetic	mg
Name of local anesthetic used (from Research Nurse)	
Time of skin incision	:
Needle Type (*e.g.*, Temno)	
Needle diameter	gauge
Needle Length	cm
Time guide needle introduced	:
Time guide needle placement confirmed	:
Time biopsy needle introduced	:

3. Biopsy Storage

Date/time biopsy specimen(s) placed at -80°C (or lower) ____/__/____ ____:____ ____ºC

4. Shipping to FNLCR (optional)

Date and time samples shipped _____ ____:____

Tracking information _____

****Attach copy of Shipping Manifest**

5. Notes, including any deviations from the SOP:

6. Laboratory Director/Supervisor Review of Batch Record

Laboratory Director/Supervisor: _____ (PRINT)

_____ (SIGN)

Date: ____/____/____

APPENDIX 2: PD SAMPLE SHIPPING MANIFEST

From:

Phone:

E-mail:

PD Sample Shipping Manifest

In Package	Item No	Patient/ Specimen ID	Clinical Protocol	Description	Primary Diagnosis	Site of Biopsy	Time Point Scheduled	Collection Date	Collection Time
	Example	*1234-xx999-1023-500*	*12-C-0000*	*Full biopsy*	*Melanoma*	*Right forearm*	*Pre-dose D1*	*06/12/12*	*08:50*
	Example	*1234-xx999-1023-501*	*12-C-0000*	*Half biopsy*	*Melanoma*	*Right forearm (same lesion)*	*Cycle 1, D8*	*06/20/12*	*16:05*
☐	1							/ /	
☐	2							/ /	
☐	3							/ /	
☐	4							/ /	
☐	5							/ /	
☐	6							/ /	
☐	7							/ /	
☐	8							/ /	
☐	9							/ /	
☐	10							/ /	

Verification of Contents **Signature** **Date**

Contents Verified Collection Laboratory _____ / /

Contents Verified FNLCR PD Central Receiving _____ / /