Title:	Shipping and Handling of Fresh Autopsy/Post-Mortem Specimens (non-frozen) for Generation of Preclinical Models				Page 1 of 20
Doc. #:	SOP-PDM10107	Revision Date:	01/25/2022	Revision:	--

Biological Testing Branch, Developmental Therapeutics Program

National Cancer Institute at Frederick, and

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

Frederick National Laboratory for Cancer Research

Clinical and QA Reviewer: Michelle A. Eugeni

BTB Approval: Melinda Hollingshead

NCI PDM Repository Approval: Yvonne A. Evrard

SOP Developed in Collaboration with:

University of Nebraska Medical Center - Eppley Institute Rapid Autopsy Program, Drs. Michael Hollingsworth, and Paul Grandgenett

Johns Hopkins University Legacy Gift Rapid Autopsy Program, Drs. Jody Hooper, Robert Anders, and Angelo De Marzo

Change History

Revision	Approval Date	Description	Originator	Approval
--	01/25/2022	New Document. Adapted internal-use SOP-PDM10107 for posting to public website for general recommendations on use of post-mortem tissue for patient-derived model development. Removed specific references for communication with the NCI PDMR.	MAE	YAE

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1.0 PURPOSE

SOP outlines efforts for collection, packaging, and overnight shipping of post-mortem tissue for patient-derived model development.

Standardize the method for collecting, preparing, and shipping fresh autopsy/post-mortem material for use in patient-derived model (PDM) generation. The tissue collections will be used for direct implantation into immune-compromised mice to generate patient-derived xenografts (PDX) and/or growth in cell culture media to generate primary tissue-derived in vitro cell models that will be distributed through the NCI PDM Repository (PDMR).

Methodology and procedures described in this SOP and utilized by the Biological Testing Branch (BTB, DTP, DCTD, NCI-Frederick) to generate models for the NCI PDMR are provided to serve as general guidance and recommendations. Rapid autopsy/post-mortem programs can vary widely in their implementation and operation based on the research and clinical team integration, institutional policies, and state regulations. The information provided in this SOP should therefore be adapted as needed to adhere to institutional and state specific requirements or restrictions.

2.0 ABBREVIATIONS

BTB	=	Biological Testing Branch
DCTD	=	Division of Cancer Treatment and Diagnosis
DTP	=	Developmental Therapeutics Program
FNLCR	=	Frederick National Laboratory of Cancer
HBSS	=	Hank's Buffered Salt Solution
NCI-F	=	National Cancer Institute at Frederick
PBS	=	Phosphate Buffered Saline
PDM	=	Patient-Derived Models
PDMR	=	Patient-Derived Models Repository
PDX	=	Patient-Derived Xenograft
PDX ID	=	Randomly generated ID assigned to PDX specimens of registered patients and used for internal tracking at NCI-F/FNLCR; it assists in the delinking process and minimizes the chance of PII issues
PII	=	Personally Identifiable Information
RAP#	=	Enrolling site Rapid Autopsy/Post-Mortem Identifier (not linked to patient medical record ID)
SOP	=	Standard Operating Procedure

3.0 ROLES AND RESPONSIBILITIES

Clinical PI/Project Leader — The Clinical PI/Project Leader, directs specimen collection operations, supervises technical personnel, and is responsible for the proper performance of all specimen collection and shipping procedures. Oversees the personnel who follow the SOPs and is responsible for ensuring the personnel have sufficient experience to handle clinical specimens.

Clinical Specimen Support Lab Personnel — Clinical Specimen Support Lab personnel work under the guidance of the Clinical PI/Project Leader. These staff ensure specimen collection and shipping are performed in accordance with the current SOP(s), as well as any other procedures conducted by a clinical site.

3.1 It is the responsibility of the Clinical Specimen Support Lab Personnel to confirm scheduled specimen collection time points, prepare all labels and data collection sheets in advance, check documentation for accuracy, and verify the required specimen collection jars/media, supplies, and equipment are available for successful collection and handling of specimens.

3.2 It is the responsibility of the Clinical Specimen Support Lab Personnel to ensure timely transport and processing of the specimens, enter and review the required collection and processing data, and archive all data sheets in the appropriate files.

3.3 Clinical Specimen Support Lab Personnel following this SOP are required to be certified in working safely with bloodborne pathogens in research laboratories in accordance with OSHA Bloodborne Pathogen Standard (29 CFR 1910.1030).

3.4 The Clinical Specimen 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.

3.5 All specimens must be shipped **on the same day as collection or at the soonest available shipping time given the potential timing of specimen collection (e.g., after regular business hours)**. A completed Shipping Manifest and signed Specimen Chain of Custody sheet must be included with each shipment (Appendix 1).

4.0 MATERIALS REQUIRED

Note: As much as is reasonably possible, separate sets of sterile disposable or non-disposable instruments, petri dishes or containers should be used for excision and manipulation of excised tissue from each separate anatomic site. This will help to minimize the risk of potential bacterial cross-contamination as well as the risk of intermingling of cells from different resection sites.

4.1 Autopsy Suite

4.1.1 Sterile instruments – disposable or non-disposable

- Ideally multiple sets:
  - one for the initial external to internal incision,
  - preferably one set for each separate site of tumor excision.

4.1.2 Sterile, one-time use tweezers and scalpels, multiple sets, for manipulation of excised tissue.

4.1.3 Sterile lab dressing and/or sterile petri dish for placement and manipulation of excised tissue.

4.1.4 Ice bucket with wet ice for transportation of specimen jars.

4.1.5 Hydrogen peroxide (H2O2) solution 1.4% (e.g., Clorox Part# 30829).

4.1.6 Sterile 0.9% saline or HBSS preferred (alternatively 1X PBS, if others not available)

4.1.7 Multiple 50-mL tubes or 6-well plates or similar containers for HBSS/0.9% saline/PBS and H2O2.

4.2 **Specimen collection kit**

4.2.1 Up to four (4) Sterile, 50-mL wide-mouthed Nalgene jar containing CO_2 Independent Medium (Gibco, Cat#: 18045-088) with antibiotics/antimycotics (Primocin®); do not use after expiration date. ***Store at 5°C±3°C**

4.2.2 Foam core packs. ***Store at -20°C or 4°C, see SOP Section 6.2.1 for guidelines.**

4.2.3 Up to four (4) Specimen jar labels

4.2.4 Up to four (4) Parafilm Strips

4.2.5 Bubble wrap

4.2.6 Specimen bags

- Up to four (4) for packing specimen jars (with biohazard label)
- One plain, Ziploc bag to protect Shipping Manifest upon return shipment

4.2.7 Styrofoam box

4.2.8 Shipping cardboard box

4.2.9 FedEx Priority Overnight return label

5.0 OPERATING PROCEDURES

5.1 General limitations:

5.1.1 Do not collect samples from patients with confirmed active or with a history of bloodborne pathogens such as: human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV).

- NOTE: This is a safety restriction applied at NCI-Frederick/FNLCR, consult with your institution to determine allowable bloodborne pathogens.

5.1.2 The goal is to be as sterile as possible within the limitations of the autopsy suite/pathology laboratory setting and minimize post-mortem interval time (time of death to time of tissue collection).

- Wherever possible, sterile technique should be used to limit environmental contamination post-mortem including (1) sterile preparation of the exterior of the body, (2) use of a sterile field for placement of and/or manipulation of the tissue specimens, (3) use of single-use sterile disposable instruments for specimen collection and handling or multiple sets of non-disposable sterile instruments, and (4) containers for the rinse solutions (HBSS/sterile 0.9% saline/PBS and H2O2).

5.1.3 Systemic infections present in the participant pre-mortem, diagnosed or undiagnosed, may contribute to downstream contamination and cannot be anticipated.

- If the collecting site is aware of the pre-mortem infection, it is helpful for notification to be provided so that appropriate antibiotic coverage can be implemented once the specimens are received.
- If no infection is indicated pre-mortem but upon specimen receipt contamination of any kind is suspected, an aliquot of the transport media should be sent for culture and sensitivity so that steps can be implemented to ameliorate.
- Any signs of contamination that occur once the specimens are in culture or implanted will be addressed and managed as they present in the collected specimens and preclinical models.

5.1.4 During initial set-up of a program to develop models from autopsy/post-mortem specimens, it may be useful to culture an aliquot of transport media from each set of specimens received to allow for evaluation of possible contamination and to QC the processes.

- Once procedures are fully established, an aliquot of the transport media can be obtained at the time of specimen processing and saved for future culture if evidence of contamination presents at a later point in time.
- The implanted animals can also be prophylactically treated with medicated feed for the first two weeks after tumor tissue implant.
- If fungal outgrowth is noted in the tissue culture, the implanted animals can be placed on an antifungal therapeutic such as Fluconazole as a preventative measure.

5.1.5 Shorter post-mortem intervals (time of death to time of tissue collection) will improve tissue viability for development of preclinical models and minimize post-mortem bacterial contamination.

5.1.6 Components of the abdominal viscera (especially the large and small intestine) should be the last portion of the trunk that is autopsied, as permitted per institutional standards and procedures, to minimize cross-contamination with microbes that replicate within these organs pre- and post-mortem.

5.2 Processing limitations:

5.2.1 Specimens can be collected from both primary and metastatic tumors.

- Please collect each site into a separate media jar.
- Media jars should be numbered to correspond to the Shipping Manifest and/or labeled with specific collection site (e.g., "upper lobe of left lung")

5.2.2 Autopsy suite/tissue handling procedures listed in this SOP are for "best" conditions. Depending on individual site facilities and required processes for tissue acquisition, not all procedures may be achievable.

5.2.3 The maximum size for an individual tissue piece should be 9 mm (3/8 in) in its longest dimension; larger pieces should be cut into smaller pieces ≤ 9 mm.

- When feasible, multiple smaller pieces are preferred to increase odds of creating a patient-derived model.
- In addition, the ratio of transport media to tissue specimen should be at least 10:1 or no more than ¼ of the jar depth should contain minced tumor tissue.

5.2.4 Whenever possible, specimens **are to be shipped on the day of collection** for receipt at the receiving in vivo/in vitro laboratories the day after collection.

5.2.4.1 Tumor tissue specimens **must** be placed in pre-chilled media jars and shipped with pre-chilled foam core packs.

5.2.4.2 Due to the uncertainty of collection times for autopsy/post-mortem procedures, material may be collected over a weekend. **Important: If a post-mortem collection occurs on a Friday,** specimens may still be sent by FedEx on the first available pick-up for arrival on Saturday, but this must be coordinated with the receiving laboratories prior to specimen shipment.

5.2.5 Each shipping box should contain its own Shipping Manifest, Chain of Custody sheet (Appendix 1) and tumor specimens from a single patient.

5.3 Notification of "On-call" for Upcoming Autopsy/Post-Mortem Specimen Collection for the Receiving Laboratory

5.3.1 Email the receiving laboratory at the earliest possible time if informed in advance of a potential procedure, even if the window is 2-3 weeks.

- It is understood that the availability of detailed information will be limited by the level of interaction of the site's rapid autopsy/post-mortem team with the hospital or hospice providers but any amount of advance notice that can be provided is helpful.
- Include pertinent information in the email to assist with preparation for model development such as: the approximate time window (e.g., 1-2 weeks), general patient diagnosis (e.g., pancreatic adenocarcinoma), and gender.

Important: On the day of shipment an e-mail must be sent with the FedEx shipping notification (SOP Section 6.3) and the completed Worksheet A (Appendix 2) confirming informed patient consent to the site's local research protocol for rapid autopsy/post-mortem specimen collection. This will allow adequate time and information for the receiving laboratory to prepare for specimen receipt. **Important:** Specimens should not be processed until the attestation of patient consent i.e., Worksheet A, is received (consult with your local IRB on timing of consent and processing requirements).

5.3.2 Final limited data i.e., Worksheet B, should be completed to the best of the enrolling site's ability from the patient's medical records and sent to the receiving laboratory within 10-14 days of the specimen collection. Alternatively, a copy of the redacted patient autopsy report can be provided.

5.4 Day of Autopsy/Post-Mortem Tissue Specimen Collection

5.4.1 Prepare the specimen jar labels including the in-house rapid autopsy/post-mortem identifier (RAP#). **Important:** PDM specimens should never be labeled with PII, including but not limited to medical record numbers, date of birth or patient initials. If labeling containing PII is needed for site purposes at the time of the specimen collection, this labeling should be removed in its entirety prior to the application of the final labels for the receiving laboratory.

5.4.2 Remove the provided tumor collection jars with media from the cold room/refrigerator storage and apply the labels. Always maintain the jar(s) on wet ice until packaging for shipment.

5.4.3 Leave the rest of the Shipping Kit items in the cold room/refrigerator or freezer (as designated) until ready to package the specimen for shipping.

5.4.4 Ensure that FedEx Priority Overnight shipping has been arranged and/or that FedEx will check the designated pick-up location on the day of the planned shipment.

5.5 Autopsy/Post-Mortem Tissue Collection Process for PDM Development

Note: The following steps should be followed to the extent feasible based on the institutional policies and procedures at the individual enrolling sites.

5.5.1 Set-up containers with H2O2 solution and sterile 0.9% saline, HBSS, or PBS to facilitate the process flow once the autopsy/post-mortem procedure begins (details in Step 5.5.5).

5.5.2 Set-up a sterile field and/or sterile petri dishes for placement of the post-dipped, excised tissue (details in Step 5.5.5).

5.5.3 Clean and disinfect the exterior surface of the body before opening with sterile instruments, as feasible.

5.5.4 Use sterile instruments for tissue excision. A separate set of instruments, disposable or non-disposable, should be used for each primary/metastatic lesion collected. Areas of obvious necrosis or hemorrhage should be avoided.

5.5.5 Dip excised tissue into the H2O2 solution and hold for 1 minute (Appendix 3), then dip twice in sterile 0.9% saline, HBSS, or PBS and place on a sterile surgical field or petri dish. Sterile 50-mL tubes or 6-well plates can be used for these washes.

- Two separate containers per resection site can be used: 1 with H2O2 and 1 with sterile saline, PBS, or HBSS. These would be used in a production line fashion with a separate set of containers used for each resection site.
- Alternatively, one sterile container can be used for each resection site.
    - Initially fill the container with H2O2 for the 1-minute dip.
    - The H2O2 can then be disposed of and the same container filled with sterile 0.9% saline or HBSS (alternatively PBS) for the rinses.
    - A different container should be used for each resection site.
- Maintaining separate containers for each resection site minimizes the risk of cross-contamination of tumor cells and/or bacteria that may be present.

5.5.6 With a new set of sterile instruments, finely mince the tissue on the sterile field or petri dish, then transfer to the transport media. The mincing helps ensure the tumor tissue is evenly exposed to the nutrients, antibiotics and antimycotics in the media.

Note: A new sterile scalpel and tweezers should be used for each excised tissue piece on a different section of the sterile field or a separate petri dish.

5.5.7 Place tissue from a single tumor site (primary or metastatic) into an individual pre-chilled, jar containing sterile media. Media jars should be kept on wet ice during this entire process.

- Each kit comes with up to 4 media jars; more than one kit should be used for shipment if the number of primary and metastatic sites is >4.
- Label every jar with the RAP#.

5.5.8 Cross-identify the tissue collection site on media the jars and Shipping Manifest:

- Either number the jars corresponding to the line items in the Shipping Manifest (1-4), and/or
- Label the jars with site of specimen collection (e.g., colon-rectosigmoid) that corresponds with the site of specimen collection listed on the shipping manifest.

5.5.9 If possible, please note on the Shipping Manifest (Appendix 1) the estimated time elapsed from death to tissue collection as an estimate of post-mortem interval time.

6.0 PACKAGING OF TUMOR SPECIMENS

Important:

- Whenever possible, specimens **are to be shipped on day of collection** for receipt at the receiving laboratory the day after collection.

6.1 Complete a Shipping Manifest (Appendix 1) for each set of specimens collected.

6.1.1 At the top of the Shipping Manifest, enter the enrolling site RAP# for the patient. The PDX ID box (gray text) will be completed by the receiving laboratory upon receipt for internal tracking.

6.1.2 The jar numbers and location of tissue collection should be clearly identified. In the below example, two separate lesions in the pancreas were collected in Jar#1 and Jar#2 and two separate metastatic sites were collected in Jar#3 and Jar#4.

Rapid Autopsy Material		
Jar #	Tissue Type	Site of Autopsy Material
1	☒ Primary Tumor Site ☐ Metastatic Site ☐ Unknown	pancreas #1
2	☒ Primary Tumor Site ☐ Metastatic Site ☐ Unknown	pancreas #2
3	☐ Primary Tumor Site ☒ Metastatic Site ☐ Unknown	Diaphragm nodules.
4	☐ Primary Tumor Site ☒ Metastatic Site ☐ Unknown	Mesenteric Fat nodules

6.1.3 Sign and date the first row of the Chain of Custody document (Appendix 1, page 2) as the 'Clinical Center'.

6.2 Packaging Instructions

6.2.1 Just before packaging, open the Tumor Shipping kits and remove the foam core packs from the freezer or refrigerator.

6.2.1.1 Guidelines for foam core pack shipping temperatures:

- A judgment call is required by the shipping sites in preparation for upcoming shipments. **Please review anticipated overnight temperatures for your area the day before specimen shipment** and be sure the foam core packs are stored at the appropriate temperatures. This may require one foam core pack to be removed from the freezer in advance so please assess anticipated weather conditions the day before the scheduled collection. Specimens will be packaged per the below table to ensure the best conditions for viable tissue maintenance within the 24-hour shipment window.

Average Overnight temperature at shipping site	Bottom foam core pack	Top foam core pack
40°F (5°C) or higher	-20°C	-20°C
39°F or lower (4°C or lower)	-20°C	4°C

6.2.1.2 The packing process should go quickly – do not leave contents or the chilled foam core packs at room temperature for a prolonged time.

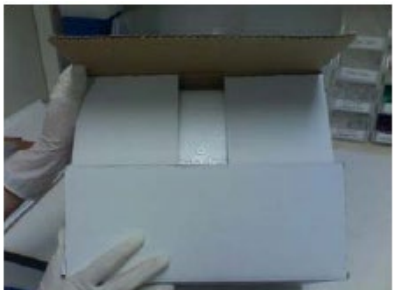
6.2.2 Turn the lid tightly on the tissue jar then wrap the parafilm around the jar where the lid and the jar meet. Wrap **each jar** in the provided absorbent green pad, and then seal it in the provided biohazard bag.

Important: The applied specimen label should be on the body of the collection jar. Do not apply the label across the lid of the jar as this may prevent a full seal of the jar when the parafilm is applied and may result in leakage.

6.2.3 Place one foam core pack on the bottom of the Styrofoam shipping box. Wrap up to 4 media jars in the provided bubble wrap, then place them on top of the foam core pack. Finally place the second foam core pack on top.

6.2.4 **Seal the completed Shipping Manifest** with the Chain of Custody section **signed** on line #1 (Appendix 1) **in the clear zip lock bag** and then place it on top of the foam core packs.

6.2.1 Close the Styrofoam box and seal the cardboard shipping box.



6.3 Shipping Instructions

6.3.1 After the cardboard box is sealed, attach the provided return FedEx Priority Overnight shipping label to the outside of the box for shipment; **do not obscure the UN3373 label.**

6.3.2 **Important**: Patient specimens that are being shipped are "viable" specimens. Do not leave boxes containing specimens on shipping docks for prolonged lengths of time as a severe temperature drop or rise could compromise the viability of the specimens.

6.3.3 Send an e-mail on the day of shipment to the receiving laboratory.

6.3.3.1 The **subject line** should state: "PDM Autopsy/Post-Mortem Specimen Shipment Notification"

6.3.3.2 For each shipping box being sent include the following information:

- FedEx Tracking Number(s)
- RAP#
- Number of individual media jars with tissue from the patient included in the box
- Finalized copy of Worksheet A. This is needed to verify patient consent was received by the enrolling institution for the specimen collection.
- Scanned copy of the final Shipping Manifest(s) for the specimens.

APPENDIX 1: SHIPPING MANIFEST AND CHAIN OF CUSTODY

RAP number

PDX ID (Completed by Receiving Lab)

1. Shipping Manifest

Include a copy of the manifest and signed, chain of custody section with every collection. If >4 tissue pieces are collected from a patient, use a 2nd shipping box and complete a separate shipping manifest for that box; both will be labeled with the same RAP#.

Basic Patient Information				
Collection Date	**Post-mortem Interval** (Approx. Time Elapsed from Death to Collection)	**Patient Gender**	**Diagnosis/Disease Code** (standardized disease code)	**Histological Diagnosis/Sub-Type**
		☐ Male ☐ Female		

Rapid Autopsy/Post-Mortem Specimens		
Jar #	**Tissue Type**	**Site of Autopsy/Post-Mortem Specimen Collection**
1	☐ Primary Tumor Site ☐ Metastatic Site ☐ Effusion ☐ Unknown	
2	☐ Primary Tumor Site ☐ Metastatic Site ☐ Effusion ☐ Unknown	
3	☐ Primary Tumor Site ☐ Metastatic Site ☐ Effusion ☐ Unknown	
4	☐ Primary Tumor Site ☐ Metastatic Site ☐ Effusion ☐ Unknown	

APPENDIX 1 - CONTINUED

2. Chain of Custody Signatures

Prior to shipping the Clinical Center Specimen Handling personnel should verify contents of and sign and date on line 1 below to verify contents of container.

Task	Responsible Party	Signature	Date
1. Shipment of tumor specimen (with appropriate foam core pack temperatures)	Clinical Center		/ /
2. Receipt of specimen: log receipt, verify specimen(s) and shipping conditions. Remove RAP ID from all documentation and replace with PDX ID.	Receiving Personnel		/ /
3. Portion of specimen received by in vitro lab for in vitro use and transfer of portion to in vivo lab for implantation. Mark N/A if not provided to lab.	Laboratory Personnel		/ /

APPENDIX 2: REGISTRATION FOR RAPID AUTOPSY/POST-MORTEM SPECIMEN COLLECTION FOR <NAME OF EFFORT>

Worksheet A (page 1 of 1)

Documentation of Consent and Registration

☐ I confirm that the patient has signed an informed consent allowing collection of autopsy/post-mortem specimens for distribution to the <RECEIVING LABORATORY> allowing for its use in creation of preclinical models, genetic characterization, and distribution of any derived materials and de-identified medical information to the research community.

Local Protocol #: _____

PI or designee name (please print):

PI or designee signature:

Clinical Center:

Date of signature *(mm/dd/yyyy)**:***

Rapid Autopsy/Post-Mortem Identifier (RAP#):

Registration Information

Specimen Collection Date:	**Biological Gender:** ☐ Male ☐ Female	
Date of Primary Pathological Diagnosis:	**Age at Time of Primary Diagnosis:**	**Age at Collection:**

Detailed Diagnosis or Diagnosis code*:

****For example:***

- ***CTEP Simplified Disease Classification (SDC)*: http://ctep.cancer.gov/protocolDevelopment/docs/SDCv10_M10.xls**
- ***OncoTree code*: http://oncotree.mskcc.org/**
- ***WHO International Classification of Disease in Oncology***

Diagnosis and/or subtype (if applicable, e.g., adenocarcinoma, undifferentiated):

Tumor Stage/Grade (if applicable, e.g., Stage III, T3N0M1, Gleason score, Fuhrman Grade)

Location of known metastases:

Tumor Biomarker Information (if available and permitted per patient consent, local IRB and/or HIPAA policy, e.g., IHC results [ER+], genetic screening [BRAF-V600E], FISH analysis [nuc ish 8q24(MYCx2)]):

RAP#: _____

Complete this form to the best of your ability. Any information unavailable in the patient record should be marked as "Don't Know," "Unknown" or write-in "Unknown."

Worksheet B (page 1 of 2)

Previous Tobacco Exposure

Was the subject a:

- ☐ Current Smoker
- ☐ Former Smoker
- ☐ Never Smoked
- ☐ Information not available

If 'Current or Former smoker, please complete the following based on the information available.

Total pack/years*: _____

*This is calculated by multiplying the number of packs smoked per day by the number of years the person has smoked (most US packs contain 20 cigarettes)

Race and Ethnicity

Racial Categories (select one or more, as applicable)

- □ American Indian/ Alaska Native
- □ Asian
- □ Native Hawaiian or Other Pacific Islander
- □ Black or African American
- □ White
- □ Unknown
- □ Subject declined to provide

Ethnicity (select one):

- □ Hispanic or Latino
- □ Not Hispanic or Latino
- □ Unknown
- □ Subject declined to provide

RAP#: _____

Complete this form to the best of your ability. If any of the following information is not available in the patient record, simply write-in "Unknown." If the patient was not on therapy at time of death or had no previous therapies, indicate "No Current Therapy" or "No Previous Therapy" in the appropriate sections.

Worksheet B (page 2 of 2)

Systemic treatments (include chemoradiation related to current diagnosis. Include radiotherapy related to current diagnosis only if administered to area of collected tissue). If unable to list specific agent names, list by agent class, mechanism of action or indicate 'Clinical Trial', as appropriate.

Therapy	Date Therapy Started (MM/YYY or YYYY)	Best Response* (SD/CR/PR/ PD, Non-Evaluable, Not Assessed)	Treatment Duration (months or cycles)	Prior or Current** Treatment	Comments
1				□ Prior □ Current	
2				□ Prior □ Current	
3				□ Prior □ Current	
4				□ Prior □ Current	
5				□ Prior □ Current	
6				□ Prior □ Current	
7				□ Prior □ Current	

* Best Response as assessed by RECIST; Indicate "N/A" if patient not assessed or it was not documented. If Best Response is assessed by other criteria, please indicate criteria used in Comments.

**Current Treatment includes any treatment received within 2 weeks prior to patient death.

APPENDIX 3: RATIONALE FOR TISSUE COLLECTION PROCESS

Initial rapid autopsy/post-mortem specimen collections involved direct placement of the tumor tissue into CO_2-independent media containing a mix of antibiotic and antifungal agents. Specimens were shipped overnight for arrival the next day or, if unable to be shipped for next day arrival, stored in the provided sterile media and held at 4°C for shipment at the next soonest available overnight delivery pick-up. Most samples were received within 24 hours post-collection, a smaller subset was received 48-96 hours post-collection. Upon receipt, specimens were rapidly processed with a portion of the received tissue cultured in vitro to develop patient-derived cell lines and an aliquot of the transport media cultured to test for sterility. The remaining tissue was directed to in vivo for implantation.

Early in vitro culture results demonstrated frequent presence of extensive bacterial contamination including but not limited to Pseudomonas aeruginosa, Enterococcus faecalis, Staphylococcus epidermidis, Streptococcus intermedius, Lysinibacillus sphaericus, and Bosea massiliensis. Fungal contamination was also a frequent occurrence. These bacterial and fungal contaminants were present irrespective of the anatomic site of tumor tissue resection or length of time between collection and in vitro culture. Salvage and mediation were attempted with Normocure™, Primocin®, gentamicin, and Fungizone™ for a variety of incubation periods but this was predominantly ineffective or exceedingly toxic to the cells.

In vivo, all animals receive antibiotic feed for approximately 2 weeks post-tumor tissue implant. Despite the confirmed presence of the above contaminants in vitro, there was minimal overt animal toxicity. Based on this initial experience, it was determined that preliminary processing of the resected tumor tissue prior to placement in the sterile transport media would be necessary to attempt to mediate contaminants that may be present on the external surface of the collected tumor tissue. In coordination with the Biological Testing Branch (DTP, DCTD, NCI-Frederick) and the UNMC RAP team, procedures to (1) process the resected tissue through a series of dips and rinses to remove an adequate amount of the surface contaminants with minimal impact to the deeper tissue and subsequent cell viability and (2) increase overall sterile technique for these types of research autopsy/post-mortem tumor collections were implemented.

The utilization of H_2O_2 solution was assessed to likely be effective for surface contaminant removal without being exceedingly toxic to the tissue when utilized as detailed in SOP Section 5.5.5. This is followed by a sterile 0.9% saline HBS, or PBS S rinse to ensure that the H_2O_2 will not persist on the tissue, diffuse into the transport media, and further impact cell viability. Additionally, the transport media formulation was adjusted to include Primocin® and Normocure™ to mediate potential residual pathogen issues while the specimen was in transit. Further emphasis was also placed on sterile handling of the specimens and use of sterile instruments during the initial resection and throughout the specimen processing prior to placement in the sterile transport media; this was implemented as much as possible based on each enrolling site research team's access to the autopsy suite and institutional policies.

Following implementation of the new tissue processing steps and revision of the transport media additives, almost complete mediation of fungal and bacterial contamination was achieved. Subsequent revisions to further mediate contaminants and maximize cell viability have included:

- CO_2-independent media with Primocin® only,
- Treatment in vitro and in vivo with Fungin™, Primocin® and/or Normocure™ as needed,

- Utilization of increased sterile technique during the autopsy/post-mortem procedure at enrolling sites when feasible including use of a sterile field for placement of the resected tissue for processing, use of separate sterile instruments between each site of resection as well as for processing the tumor tissue prior to placement into the sterile transport media. This is implemented as feasible based on each enrolling site's access to the autopsy suite and institutional policies.

It should be noted once again, that all method development for collections was based on the need to send the tumor material by overnight shipping from the collecting clinics to NCI-Frederick, MD. It is possible if same-day use of the tumor material for patient-derived model development can occur that some of these additional methods to decrease bacterial and fungal growth may be less critical.