| Title: | Shipping and Handling of Viably Cryopreserved Autopsy/Post-Mortem Specimens for Generation of Preclinical Models | | | | Page 1 of 20 |
|---|---|---|---|---|---|
| Doc. #: | SOP-PDM10108 | 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

# 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 specimens for patient-derived model development. Removed specific references for communication with the NCI PDMR. | MAE | YAE |

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## 1.0 PURPOSE

Standardize the method for collecting, preparing, and shipping viably cryopreserved autopsy/post-mortem specimens for use in patient-derived model (PDM) generation. Ideally these tissues will be from both the primary tumor site and metastatic lesions so PDXs from multiple locations can be developed for study. The tissue collections will be used for direct implantation into immunocompromised mice to generate patient-derived xenografts (PDX) and/or to culture in cell culture media to generate primary tissue-derived in vitro cell models that will be distributed through the NCI PDM Repository.

We have performed a limited comparison of carcinoma PDX take-rates developed from matched non-cryopreserved and cryopreserved tissues. This data set demonstrated that development of PDXs from cryopreserved primary tissue specimens have decreased cell viability resulting in a significantly lower take rate than those attempted utilizing non-cryopreserved primary tissue specimens. This SOP has been developed and utilized by the Biological Testing Branch (BTB, DTP, DCTD, NCI-Frederick) to provide general standards and procedures for rapid autopsy/post-mortem (RAP) tissue specimen collection, cryopreservation, and shipment to be used when collection of non-cryopreserved specimens is not feasible due to any number of circumstances. Whenever possible, non-cryopreserved primary tissue specimens are always preferred for use in the development of PDXs.

## 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 Balanced Salt Solution |
| NCI-F | = | National Cancer Institute at Frederick |
| PBS | = | Phosphate Buffered Saline |
| PDM | = | Patient-Derived Models |
| 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 sample 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 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 all 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** **Viably cryopreserved specimens should NOT be shipped the same day as collection** as they need to go through the freezing procedure.

**3.6** Confirm FedEx Priority Overnight shipping has been arranged for **the date of choice for shipment Monday - Wednesday**; specimens can be held at -80°C for several days [less is better] before shipping if the next shipping day would result in the receiving laboratory receiving the specimens on a weekend.

**3.7** A completed Shipping Manifest and signed Specimen Chain of Custody sheet must be included with each shipment (Appendix 1).

**3.8** Provision of associated Patient Limited Medical Information (Appendix 2) or redacted autopsy report should be received within 10-14 days of shipment of the specimens.

## 4.0 MATERIALS REQUIRED

**Note:** As much as is reasonably possible, separate sets of sterile disposable or non-disposable instruments and petri dishes 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

4.1.1.1 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 (one set per tumor/metastatic site).

**4.1.3** Sterile drapes/surgical fields
**Optional:** Sterile petri dish for manipulation of individual excised tissues

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

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

**4.1.6** 2-mL cryovials

**4.1.7** Laboratory marker and tube labels (e.g., Sigma-Aldrich, Cat# Z359122)

**4.1.8** Tube holder

**4.1.9** Microcentrifuge tube labels

**4.1.10** Freezing media: RPMI-1640 media containing 20% fetal bovine serum and 10% DMSO
- Hold on wet ice (stored at 4°C)
- Ideally, the DMSO is added the same day as it is used.
- The RPMI-1640 + 20% FBS can be prepared in advance and stored at 4°C.

**4.1.11** Ice bucket with wet ice for transportation of specimen jars

**4.2** Laboratory (freezing of specimens)

**4.2.1** Mr. Frosty Freezing container (Nalgene, Cat# 5100-0001)

**4.2.2** Isopropanol

**4.2.3** -80°C freezer

**4.3** Shipping

**4.3.1** Tube box

**4.3.2** ZipLock bag to protect Shipping Manifest upon return

**4.3.3** Sufficient dry ice for 3-d shipment

**4.3.4** Styrofoam box

**4.3.5** Shipping cardboard box

**4.3.6** FedEx Priority Overnight return label

## 5.0 OPERATING PROCEDURES

### 5.1 General limitations:

**5.1.1** Do not collect autopsy/post-mortem specimens from patients known to actively have or have a documented history of having the following bloodborne pathogens: 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 (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 for implant and culture.
- 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** Shorter post-mortem intervals (time of death to time of tissue collection) will help improve tissue viability and minimize post-mortem bacterial contamination.

**5.1.5** 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.
- Details on the Shipping Manifest should correspond exactly to the labeling on the cryovials.
- If matched fresh and viably cryopreserved specimens are collected, the site of resection details should correspond between the fresh specimen and the matched cryopreserved specimen.

**5.2.2** Autopsy suite/tissue handling procedures listed in this SOP are for “best” conditions; depending on individual site set-up for tissue acquisition not all may be achievable.

**5.2.3** The maximum size for individual pieces for shipping should be ~2-3 mm3 each; 3-5 pieces of this size can be placed in an individual cryo-vial for freezing.

**5.2.4** Each shipping container should contain its own Shipping Manifest and Chain of Custody sheet (Appendix 1). Important: Each shipping box should contain tumor specimens from only one patient.

### 5.3 Notification of "On-call" for Upcoming Autopsy/Post-Mortem Material 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, hospice, or care providers but any amount of advance notice that can be provided is helpful.

  **Note:** When possible, the plan to send both fresh and cryopreserved material should be included in the on-call notification email.

- Include pertinent information in the email to assist in laboratory preparation, 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 email must be sent with the FedEx shipping notification (SOP Section 7.3) and the completed Worksheet A (Appendix 2) so the receiving laboratories can 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).

### 5.4 Day of Autopsy/Post-Mortem Specimen Collection

**5.4.1** Prepare specimen tube 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.

**5.4.2** Have screw-capped 2-mL cryo-vials and labels ready for collected tissue; place freeze medium on ice; multiple tubes can be collected for each tissue collection location.

**5.4.3** Viably cryopreserved specimens should **NOT** be shipped the same day as collection as they need to go through the freezing procedure.

- Arrange FedEx Priority Overnight shipping for **the following day or the date of choice for shipment Monday – Wednesday.** Delivery to the receiving laboratory as soon as possible is preferred to ensure tissue is handled and stored under consistent conditions.

- Specimens can be held at -80°C for several days before shipping if the next shipping day would result in the receiving laboratory receiving the specimens on a weekend.

## 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 of the enrolling site.

**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 of 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 4), 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 0.9% saline, HBSS, or PBS. 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 on the sterile field or petri dish, cut the tumor tissue into 2-3 $mm^3$ fragments (~30 mg).

5.5.6.1 Place the number of cryovials needed for the available fragments (3-5 fragments/tube) into a tube rack; keeping the rack and vials on wet ice.

5.5.6.2 Add the 3-5 fragments to each tube.

5.5.6.3 Add 1-mL of freeze medium to each tube without exceeding the tube fill volume.

**5.5.7** Seal the cryovials well, wipe with disinfectant, and place into wet ice.

**5.5.8** Label vials with RAP# and tissue source.

- For example, if 10 fragments are collected from the colon of RAP ID #123 and split into two vials, both vials would be labeled “RAP#123, colon”. The shipping manifest would note that 2 vials are being shipped for a colon resection.

## 6.0 CRYOPRESERVING RAP MATERIAL

**6.1** If using a step-rate freezer:

**6.1.1** Follow the manufacturer's instructions to operate stepped rate freezer. The Biological Testing Branch (DCTD, NCI-Frederick), who generates models for the NCI PDMR, current parameters are:

- 1°C/minute down to -4°C
- -25°C/minute down to -40°C
- +15°C/minute up to -12°C
- -1°C/minute down to -40°C
- -10°C/minute down to -90C

**6.1.2** Vials can be held up to 3 days at -80°C before shipping or transferred to the vapor phase of a liquid nitrogen storage tank if there will be a longer time gap before shipping.

**6.2** If using a slow rate freezing container, such as a Mr. Frosty:

**Note:** Follow manufacturer's instructions exactly paying close attention to whether the container should be pre-chilled or held at room temperature before loading.

**6.2.1** For isopropanol-based devices (e.g., Mr. Frosty) it is important the isopropanol be replaced regularly, and the container be filled to the manufacturer's fill line.

**6.2.2** Room-temperature isopropanol should be placed in the base of the cryo-container and the tube holder placed on top.

**6.2.3** Transfer the specimen-containing cryovials from the ice-bucket into the cryo-container, screw on container lid, and place at -80°C overnight. Vials can be held up to 3 days at -80°C (less is preferred) before shipping or transferred to the vapor phase of a liquid nitrogen storage tank if there will be a longer time gap before shipping.

## 7.0 PACKAGING OF TUMOR SPECIMENS

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

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

**7.1.2** The cryovial numbers and location of tissue collection should be clearly identified. In the below example, specimens from 2 different liver lesions have been obtained (ff and B) and 2 vials for each are being shipped. In Vial Label row three, a pancreas specimen (Pancreas A) has been obtained and only 1 vial is being shipped.

- If only one 81-slot box of freezer specimens is being sent, then column 4 (Box) would indicate 1 of 1.
- The Vial Position in the Box is based on a standard 81-Slot Box layout (Appendix 3)

| Rapid Autopsy Material (viably cryopreserved) | | | | | |
|---|---|---|---|---|---|
| **Vial Label (exactly as written on label)** | **Vial Contents** | **# Vials** | **Box** | **Vial Position in Box** | **Tissue Type** |
| RAP 199 Liver ff | Cryopreserved fragments of Originator material from liver Section ff, patient #199 | 2 | 1 of 2 | A1-A2 | ☐ Primary Tumor Site<br>☒ Metastatic Site<br>☐ Unknown |
| RAP 199 Liver B | Cryopreserved fragments of Originator material from liver, Section B, patient #199 | 2 | 1 of 2 | A3-A4 | ☐ Primary Tumor Site<br>☒ Metastatic Site<br>☐ Unknown |
| RAP 199 Pancreas A | Cryopreserved fragments of Originator material from pancreas, Section A, patient #199 | 1 | 1 of 2 | A5 | ☒ Primary Tumor Site<br>☐ Metastatic Site<br>☐ Unknown |
| RAP 199 Lung E | Cryopreserved fragments of Originator material from lung, Section E, patient #199 | 2 | 1 of 2 | A6-A7 | ☐ Primary Tumor Site<br>☒ Metastatic Site<br>☐ Unknown |
| RAP 199 Lung F | Cryopreserved fragments of Originator material from lung, Section F, patient #199 | 1 | 1 of 2 | A8-A9 | ☐ Primary Tumor Site<br>☒ Metastatic Site<br>☐ Unknown |

**7.2 Packaging Instructions**

**7.2.1** Place sufficient dry ice inside the internal styrofoam box to last 3 days. Ensure box is clearly marked that it contains dry ice per institutional guidelines and that there is a UN3373 label on the outside.

**7.2.2** The cryotubes can be placed in a 50-mL conical tube and nested inside the dry ice for shipping. Alternatively, place them in a cryotube box and nest the box in the dry ice for shipping. Either way, the sample container should be surrounded by dry ice, not sitting on top of it.

**7.2.3** Close the styrofoam container.

**7.2.4** Seal the completed Shipping Manifest with the Chain of Custody section signed on line #1 (Appendix 1) in the clear zip lock bag and place it on top of the styrofoam lid to protect it from condensation.

### 7.3 Shipping Instructions

**7.3.1** Ship specimens on dry ice by FedEx Priority Overnight. Once 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.**

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

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

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

- FedEx Tracking Number(s)
- RAP#
- Number of individual cryo-vials with tissue from the patient included.
- If not previously sent, a finalized copy of Worksheet A. This is needed to verify patient consent was received by the institution for the 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 signed manifest, chain of custody section with every collection. If tissue specimens from >5 locations from one patient are being sent, complete an additional separate shipping manifest; both will be labeled with the same RAP#.

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

| **Rapid Autopsy/Post-Mortem Specimens (viably cryopreserved)** | | | | | |
|---|---|---|---|---|---|
| **Vial Label** (Exactly as written on label) | **Vial Contents** (Site of Resection) | **# Vials** | **Box** (e.g., 1 of 1, 1 of 2, etc.) | **Vial Position in Box** (A1, B2, etc.) | **Tissue Type** |
| | | | | | ☐ Primary Tumor Site<br>☐ Metastatic Site<br>☐ Unknown |
| | | | | | ☐ Primary Tumor Site<br>☐ Metastatic Site<br>☐ Unknown |
| | | | | | ☐ Primary Tumor Site<br>☐ Metastatic Site<br>☐ Unknown |
| | | | | | ☐ Primary Tumor Site<br>☐ Metastatic Site<br>☐ Unknown |
| | | | | | ☐ Primary Tumor Site<br>☐ Metastatic Site<br>☐ 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 | Number of Vials | Signature | Date |
|---|---|---|---|---|
| 1. Shipment of tumor specimens (dry ice) | Clinical Center | Total # Shipped: _____ | | / / |
| 2. Portion of specimens received by PDC in vivo lab for implantation. Mark N/A if not provided to lab. | Receiving Personnel | Total # Received: _____ | | / / |

**Important:** If multiple **different** patient specimens are being sent in the same cryovial box, separate shipping manifests must be completed for each patient. Leave one empty row in the cryovial box between the different patient specimens.

## 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 be 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<br>□ Current | |
| 2 | | | | □ Prior<br>□ Current | |
| 3 | | | | □ Prior<br>□ Current | |
| 4 | | | | □ Prior<br>□ Current | |
| 5 | | | | □ Prior<br>□ Current | |
| 6 | | | | □ Prior<br>□ Current | |
| 7 | | | | □ Prior<br>□ 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: 81-SLOT BOX LAYOUT**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 | A9 |
| B1 | B2 | B3 | B4 | B5 | B6 | B7 | B8 | B9 |
| C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | C9 |
| D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 |
| E1 | E2 | E3 | E4 | E5 | E6 | E7 | E8 | E9 |
| F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 |
| G1 | G2 | G3 | G4 | G5 | G6 | G7 | G8 | G9 |
| H1 | H2 | H3 | H4 | H5 | H6 | H7 | H8 | H9 |
| I1 | I2 | I3 | I4 | I5 | I6 | I7 | I8 | I9 |

## APPENDIX 4: 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-derivedcell 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 the 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, HBSS, or PBS 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.