

Fresh Tumor Collection and Handling for Generation of Patient-Derived Models		
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## 1.0 PURPOSE/SCOPE

This Standing Operating Procedure (SOP) describes the procedures for collecting, preparing, and shipping fresh core needle biopsies or fresh tissue specimens from Clinical Centers for use in patient-derived model (PDM) generation such as patient-derived xenografts (PDX) and primary tissue in vitro cell models. This SOP was developed by NCI-F/FNLCR in collaboration with the Developmental Therapeutics Clinic, National Cancer Institute and NCI-designated Cancer Centers participating on an NCI multicenter Tissue Procurement Protocol. This SOP is used/performed by the Clinical Centers collected patient blood samples and the Biological Testing Branch (BTB) at NCI-Frederick, Frederick National Laboratory for Cancer Research.

## 2.0 SAFETY

All fresh human tissue, including whole blood and its purified components, are handled under Biosafety Level 2 (BSL2) conditions. All work is conducted in a biological safety cabinet (BSC) using personal protective equipment and avoiding the use of sharps where possible. All materials potentially exposed to the human material is treated with a 10% bleach solution for a minimum of 10 minutes, double bagging for autoclaving or incineration. Consult with your facility safety professionals regarding the safe handling of BSL2 studies.

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### 3.0 MATERIALS & EQUIPMENT

#### 3.1 Tissue Collection

- 3.1.1 Ice bucket with wet ice for transportation of specimen jars
- 3.1.2 Sterile glass microscopy slides/petri dish (optional, surgical suite)
- 3.1.3 Sterile, one-time use tweezers (optional, surgical suite)
- 3.1.4 Sterile, 50-mL wide-mouthed Nalgene jar containing sterile CO<sub>2</sub> Independent Medium (Gibco, Cat#: 18045-088) \*Store at 5°C±3°C.
  - Include antibiotics in media. Recommend either 30 µg/mL Zosyn or 100 µg/mL primocin; selected due to common use in patients undergoing chemotherapy. For tissue from primary head & neck, vaginal, and bladder cancers, the addition of fungizone is also recommended.
  - Due to addition of antibiotics, an expiration date of 30-days should be applied to the media.

#### 3.2 Shipping

- 3.2.1 Two temperature-controlled gel packs. (See SOP Step 5.2.2 for storage temperature)
- 3.2.2 Temperature Monitors for shipping
  - The NCI PDMR uses TempTale4 monitors (Sensitech)
- 3.2.3 Specimen jar labels
- 3.2.4 Absorbent material
- 3.2.5 Parafilm
- 3.2.6 Bubble wrap
- 3.2.7 Two ZipLock bags
- 3.2.8 Styrofoam box
- 3.2.9 Shipping cardboard box

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## 4.0 OPERATING PROCEDURES

### Important:

- Processing limitations:
  - Tumor specimens should be an 18-g needle biopsy or resected material at least 4x4x4 mm in size; 4 mm is equivalent to the thickness of two nickels stacked together.
  - The maximum size for an individual piece placed in the media should be 9 mm (3/8 in) in its longest dimension.
  - The ratio of transport media to tissue sample should be at least 10:1.
  - Use of surgical markers is common to outline the area of tissue for resection; however, this marker is often toxic to cells. If used, be sure shipped tumor specimen does not have marker dye.
- Tumor tissue specimens **MUST** be placed in pre-chilled media jars and shipped with pre-chilled cold packs. See Appendix 2 for examples of shipment using -20°C cold packs versus 4°C cold packs mid-summer; target temperature is 0°-8°C.
- All samples **MUST** be shipped on day of collection and received within 24-h of collection at the processing laboratory for implantation into mice.

### 4.1 Day of Tumor Tissue Collection, Just Prior to Specimen Receipt

- 4.1.1 Prepare a specimen tube label with the specimen ID.
- 4.1.2 Take a sterile wide mouth jar with media from the cold room/refrigerator where it is stored and apply a label. Keep the jar on ice.
- 4.1.3 Leave the cold packs in the cold room/refrigerator or freezer (as designated) until ready to package the specimen for shipping.
- 4.1.4 If shipping material, ensure that overnight shipping has been arranged.

### 4.2 Fresh Tumor Tissue Collection

- 4.2.1 Clinical Specimen Support Lab Personnel should arrive at the collection site early enough to allow sufficient time to set up supplies, collect relevant clinical information, and ensure rapid transport of specimens to the laboratory for preparation for shipping.
- 4.2.2 At the surgical suite, one labeled, pre-chilled, sterile jar containing media should be ready for specimen collection (on wet ice in ice bucket or maintained at 5°C±3°C). Sterile glass slides and tweezers may be used in the surgical suite specimen preparation areas to aide in transfer of the fresh tissue to the jar.
- 4.2.3 During specimen collection, **two key steps to successful** generation of PDMs will be (1) minimizing the time between specimen collection and placement into the chilled, sterile media and (2) ensuring sterility of the specimen and media during this process.

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#### 4.2.4 **18/g Needle Biopsy collection:**

1. The preferred collection method is for the interventional radiologist/surgeon to place the biopsy directly into the chilled, sterile media solution.
2. The interventional radiologist/surgeon can place the biopsy onto a sterile slide/petri dish (for optimal collection the slide/petri dish should be pre-chilled). The tissue should then immediately be walked into the sample preparation area and placed into the chilled, sterile media solution. Use a sterile tweezers to transfer tissue into the chilled, sterile media solution.

#### 4.2.5 **Resected Tissue Collection:**

We understand that collection of resected tissue into the sterile, chilled media jar may not be possible in the surgical suite.

3. If the tissue is at ambient temperature for longer than 30 minutes following excision, make a note in the Shipping Manifest (Appendix 1) of the approximate time between collection and placement in the chilled media jar.
4. Sterility issues with transported, resected tissue. Assume the exterior of the tissue is no longer sterile. Use a sterile scalpel or scissors and excise tumor pieces (maximum 9 mm/side) from the interior of the resected tissue and transfer to the sterile media jar with a sterile tweezers.
5. Use of surgical markers is common to outline the area of tissue for resection; however, this marker is often toxic to cells. If used, be sure the shipped tumor specimen is free of marker dye.

## 5.0 **PACKAGING OF TUMOR SPECIMENS FOR OVERNIGHT SHIPMENT**

### **Important:**

- For overnight shipping: All tumor specimens should be shipped on the day of collection and received within 24-h of collection at the processing laboratory for implantation into mice.

**5.1 Complete a Shipping Manifest** ([Appendix 1](#)) for each specimen collected on the day of shipment.

### **5.2 Packaging Instructions**

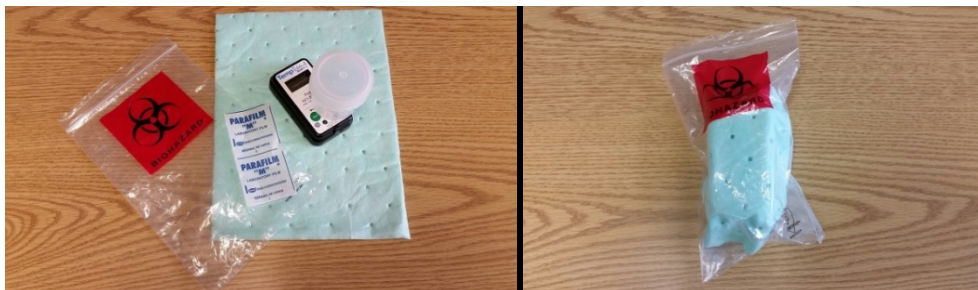
- 5.2.1** Just before packaging, open the Tumor Shipping kits, and remove the gel packs from the freezer or refrigerator.
- 5.2.2** Guidelines for gel pack shipping temperatures are outlined below based on 3-years of temperature monitoring by the PDMR of shipments from across the U.S. and Canada ([Appendix 2](#)).

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

**5.2.3** The packing process should go quickly – do not leave contents at room temperature for a prolonged time.

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- 5.2.4** Turn the lid tightly on the tissue jar then seal the lid with parafilm. Set the tissue jar directly on top of an activated temperature monitor and wrap both items in the absorbent material and then seal them in a ZipLock bag labeled with a biohazard sticker.



- 5.2.5** Wrap the ZipLock bag in bubble wrap.
- 5.2.6** Place one gel pack on the bottom of the Styrofoam shipping box (reference guidelines in 5.2.2 for placement location and temperature of gel packs). Set the bubble-wrapped ZipLock bag containing the tissue jar and temperature monitor on top of the first gel pack, and then place the second gel pack on top.



- 5.2.7** Place the completed **Shipping Manifest** with the Chain of Custody section signed on line #1 ([Appendix 1](#)) in a sealed zip lock bag and then place on top of the gel packs. Close the Styrofoam box and then seal the cardboard shipping box.



### 5.3 Shipping Instructions

- 5.3.1** Once the cardboard box is sealed, attach a return shipping label to the outside of the box; **do not obscure the UN3373 label.**
- 5.3.2** **Important:** Patient samples that are being shipped are “live” specimens. Do not leave specimen boxes on shipping docks for prolonged lengths of time as a severe temperature drop or rise could compromise the viability of the patient specimens.

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## APPENDIX 1: SHIPPING MANIFEST AND CHAIN OF CUSTODY

<b>Specimen number</b>
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### 1. Shipping Manifest

Include a copy of the manifest and signed, chain of custody section with every patient-derived model collection.

To be completed by the Clinical Center						
Tissue Type	Collection Date	Collection Time with time zone	Patient Gender	Site of Biopsy/Resection	Histological Diagnosis (e.g., Colon ca, Breast ca, etc.)	CTEP SDC code*
<input type="checkbox"/> Biopsy <input type="checkbox"/> Resection <input type="checkbox"/> _____		<input type="checkbox"/> EDT <input type="checkbox"/> CDT <input type="checkbox"/> MDT <input type="checkbox"/> PDT	<input type="checkbox"/> Male <input type="checkbox"/> Female			

\*[http://ctep.cancer.gov/protocolDevelopment/docs/SDCv10\\_M10.xls](http://ctep.cancer.gov/protocolDevelopment/docs/SDCv10_M10.xls)

### 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 (cold packs)	Clinical Center		/ /
2. Receipt of specimen: log receipt, verify specimen(s), and verify shipping conditions. If deidentification is required, remove provided ID label and replace with anonymized research ID.	Research Site: Receiving/ Honest Brokers		/ /
3. Receipt of specimen for research use.	Research Site: Laboratory		/ /

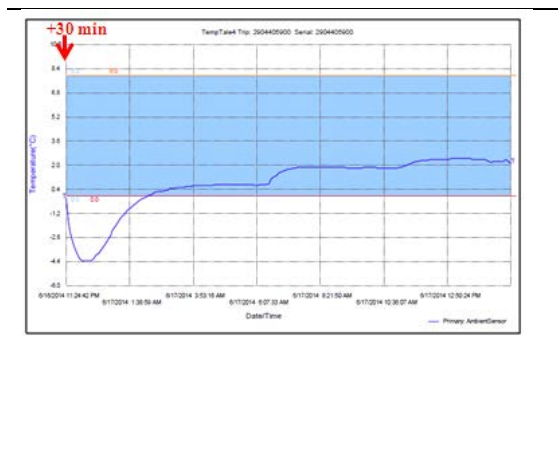
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## APPENDIX 2: OVERNIGHT SHIPPING TEMPERATURE MONITORING

Target Shipping temperature to obtain viable tumor tissue: 0°C-8°C. Temperature monitors used begin recording temperatures 30-min after activated.

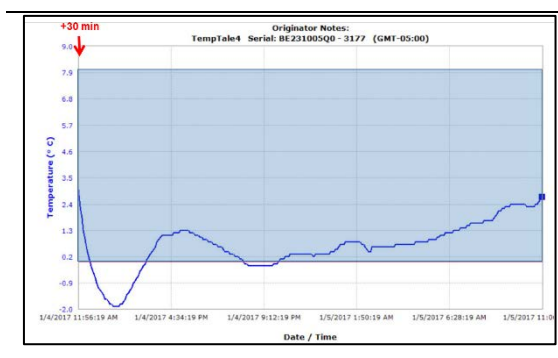
### 1. Summer Shipment: Two -20°C gel packs (recommended shipping conditions)

- California to Maryland: June
- CA regional temp max, 24°C
- MD regional temperature max, 33°C
- 15 h 45 m monitoring period, 189 data points
- Min: -4.3°C
- Max +2.5°C
- Temperature was <0°C for less than 3 hours; given the time it would take to cool 50-mL media to <0°C and then cool the tumor sample we deemed these shipping conditions optimal.



### 2. Winter Shipment: One 4°C and one -20°C gel pack (recommended shipping conditions)

- Iowa to Maryland: January
- IA regional temp min overnight, -9°C
- MD regional temp min overnight, 1°C
- 23 h 17 m monitoring period, 279 data points
- Min: -1.9°C
- Max +3.0°C



### 3. Example: Summer Shipment with two 4°C gel packs (NOT recommended)

- California to Maryland; June
- CA regional temp max, 29°C
- MD regional temperature max, 33°C
- 17 h 35 m monitoring period, 211 data points
- Min: +9.3°C
- Max +19.3°C
- Even with a 30-min delay in recording time, the internal air temperature of the shipping box never reached below 8°C.

