SOP_MTL-1.1 Processing Solid Patient Tissue Specimens

A. Purpose: To provide aseptic techniques and instructions to process solid patient tissue specimens for the generation of viably frozen, snap frozen, and FFPE specimen derivatives.

B. Scope: The procedure described here can be used to process all solid tissues, including bone (if it is soft enough to be cut with a scalpel) from human origin.

C. Definitions:

Derivative: Each of the aliquots generated from the original tissue specimen collected from a tissue donor

DMEM: Dulbecco's modified Eagle medium

DMSO: Dimethyl sulfoxide

EtOH: Ethanol

FBS: Fetal bovine serum

FFPE: Formalin-Fixed Paraffin-Embedded

Formalin: 10% neutral buffered formalin

Freezing media: 50% FBS, 40% DMEM, 10% DMSO

RPMI: RPMI-1640 - Cell culture medium developed by Moore et al. at Roswell Park Memorial Institute

D. Materials and Reagents: In quantities to process one patient sample

Name	Quantity	Cat number	Sterility status for use
1000 µL Pipette tips (+pipette)	1-3	1000 µL: 05-403-18, Eppendorf	Sterile
2 mL Cryovials	1-10	W985865, Wheaton	Sterile
70% Ethanol spray bottle	1	LC222102, Fisher scientific	Non-sterile
Cutting board/petri dish	1	NA / FB0875712, Fisher scientific	Clean with bleach and 70% EtOH/Sterile
Formalin	15 mL	22-050-105, Fisher scientific	Non-sterile
Formalin container	1	Any container with a sealed lid	Non-sterile
Freezing media	2-10 mL	SOP_MTL-1.2 Freezing Media	Sterile
Glass Pasteur pipets	1	22-230-482, Fisher scientific	Sterile
Human tissue specimen	1	NA	Non-sterile
Labels	1-10	B-490, Brady	Non-sterile
Liquid nitrogen + thermo-flask container	Enough to cover	11-670-25C, Fisher scientific	Non-sterile

	tubes		
Paper towels	Several	10714-002, VWR	Non-sterile
Razor blade/scalpel	1	55411-050, VWR	Sterile
Regular forceps	1	RS-5139, Roboz Surgical	Sterile
Securline marker (ethanol resistant)	1	14-905-30, Fisher scientific	Non-sterile
Slow freezing container	1	Nalgene cryo-freezing, Biocision CoolCell	NA
Specimen paperwork	1	NA	NA
Tape	1	15-901-10R, Fisher scientific	Non-sterile
Tissue cassettes	1	89199-446, VWR	Non-sterile

E. References:

SOP_MTL-1.2 Freezing Media

F. Procedures:

General Considerations:

- Samples are usually delivered in 15 mL tubes with RPMI. Keep the sample at 4°C until ready to process it.
- Review the paperwork that comes with the sample to be sure the barcode on the tube matches the information on the paper.
- Sample processing should be done inside the cell culture hood, following standard sterile procedures.
- Based on tissue availability the priority to generate the different derivative types is as follows: viably frozen > snap frozen > FFPE.
- The priority for processing the tissue is as follows: snap frozen > viably frozen > FFPE.
- One derivative for viably frozen tissue = 12-13 tissue fragments (1-2 mm^3 each).
- One derivative for snap frozen tissue = 1-4 tissue fragments (1-2 mm^3 each). This tissue is only useful for DNA extraction.
- One derivative for FFPE = 1 nickel-thick cross-section. If the tissue is very large then two sections may be collected in one cassette.
- In order to keep the tissue as sterile as possible, all steps will be done inside the cell culture hood. Use aseptic techniques throughout the procedure. All items going into the cell culture hood should be sprayed with 70% ethanol and wiped down with a paper towel.

Special considerations for each pathological type of tissue specimen:

a) Tumor and Metastatic Samples:

i. Aim to get as many viably frozen derivatives as possible.
ii. If there is enough tissue, make snap frozen derivatives. Example: there is enough tissue for two viably frozen derivatives and a small amount left over, snap freeze the left-over tissue.
iii. While processing the sample remove grossly fatty areas.

b) Breast Normal:

i. All normal tissue should be processed as viably frozen derivatives.
ii. The collected tissue fragments can be larger than the usual 1-2 mm^3.
iii. While processing the sample remove any black/cauterized tissue.

c) Skin Normal:

i. All normal skin samples should be processed as snap frozen derivatives.
ii. If the pathological status (tumor vs normal) is not clear, consult with the core director before processing the sample.

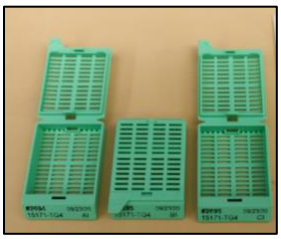
1. Crosscheck each tube barcode against the paperwork to be sure that they match. Do not process the specimens until all discrepancies are clarified.
2. Evaluate the size of the specimen and determine how many cryovials are needed and whether there will be sufficient material for an FFPE derivative.
3. Place the appropriate amount of freezing media in the 37°C bead bath.
4. Using the vacuum and glass Pasteur pipettes in the hood, vacuum off the media from the tissue specimen tube (be **very** careful not to aspirate any of the specimen).
5. Transfer all of the tissue to the cutting board/petri dish by carefully tilting the tube. Use forceps to remove any tissue stuck in the tube.
6. Cut the tissue into fragments (1-2 mm^3 each). Cut a nickel-thick cross-section of tissue for FFPE if there is enough tissue.
7. Process the tissue by derivative type:
   - 7.1. Snap frozen:
     - 7.1.1. Place the tissue fragments individually around the inner wall of a Cryovial.
     - 7.1.2. Add a label and place it directly into the liquid nitrogen (tissue needs to be media-free).
     - 7.1.3. Transfer to the appropriate box for storage at -80°C.
   - 7.2. Viably frozen:
     - 7.2.1. Aliquot 12-13 tissue fragments (1-2 mm^3 each) into the appropriate number of Cryovials.
     - 7.2.2. Add 1.25 mL of freezing media to each Cryovial and add a label. Be sure that all tumor pieces are in the media and not stuck to the side of the tube.
     - 7.2.3. Place the Cryovials in the slow freezing container.
     - 7.2.4. Place the slow freezing container at -80°C overnight. Transfer the viably frozen tissue vials to liquid nitrogen storage within one week.
   - 7.3. FFPE:
     - 7.3.1. Place the tissue in a pre-printed tissue cassette.
     - 7.3.2. Place the cassette in a container with formalin.
     - 7.3.3. After the tissue has been in formalin for 14-24 hr., pour the formalin into the proper waste container, blot off remaining formalin, and refill the container with 70% EtOH.
8. Fill out the tissue specimen paperwork with the amount and type of aliquots (viably frozen, snap frozen, FFPE) made from the human specimen.
9. Collect all supplies and clean the cell culture hood with 70% EtOH.
10. Place the forceps in the dirty tool bin.

11. Place the cutting board in 10% bleach.
12. Store remaining freezing media at -20°C.

G. Revisions log:

Version	Revision Date	Section Revised	Notes
1	10.23.2020	All	SOP created
2	02.03.2021	D	Format updated

H. Appendix:



H.1 Pre-printed tissue cassette.