

Tissue Acquisition and Processing

SOP 400-10

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	Signature	Date
Originator/Reviser:	_____	_____
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SOP for the UACC Biospecimen Repository	
Kepler	

1. PURPOSE & SCOPE

To define procedures relating to the collection, processing and transport of biospecimens from Banner University Medical Center operating room (OR) to the UACC Biorepository laboratory. Procedures are designed to minimize variability and optimize the collection and preservation of high quality biospecimens.

2. DEFINITIONS

TissueMetrix (AIM) is the biospecimen information management system administered through the University of Arizona Cancer Center.

3. REFERENCES

- SOP 400-5 Steps to Enroll Subjects and Bank Biospecimens
- SOP 400-9 Information Management
- SOP 400-XX Tissue processing & Embedding
- SOP 400-XX H & E Staining
- “Bloodborne Pathogen Exposure Control Plan.” University of Arizona. Revised August 2003. Reference 29 CFR 1910.1030.
- SOP 400-10 Appendix I – Collection Contacts
- SOP 400-10 Appendix II – Transport cart checklist

4. RESPONSIBILITIES

- 4.1. TACMASR Laboratory research personnel who are preparing samples for banking are responsible for following the procedures in the SOP and confirming that each step has been properly executed.
- 4.2. Deviations are to be documented on the Collection Event Form.
- 4.3. Unexpected events are to be reported to the Coordinator, Lab Manager, and TACMASR Director.
- 4.4. The Coordinator and Lab Manager are responsible for training and daily oversight of personnel who perform tissue preparation procedures.

5. SAFETY AND CAUTIONARY NOTES

- 5.1. Universal precautions and sterile technique should be followed. At minimum this should include the use of gloves, eye protection and lab coat.
- 5.2. All equipment and surfaces, forceps, dissecting board, that come in contact with tissue must be decontaminated with Converge upon the completion of tissue processing.
- 5.3. Used scalpels should be disposed of in the sharps container located on the transport cart.
- 5.4. Scrubs must be worn to enter the OR. Before entering the OR suite ~~Once in the frozen section room~~ a disposable surgical cap, mask and shoe covers are available to don before entering the actual OR suite.
- 5.5. Acquisition of tissue is prioritized as follows:
 - 5.5.1. Frozen OCT blocks,
 - 5.5.2. Other institutional studies
 - 5.5.3. Formalin-fixed paraffin embedded (FFPE) tissue blocks.
- 5.6. Tissue can only be grossed and handed off to Biorepository personnel for banking by a pathology assistant, pathology resident or pathology attending.

- 5.7. Tissue should be snap frozen as soon as possible to preserve the morphology, protein biomarkers and RNA.
- 5.8. Each tissue specimen is labeled with the collection bar code.
- 5.9. Complete the corresponding collection event log.

6. MATERIALS AND EQUIPMENT

- 6.1. TissueMetrix Collection Event form and the sequential Collection ID barcodes that are linked to the PTID. Refer to SOP 400-9: Information Management for details regarding the TissueMetrix labeling system.
- 6.2. Collection Cart
- 6.3. Lead marking pencil, permanent marking pen, forceps (VWR or equivalent)
- 6.4. Metal Dewar bowl and stainless steel beaker
- 6.5. Dewar flask filled liquid nitrogen
- 6.6. Dry Ice in a Styrofoam box
- 6.7. Isopentane (2-methyl-butane) VWR JTQ223-8
- 6.8. OCT compound and cryomolds (Leica)
- 6.9. Specimen bags (Bitran)
- 6.10. 1.5ml cryovials (USA Scientific)
- 6.11. RNAlater (Ambion)
- 6.12. Igloo cooler
- 6.13. Styrofoam box with wet ice

7. COLLECTION PROCEDURE

- 7.1. Patients are consented in clinic at their pre-surgery appointment. Clinic staff will notify the Biorepository of consented patients with their surgery date. Time of surgery can be determined by viewing the OR schedule within ~~EPIC~~. Cerner
- 7.2. Obtain a new Collection Event Form, Biospecimen Labels and current Box Logs in preparation for a potential collection. Refer to SOP 400-9 that describes the use of the collection event form.
- 7.3. Prepare the transport cart. Fill a Styrofoam box with dry ice, located in the cryobox in room 0915. Fill the liquid nitrogen Dewar with liquid nitrogen, located in room 0915. Ensure the working solution of Isopentane, kept in a glass bottle on the freezing cart, has at least 150ml of solution in it. It can be replenished when low from the stock bottle of Isopentane stored in the flammables cabinet under the fume hood in room 0915.
- 7.4. Verify the cart contains the rest of the items needed for collection. See Appendix II for details.
 - 7.4.1. Monitor when a surgical procedure has started from the OR status board within ~~EPIC~~. Cerner Once the surgery has started, telephone into the appropriate OR room to alert the circulating nurse know the patient is consented for tissue collection. When calling in, state your name, that you are from Tumor Bank, the physician's name, and the patient's name. Give the OR nurse the pager number, ~~8577.531-5974~~
- 7.5. When the OR pages that the specimen is ready for pickup, note the time of page on the collection event form. Call the OR to verify that you received their page, the tissue has been excised and you will be in to pick it up.
- 7.6. Proceed with the transport cart to the frozen section room. Once inside there, prepare to enter the OR by donning protective apparel in the following order: surgical cap, surgical mask and shoe covers. Enter the OR suite and go directly to the specified OR room.
- 7.7. Cautiously enter the operating room. Do not touch anything sterile. Stand well back from the operation in progress, as close to the perimeter wall as possible. Locate the circulating nurse. He or she will hand over the tissue in a biospecimen container. Verify the label on the container is the correct patient and specimen.

- 7.8. Exit the OR suite and return to the frozen section room. Record the time of tissue collection on the collection event form.
- 7.9. If the surgeon has called for a frozen section, a pathology resident will have been called by the OR nurse. In this case, tissue for banking may be obtained there in the frozen section room AFTER the frozen sectioning is completed and the Pathologist or Resident hands off the tissue designated for banking. If no frozen section has been called for, place the specimen container in the bottom drawer of the transport cart and proceed to the morgue for grossing.
- 7.10. Pathology will determine if there is enough tumor for banking. The size of the specimen provided to the biorepository depends on the amount and specimens banked. Tissue pieces should be no larger than 5mm thick by 20mm wide for optimal fixation (snap freezing or formalin).
- ~~7.11.~~ Tissue specimens are prioritized as follows: OCT frozen tissue, FFPE, Fresh frozen.
In addition to the tumor specimen, a matching normal piece of tissue can be requested if possible.
- 7.12. As soon as the tissue specimens have been received (in the Frozen Section Room or the Morgue), proceed to process using materials on the transport cart. Place the piece of tissue on a small section of modeling wax and lay that on the dissecting board. Using a sterile scalpel, cut the tissue into sections if it is large enough to divide for freezing and fixing in formalin. If not freeze the entire piece in OCT.

Procedure to freeze tissue in OCT

- Pre-chill the isopentane by filling the metal dewar bowl half way with liquid nitrogen. Pour isopentane into the metal beaker so it's one third full. Set the metal beaker into the dewar bowl. Isopentane begins to thicken after 1-2 minutes forming a syrupy consistency (approximately 150 °C. It will turn white on the bottom of the beaker when chilled and ready for freezing.
- Label one cryomold and one Bitran Ziploc specimen bag for each tissue specimen banked. Use alcohol resistant marker pens to label. Place the specimen bag on dry ice for pre chilling.
- Bend the labeled end of the cryomold so that it can be picked up with the forceps.
- Place a small amount of OCT in the bottom of the cryomold then place the tissue section in the cryomold.
- Fill the cryomold with OCT compound avoiding trapping air bubbles in the OCT.
- Gently lower the tissue into the chilled isopentane.
- Leave the cryomold in the isopentane for approximately 30 seconds until frozen.
- Remove and place on dry ice while popping the frozen block out of the cryomold. Place the frozen OCT block in the pre-labeled and pre chilled specimen bag. Keep surrounded by dry ice for transport back to the biorepository and transfer to a -80 °C freezer.
- Record the time frozen on the collection event form as well as the sample type and tissue.
- Dispose of the scalpel in the biohazard container on the transport cart. The used piece of dental wax should be disposed of in the biohazard container in pathology.
- Pour the remaining liquid nitrogen back into the liquid nitrogen dewar. Pour the isopentane back into the glass bottle.
- Spray the forceps and dissecting board with Converge spray located in pathology.

Procedure to collect tissue in the new Banner hospital

- **Prepare the Igloo cooler , styrofoam box with wet ice, cryomolds, marker, OCT compound**
- **When paged, proceed to Banner Tower 1. Drop the Igloo cooler off in pathology.**

- Outside of pathology and right before entering the OR suite are masks, hat and shoes covers. Don these.
- Enter the OR suite and locate the OR of the surgery.
- Enter the OR and retrieve the specimen container from the circulating nurse at the nurses station.
- Take the container & tissue back to pathology for grossing.
- Once in pathology, don a protective coat and eyewear. Record the time the tissue was collected.
- Pathology personnel will gross the tissue and determine if there is enough tumor for banking. The size of the specimen provided to the biorepository should be no larger than 5mm thick by 20mm wide for optimal fixation.
- Tissue specimens are prioritized as follows: OCT frozen, FFPE and then Fresh frozen.
- If possible obtain a matching piece of normal tissue
- The piece of tissue to be preserved as OCT frozen is placed into the cryomold, filled with OCT compound, then placed on the wet ice in the Igloo cooler.
- Return to the Cancer Center Biorepository lab and snap freeze the OCT preserved sample using the supplies on the transportation cart.

Procedure to fix tissue in formalin for paraffin embedding

- Using a lead pencil, label a tissue cassette with the collection number on the top and the tissue type on the right hand side.
- Place the piece of tissue in the cassette and lock the cassette lid in place.
- Fill a specimen cup 2/3 with 10% neutral buffered formalin and place the tissue cassette into it.
- Record the time and date placed in formalin on the lid of the collection cup and on the collection event form.
- Place a pink Buffered formalin sticker on the side of the specimen cup. These are located in pathology by the formalin container.
- Specimens should be fixed in formalin at room temperature between 5-24 hours. Do not leave in formalin over 24 hours, after this time the tissue is overfixed and the DNA will begin to fragment.
- Transfer the cassette to 70% ethanol and store in the specimen refrigerator in the biorepository lab. Specimens can be stored in alcohol for up to 7 days before processing.
- Record the date and time transferred from formalin to ethanol on the collection event log.

Procedure to preserve tissue in RNAlater

- 1.5ml cryovials are sterilely filled with 1.0ml of RNAlater and stored on the collection cart.
- After obtaining tissue from pathology, drop a piece of tumor tissue no larger than 5mm x 5mm into a cryovial labeled with the collection number, tissue type and date.
- Repeat the same process with normal tissue.
- Store the tissue on the cart until return to the TACMASR laboratory.
- Once in the laboratory store the RNAlater vials containing the tissue in the small refrigerator located on the counter next to the entrance door.
- Tissue should remain in the refrigerator for a minimum of 24hours and no longer than 5 days.
- After that time transfer the vials to -80 Freezer F in 0963 for long term storage.
- Record the collection number on the RNAlater log hanging on door of Freezer F.
- Record the box and slot of vials placed into the -80 on the collection event log.

Biorepository OPERATING PROCEDURE 400-10 Appendix I

COLLECTION CONTACTS

OR Front Desk	694-6120
Pre-op	694-2220
OR Rooms	964-72 694-31- - (last two digits of OR room#)
OR Room 17 (exception)	964-5858
Frozen Section room (1648)	694-6480
Morgue Pathology	694-6564
Paging system	694-4480
Biorepository Pager#	8577-531-5974

Biorepository Operating Procedure 400-10 Appendix II

TRANSPORTATION CART CHECKLIST

The following is a list of items that should be routinely stocked and stored on the cart:

- Forceps
- Modeling wax
- Dissecting board
- Permanent, alcohol resistant, Marker/Sharpie
- Lead Pencil
- Biotran specimen bags
- Shallow Dewar Bowl
- Stainless steel beaker
- Liquid Nitrogen Dewar Flask
- Specimen cups
- Scalpels
- Biohazard Sharps container
- RNAlater filled cryovials

- OCT cryomolds (standard size)
- OCT Embedding media
- Tissue Cassettes
- Gloves

The following items must be added to the cart on the day of collection:

- Dry Ice
- Liquid Nitrogen
- Isopentane

IGLOO COOLER CHECKLIST

- Styrofoam box with wet ice
- Cryomolds
- OCT embedding media
- Tissue Cassettes
- Specimen container
- Permanent marking pen
- Lead pencil