1. PURPOSE

1.1. The purpose of this procedure is to outline the process for collecting, processing and transporting the highest quality patient tumor, tissue, and/or blood specimens to the TCRB BioBank sites.

2. SCOPE

2.1 This procedure is applicable to all individuals who perform a function related to the procurement processing and shipment of any patient tumor/biopsy or blood sample that will be placed in the TCRB BioBank.

3. DEFINITIONS

3.1 ACQUIRE: TCRB's proprietary and secure database.

3.2 ASEPTIC PROCESS: Series of procedures that are performed under carefully controlled conditions and designed to prevent the introduction of or unintended transfer of microorganisms.

3.3 LN_2: Liquid Nitrogen, approximately -200°C

3.4 NBF: Neutral Buffered Formalin

3.5 OCT: (Optimal Cutting Temperature). Embedding media (Tissue-Tek) for cryopreservation and cryosectioning of tissues.

3.6 OR: Operating Room

3.7 SNAP FREEZE: Freezing tissue or cell pellet rapidly (optimally in of 10-20 seconds).

3.8 TCRB: Texas Cancer Research BioBank

3.9 TRANSPORT MEDIUM: A sterile isotonic solution into which a viable tissue sample is submerged during transport.

3.10 WET ICE: Crushed ice with a little water added to make slush.

3.11 W-G: Wright-Geimsa; hematology stain used on smears, used herein primarily on smears of cell concentrates from pleural and ascities fluids.

4. RESPONSIBILITY

4.1 The individual collecting and/or transporting the tumor tissue sample is responsible for:

4.1.1 Following the outlined process when collecting and transporting tumor tissue to the TCRB BioBank.

4.1.2 Contacting the appropriate personnel at the receiving BioBank site, for any concerns or issues that arise during the collection and transport of tissue.

4.2 The Clinical Sampling Site referring the patient for tumor tissue procurement is responsible for:

4.2.1 Ensuring the appropriate regulatory approvals are in place to allow for collection of any patient samples to be used for research purposes.

4.2.2 Obtaining and documenting the required IRB-approved, valid consent from the patient to allow for collection of tumor tissue sample for the TCRB BioBank.

4.2.3 Ensuring the appropriate materials transfer agreements are in place to allow for transfer of clinical tissue samples to the TCRB BioBank.

4.2.4 Deidentifying and alphanumerically encoding the patient specimens collected (tumor and normal tissue). This deidentification and encoding can occur through the use of sterile containers with barcodes and labels.

4.3 The TCRB BioBank site is responsible for:

4.3.1 Training individuals who will collect and transport tissue samples to the TCRB BioBank.

4.3.2 Ensuring the appropriate materials transfer agreements and regulatory approvals are in place with the Clinical Sampling Site to allow for transfer of clinical tissue samples to the Human Genome Sequencing Center's Nucleic Acids Core or other approved site.

4.3.3 Logging in the receipt of the tissue sample(s) in the TCRB site Clinical Sample Log and Acquire.

4.3.4 Confirming proper documentation of consent is available for each patient tissue sample collected.

4.3.5 Verifying that the tissue detailed in the written requisition matches the tissue received.

5. REFERENCES AND APPLICABLE DOCUMENTS

5.1 Flowchart for Tissue Processing Requisition, Procurement, and Transport (Attachment 1)

5.2 IATA Dangerous Goods Regulations. 51st Edition. 2010.

5.3 Center for Disease Control, National Institute of Health, and the US Department of Health and Human Services. Biosafety In Microbiological and Biomedical Laboratories, 5th edition, 2007.

6. MATERIALS AND METHODS

A. Tissue Collection Process

1.1 The flow of tissue from processing requisition to procurement and shipment is shown in Attachment 1.

1.1.1 Tissue Processing Requisition

1.1.1.1. When a Physician at a Clinical Site identifies a surgical candidate who is to have tumor tissue procured, the site should inform the collector / Biobank the tentative procurement via a written requisition and confirm processing can be done.

1.1.1.2. The requisition should identify the following information:

1.1.1.2.1 Patient name and demographic details

1.1.1.2.2 Patient diagnosis details

1.1.1.2.3 Scheduled surgery details

1.1.2 Once the requisition is received, feasibility and scheduling should be determined based on personnel availability.

1.1.3 Confirmation of acquisition scheduling should be relayed to the Clinical Site.

1.2. In the event that candidate tumor tissue first comes to attention during the day of surgery, and in particular upon the arrival of the specimen at the Pathology Department, one may follow the TCRB Protocol as described herein and attempt to obtain consent after the fact.

1.2.1. This requires prior approval of the local IRB protocol.

1.2.2. Obtained tissue must be incidental to non-research tissue acquisition and excess tissue beyond that needed for clinical care purposes. The obtained tissue must be quarantined such that it can be destroyed in the event that consent is not obtained over a defined time frame (e.g. 2 years).

1.2.3. Obtained tissue may otherwise be routinely processed as described in subsequent sections, as this is not research *per se* but rather specimen manipulation and stabilization.

1.3 Informed Consent

Informed consent for tissue collection will be obtained preoperatively from each study participant by the surgical team at the time of consent for operative intervention. The consent explicitly includes permission for DNA, RNA, and protein extraction, genomic sequencing, release of de-identified clinical information, post-treatment re-contact, and release of de-identified genomic and clinical information to scientific databases. The risks of participation including potential loss of confidentiality of genomic information and discomfort associated with blood collection will be included. Additionally, relevant HIPAA protections will be included in the patient consent. The TCRB consent can be found at http://txcrb.org/forms/Consent%20Form%20for%20Distribution.pdf.

1.4 Specimen De-identification

At the time of enrollment or at some point prior to tissue being entered into the TCRB BioBank, patients will be assigned an identification number that must be unique for the Sampling Site. This identifier will be associated with all tissue samples collected, as well as all data elements acquired.

B. Tissue Targets for Collection

There are three main types of tissues being collected from each patient: Blood, normal tissue and tumor. Blood is being collected by the PaxGene tube method (described herein) and normal tissue and tumor tissue are being collected for several different types of analytical processes and storage in the TCRB Banking system for later disbursement for research investigation. There are two main processes for cyropreserving tumor and normal tissue –Snap Freezing (detailed immediately below) and OCT Embedding. In addition, techniques for isolating and processing cells from ascites and pleural fluids are detailed. An additional key objective of the TCRB is to capture viable tissue for the establishment of tumor xenografts and cell lines (described herein). A pancreatic tumor specific processing procedure is given in Attachment 6.

C. Large/Small Tissue Snap Freezing Method

1.1. Materials*

MATERIALS REQUIRED	VENDOR	CATALOG #
1. Consent form	Local TCRB compliant	n/a
2. Gloves	Various	Various
3. Biohazard bags (two)	Various	Various
4. Plastic container (sterile)	Various	Various
5. Plastic containers (multiple, non-sterile)	Various	Various
6. Sterile Disposable Forceps (or use sterile instruments in OR)	VWR*	12576-934
7. Sterile Disposable Scalpels (or use sterile instruments in OR)	VWR*	21909-656
8. Cryovials	Various	Various
9. Protocol Prefilled Formalin container (two)	Fisher Scientific*	23-032-059
10. RNAlater: RNA Stabilization Solution	Applied Biosystems	AM7021
11. Transfer Pipettes	Various	Various
12. Styrofoam coolers	Various	Various
13. Dry ice	Various	Various
14. Isopentane, Absolute Ethanol or other solvent, ACS Grade	Various	Various
15. Metal tray (or 500mL metal beaker T1062)	VWR*	62687-027
16. Crucible Tongs / Long Metal Forceps	VWR*	62460-000
17. Funnel	Various	Various
18. Freezer storage container for vials	Various	Various

* or equivalent

** LN2 may be substituted for Solvent / Dry Ice Freezing Bath or similar, depending on local guidelines. The solvent used may be absolute (100%) ethanol, 100% methanol, acetone or isopentane as long as it is at least ACS grade and has not becoming hydrated (water saturated from use; limit use of an alcohol to one month or ten freeze cycles, which ever comes first). In many institutions absolute ethanol will be preferred as it is the least toxic and easiest to handle, dispose of and transport. If using a metal beaker for freezing (#15 above) only about 100 - 150mL solvent is necessary for freezing. Any of these solvent dry ice baths with achieve a temperature of about -70°C.

1.2. Staff Notification

1.2.1. The tissue collection staff will be notified at the time of initial incision, and will be present in the operating room at completion of resection for transport of the specimens from the operating room to pathology suite for immediate processing.

1.3. Tissue Collection and Storage (see also Figure 1 and Flowcharts 1and 2 below as well as Attachment 5: Step-by-Step Photo Explanation of Specimen Collection and Processing)

1.3.1. Cryovials are labeled to indicate patient, tissue type, and preservation mechanism according to the standardized de-identified nomenclature.

1.3.2. The specimens are immediately transported by tissue collection personnel to pathology for processing.

1.3.3. The specimen is serially sectioned longitudinally into 1 cm slices. If the tissue is smaller this will not be necessary.

1.3.4 Using a new sterile disposable scalpel blade, the surplus tumor specimen will be divided into sections no larger than 1cm x 1cm x longest dimension. If multiple rectangles of tumor are produced, they will be arbitrarily labeled as tumor section A (TA), tumor section B (TB), etc....

1.3.5 A ~0.2 cm-thick (endpiece) section will be removed from each long end of a tumor specimen rectangle. The tumor specimen rectangles will then be sectioned at 0.5-cm intervals, producing approximately 1x1x0.5 cm sub-specimen cubes. As with the endpieces, a ~0.2 cm-thick intervening section will be cut between each 0.5-cm tumor section. The 0.2 cm (histology) and 0.5 cm (Biobank) sections will thus alternate.

1.3.5.1 Once sectioning is completed, each tumor rectangle will have been divided into alternating 1x1x0.5 cm cubes and 1x1x~0.2 cm sections (including endpieces).

1.3.5.2 Tumor cubes will be labeled in series as TA1, TA2, TA3, etc... if they were produced from the TA specimen; the same will be true for TB, TC, etc....

1.3.5.3 End sections will be labeled as TA1 and TAn (where n is the last number in the series)

1.3.5.4 Intervening sections will be labeled as TA1-2, TA2-3, TA3-4, etc..., depending on between which 2 tumor cubes the section was taken.

1.3.6 Each endpiece/interval section will be placed in an individual, appropriately-labeled container with at least 20 mLs of 10% neutral-buffered formalin for subsequent paraffin fixation, slide preparation and TCRB pathologist review as part of the specimen characterization and documentation. It is acceptable to take 0.5-cm thick endpieces and interval sections in the step above, divide them and submit one piece as part of the diagnostic case material while placing the other piece in an individual, appropriately-labeled 20cc container of 10% neutral-buffered formalin for inclusion in materials shipped to TCRB (for subsequent paraffin fixation, slide preparation and TCRB pathologist review), if required by local Pathology Department practice/protocol.

1.3.7 Order of Specimen Processing: To ensure that ample material is available for DNA sequencing, the first 2 tumor samples (A1 and A2, e.g.) will be snap-frozen entirely and without further processing in appropriately-labeled individual containers. If there is additional material (i.e. there are 3 or more blocks of tumor specimen), the remaining cubes of tumor tissue will each be transversely sectioned ("breadloafed") into 3 equal parts, each measuring ~1x1x0.2cm (i.e. similar to the interval sections placed in formalin), cut into 4 - 0.5x0.5cm pieces and placed into separate containers as follows:

1.3.7.1 One 2cc cryovial to be flash frozen (DNA sample).

1.3.7.2 One 2 cc screw-top tube containing L-15 (preferred), RPMI-1640, DMEM or other suitable culture medium (TXCCR sample).

1.3.7.3 One 2cc cryovial containing 1cc of RNAlater RNA stabilization Solution (RNA sample).

1.3.8 Paired normal tissue, if available, is processed as above with the following modifiers:

1.3.8.1 A 1cm x 1cm x 4cm (or less) section of grossly normal/non-tumor tissue, approximately 2 cm from the nearest tumor margin if possible, is removed.

1.3.8.1.1 Normal tissue should be as similar as possible to presumed tumor tissue of origin. For example, in colon cancer, the normal tissue should be from the adjacent colonic mucosa and not from the pericolonic fat.

1.3.8.2 End and interval segments are produced and labeled as above, yielding approximately 1x1x0.5 cm cubes of normal tissue and intervening/end sections.

1.3.8.2.1 There will only be one normal tissue section, labeled NA (Normal Tissue, section A), and only up to 4 subsections labeled NA1, NA2, NA3 and NA4.

1.3.8.2.2 If intervening tumor sections are split between case material submission and TCRB submission as detailed above, this should also be done for normal sections.

1.3.8.2.3 The tissue is divided between DNA, TXCCR and RNA samples as described and prioritized above.

1.3.9 After a minimum 2-3 minutes in a snap-freezing medium (dry ice, dry ice-solvent or liquid nitrogen freezing bath, e.g.), flash frozen tissue will be transported on dry ice to -80°C freezers for long-term storage until required for sequencing.

1.3.9.1 Note time of freezing for each cryovial.

1.3.9.2 **Note on Method of Snap Freezing:** The choice of freezing medium is arbitrary and should be the optimized for conditions/requirements at each sampling site. The only requirement is that the tissue should be completely frozen in 30 seconds or less.

1.3.9.3 Should it be the preference of the local pathology department that this tissue does not leave the department until it has been determined that diagnostic tissue is present in the case material, then storage at -80C will be in Pathology until the case is signed out or the material is otherwise released by the attending pathologist on the case.

1.3.9.4 Other arrangements by which the tissue is transported to a TCRB -80C freezer but is sequestered and available to pathology if needed may also be acceptable to local pathology departments.

1.3.10 Tissue preserved in RNAlater RNA Stabilization Solution will be incubated at 4°C for 24-48 hours, after which time the supernatant is decanted, and the tissue is stored in an ultra low -80°C freezer. Note time of initial immersion in RNAlater for each cryovial.

1.3.11 After a minimum 2-3 minutes in a dry ice isopentane or liquid nitrogen freezing bath, flash frozen tissue will be transported on dry ice to -80°C freezers for long-term storage until required for sequencing.

1.3.12 Tissue for TXCCR is rapidly shipped to TXCCR according to the TXCCR shipping protocol.

1.3.13 **Note on Warm Ischemia Time:** Warm ischemia time (WIT), defined as [(time of freezing/RNAlater immersion) – (time of specimen removal from patient)], is to be kept as low as possible and recorded as noted above for each cryovial. WITs of > 1 hour are to be avoided if at all possible. WITs of 15-30 minutes are considered optimal.

1.3.14 **Note on Deviations from Above**: We anticipate that there will be deviations from these parameters. When these deviations occur, and time is of the essence, adhere to the spirit of the protocol by trying to sample the histology of material and reserve the majority remainder for frozen tissue first and RNA/TXCCR as available. Note the deviations precisely. TCRB personnel will be able to review this information and decide later whether it disqualifies the specimen. Our goal at this stage of the process is to secure as many specimens as possible for potential TCRB research, not to exclude them.

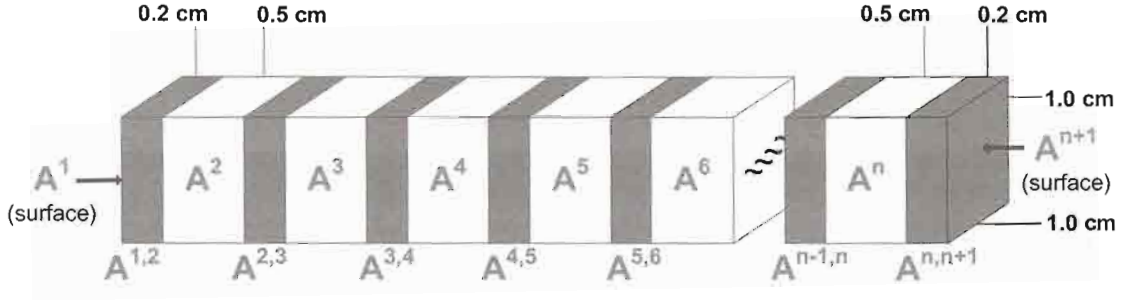
1.3.15 **Example 1: Several Fragments of Tissue**. It is possible that a single case's tissue fragments might add up to several centimeters, but consist of multiple, smaller fragments of tissue, none of which is individually 1 cm in any dimension. In this case, it would be

reasonable to label each fragment (A, B, C, etc...), take a single end-section for formalin fixation and histology (A, B, C, etc...), and submit the remaining specimens as frozen tissue (A, B) and divided among frozen, RNAlater and TXCCR (C, etc...), although if the sizes were too small one might reserve C exclusively for RNAlater and D exclusively for TXCCR, according to the submitting person's judgment.

1.3.16 **Example 2: Multiple Minute Fragments of Tissue.** It is possible that a single case's tissue fragments might add up to several centimeters, but consist of multiple small or discohesive fragments that are all less than 0.5 cm in any dimension. In this case, it would be reasonable to randomly submit multiple small fragments in formalin for histology and submit the remaining majority as frozen tissue, with RNAlater and TXCCR submitted if the amount of tissue was sufficient.

Figure 1: Large Tumor - Snap Freeze

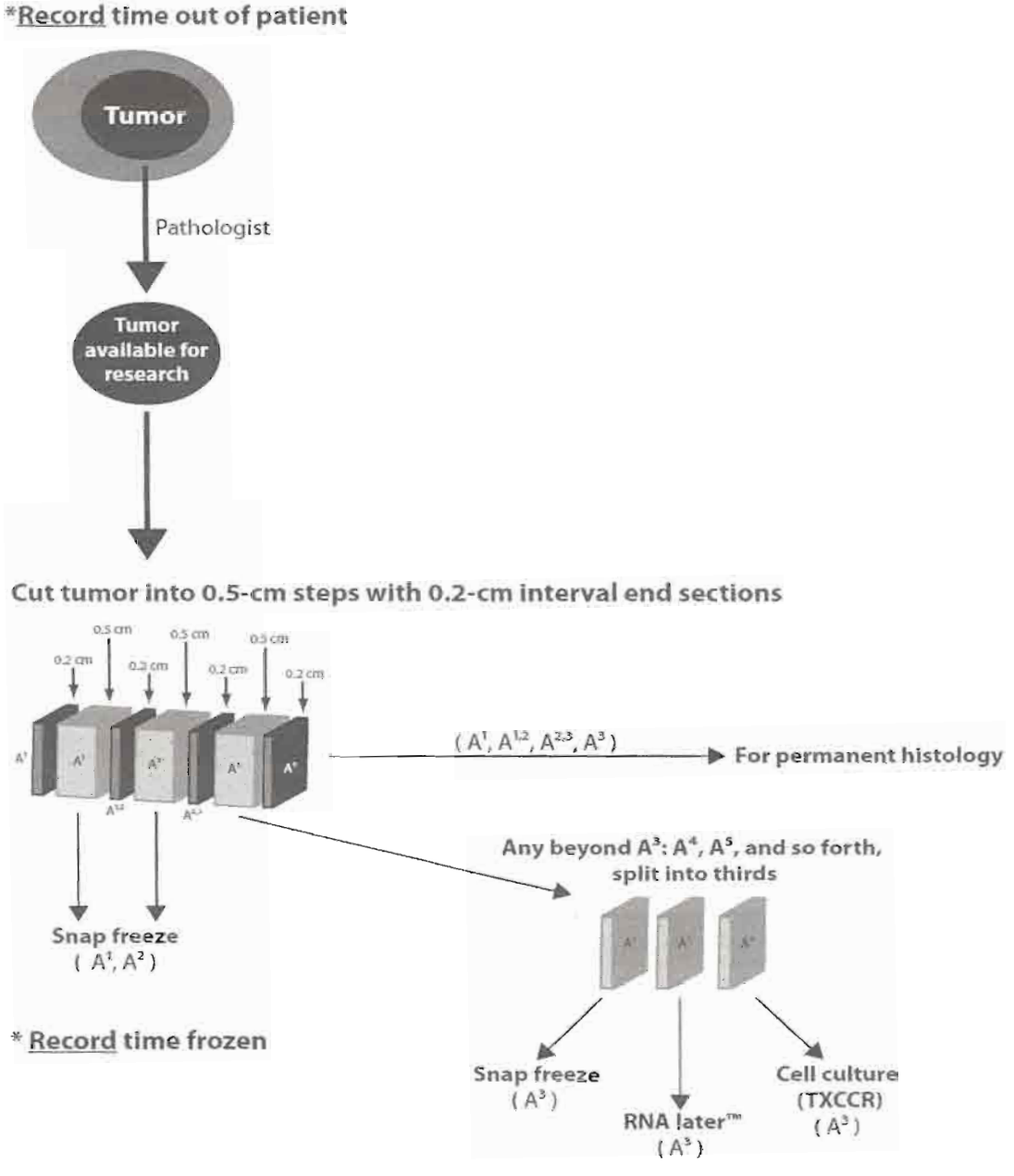
1.0 x 1.0 x **Z** cm Tumor Block



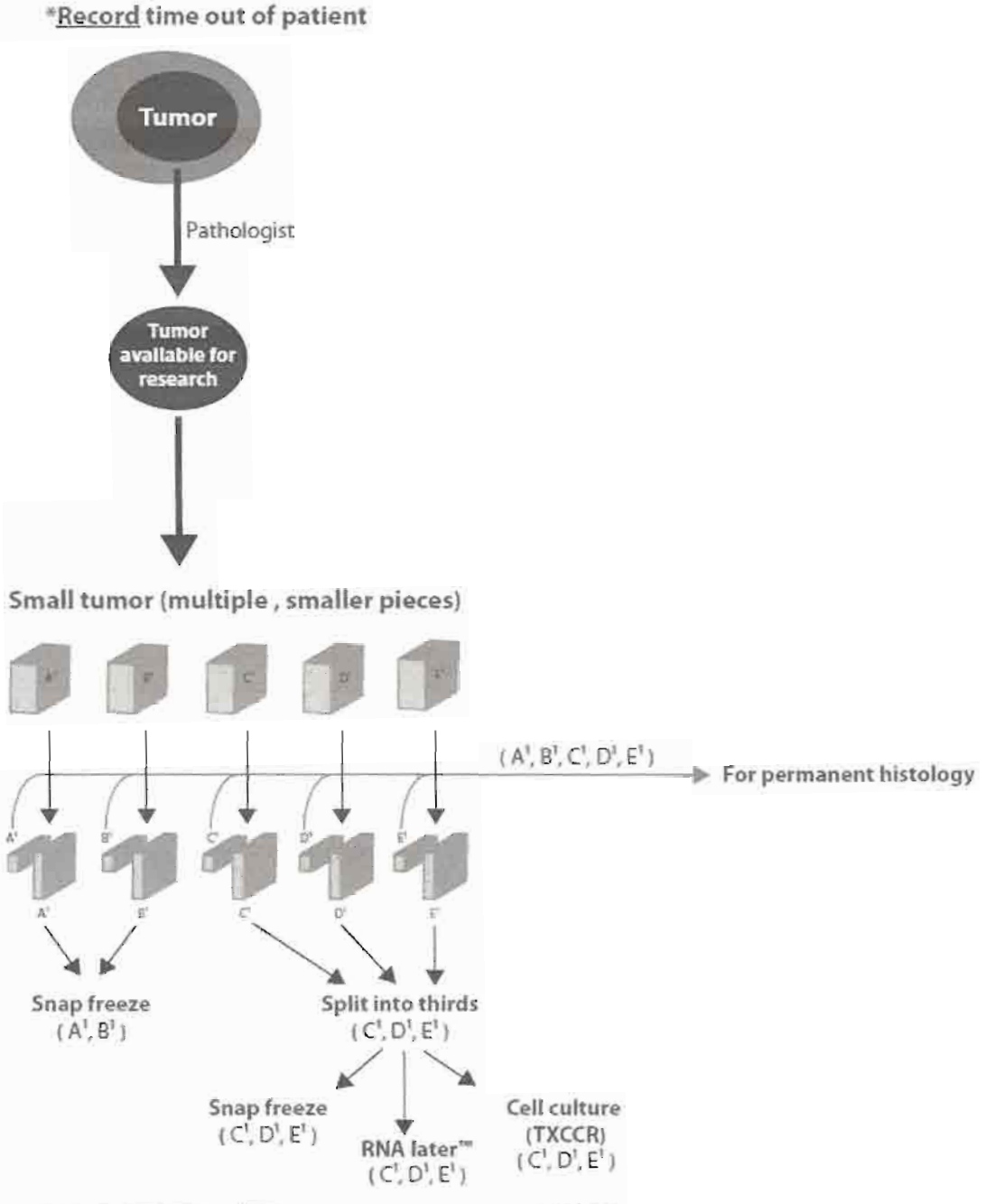
A^2 → Snap freeze – DNA (4 – 0.5 x 0.5 cm^2 pieces)
A^3 → Snap freeze – RNA (4 – 0.5 x 0.5 cm^2 pieces)
A^4 → Xenograft viable (4 – 0.5 x 0.5 cm^2 pieces)
A^5 → RNA later™
A^{6-n} → Bank

$A^{1,2}$, $A^{2,3}$, $A^{3,4}$, $A^{4,5}$ → Histology, fixed in NBF, Paraffin Embedded nominally 0.2 x 1.0 x 1.0 cm^2 pieces

Flowchart 1: Collection of Large Tumor Specimen
For normal, same except no need for more than A3



Flowchart 2: Collection of Tumor Fragments



D. Small Tissue OCT Snap Freezing Method:

1.1. Materials

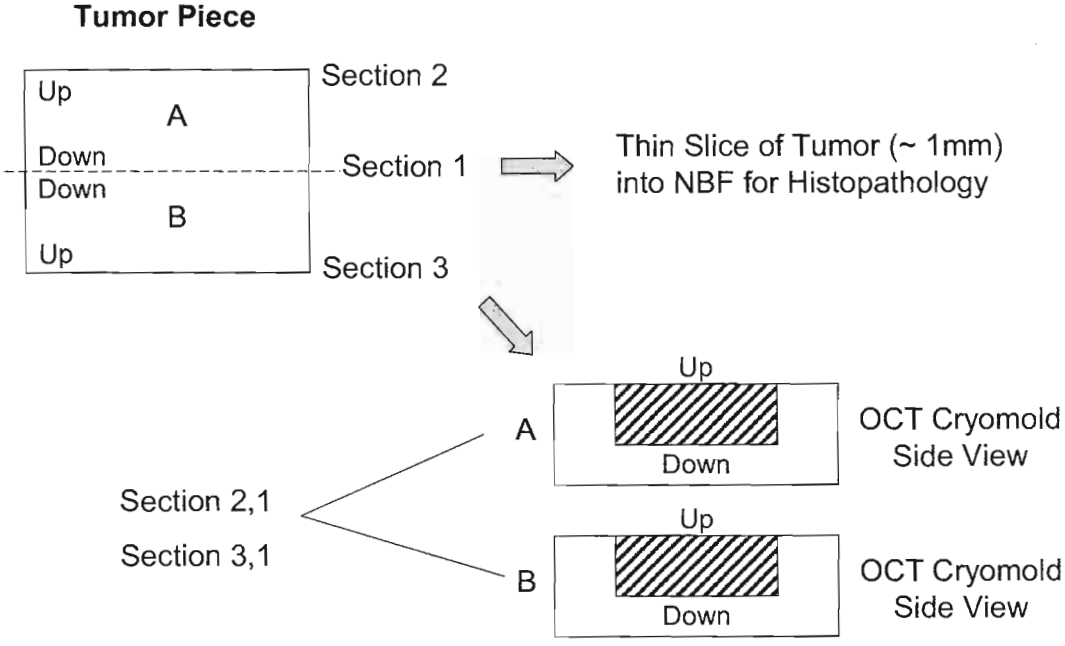
MATERIALS REQUIRED	VENDOR	CATALOG #
1. Tissue–Tek OCT (Optimal Cutting Temperature) Compound	VWR	25608-930
2. Tissue–Tek Cryomold	VWR	25608-916
3. Sterile Disposable Forceps (or use sterile instruments in OR)	VWR*	12576-934
4. Sterile Disposable Scalpels (or use sterile instruments in OR)	VWR*	21909-656
5. Sterile container such as a tissue culture Petri dish to receive and cut tissue (or use sterile towel in OR)	VWR*	60872-306
6. Cryo Marker	Various	Various
7. Pre filled Neutral Buffered Formalin (NBF)	VWR	16004-112
8. Crucible tongs/Long metal forceps	VWR	62460-000
9. CryoTemp Storage Box for storing patient samples	VWR	80077-430
10. Sterile gloves	Various	Various
11. Dry Ice	Various	Various
12. Metal Tray	VWR	62687-027
13. Specimen transport bag / Ziplock bag (quart size)	Various	Various
14. Insulated shipping container	Various	Various
15. Padded envelope	Various	Various
16. Shipping Labels (all labels MUST be placed on outer box): a. Dry Ice (UN 1845) b. Biological Substance Category B (UN3373)	Various	Various
17. Courier Label	Various	Various

* or equivalent

1.2. Procedures for Tissue Collection (see also Figure 2 below)

1.2.1. In the OR or a nearby area have the Petri dish, scalpel, forceps, cryomolds, OCT compound, and dry ice ready.
1.2.2. When the tissue is ready, work quickly noting the start and finish times.
1.2.3. Label the cryomold with patient ID (Initials), specimen ID (tumor or normal), and date with a lab marker (i.e. one that won't come off when frozen).
1.2.4. Transfer the block or pellets of dry ice to the metal tray.
1.2.5. Place a cryomold on a flat surface on the table and place a thin layer of OCT on the bottom of the mold.
1.2.6. Work with normal and tumor tissue separately. Change the scalpel and forceps between normal and tumor samples.
1.2.7. The total time between resection and tissue stabilization by snap freezing should not exceed 30 minutes and preferably not more than 15 minutes as the norm. **The faster the processing time, the greater the tissue / target integrity**
1.2.8. As depicted in Figure 2 below, cut the pea size tumor tissue into two pieces, Piece "A" and "Piece B", each of which can easily fit into an OCT cryomold using sterile instruments. A thin tissue section is removed from the "down" orientation interface between pieces A and B and this tissue section is placed in a container with NBF.
1.2.9. The down orientation A and B pieces are placed into OCT in separate molds with the down side facing the bottom of the mold.
1.2.10. Freeze pieces A and B. Optimally, freezing should be completed within 10-20 seconds.
1.2.11. Working quickly, place the tissue pieces in the proper orientation in the molds within the thin layer of OCT compound. Press down on the tissue to settle it at the bottom of the mold. (A good amount of surface area of the tissue should be available when sectioned).
1.2.12. Add more OCT to the cryomold until specimen is completely covered. (If the tissue floats to the top, push the tissue down with a scalpel/forceps so that it stays close to the bottom of the cryomold.)
1.2.13. Gently place the cryomold in the dry ice in the metal tray using long crucible tongs or the long forceps provided so that the sample freezes from the bottom to the top. Wait for the OCT to solidify completely. (If you have a large flat block of dry ice, you can place the mold on the block of dry ice. If using dry ice pellets position the cryomold with tissue in OCT carefully on the pellets without tilting to freeze the tissue.)
1.2.14. Prepare multiple tumor tissue chunks and in the same manner generating matched NBF containers and cryomolds pairs and properly label each for split large samples.
1.2.15. Before removing the molds from the dry ice, ensure the OCT is solidified completely.
1.2.16. Place the frozen samples in the specimen transport bag/Ziploc bag and place the bag in the provided box for storing samples per patient.
1.2.17. Place the container with formalin in a specimen transport bag with absorbent material. Place the container in the padded envelope provided and send to the TCRB BioBank site along with the frozen tissue.
1.2.18. Place the patient-specific box containing the OCT blocks inside another Ziploc bag prior to placing on dry ice.
1.2.19. Place the bag in an insulated cooler on dry ice.
1.2.20. Complete the appropriate sections of the TCRB Specimen Collection Form and send along with the sample.

Figure 2: Small Tumor – OCT Freezing



1.3. Procedures for Tissue Shipment

1.3.1. The frozen OCT blocks are to be shipped on dry ice and the fresh tissue in NBF at ambient temperature on the same day of collection to the TRCB BioBank site

1.3.2. Four to 6 pounds of dry ice should suffice for a small insulated shipping box (>48 hours of dry ice). Ship out Monday – Wednesday to ensure next day delivery is met (one day's latitude for errant package). Batch shipments are more cost effective if there is sufficient secure storage but samples should not be allowed to accumulate over several weeks.

1.3.3. NBF samples can be stored refrigerated in the dark over the weekend and then shipped if they are collected Thursday – Sunday.

1.3.4. Always notify in advance (with return acknowledgement request) the party you are shipping to. The notification process should be repeated upon receipt of samples.

1.3.5. Label the outer shipping container with the labels for dry ice and biological substances, along with courier routing information.

1.3.6. Complete the appropriate sections of the TCRB Specimen Collection Form and send with the sample.

E. Ascites and Pleural Fluid Collection Method

1.1. Materials

1.1.1. Materials needed to be taken into the OR (Operating Room) or procedure room in the clinic on day of tapping ascites or pleural fluid. The highest yield is often obtained in the earliest taps (taps 1-3).

MATERIALS REQUIRED	VENDOR	CATALOG #
1. Sterile evacuated container, 0.5L or 1L (If more than 1L of fluid is expected more evacuated containers are needed.)	Baxter	1A8504
2. Sterile gloves	Various	Various
3. Alcohol wipes	Various	Various
4. Anticoagulant: Sodium Citrate Solution, USP grade	Baxter*	4B7867
5. Sterile 16G needle	BD*	BD305197
6. Sterile 60ml syringe	BD*	BD309653
7. Sterile 1ml syringe	BD*	BD309602
8. Small biohazard bag (1)	Various	Various
9. Large biohazard bags (2)	VWR*	11217-126
10. Document bag 9"x12" or ziplock bag equivalent	VWR*	11217-194
11. Absorbent strips (2)	Various	Various
12. Label	N/A	N/A
13. IATA 650 Compliant Insulated shipping container	Various	Various
14. Wet ice (crushed ice w/ little water added to make a slush)	N/A	N/A
15. Parafilm	Various	Various

* or equivalent

1.2. **Procedures for Tissue Collector** (see also Flowchart 3 below)

1.2.1. Ascites or pleural fluid procurement should take place in an operating room (OR) or other similarly equipped clinical setting where an aseptic process can be conducted to remove tumor tissue or fluid containing tumor cells.

1.2.2. Individuals who will be collecting the procured tumor tissue samples will need to be trained on the procedures for appropriate tissue collection. All personnel involved will need to understand the need for maintaining sterility of the target material by performing aseptic processing.

1.2.3. Care should be taken to confirm with the physician and staff that the patient identity is correct and that the tapping procedure will yield the appropriate tumor material for processing.

1.2.4. Before going starting the collection, hands should be washed thoroughly.

1.2.5. Wear sterile gloves. Change gloves as and when needed to keep the whole process as clean as possible.

1.2.6. Request a sterile towel or drape and spread it out to have a clean work surface on a cart or table.

1.2.7. Wipe the access port of the Sodium Citrate bag with alcohol wipes and set them on the sterile towel.

1.2.8. Gather the remaining materials required to collect the tissue (i.e. evacuated container or vaccutainer, needles, and syringes) and wipe down the outer packaging with the alcohol wipes and set them on the towel.

1.2.9. The fluid will be collected into one or more 1L or 0.5L evacuated containers. As soon as each vaccutainer bottle is full and disconnected Step 1.2.11 should be performed.

1.2.10. Using the 60ml syringe and 16G needle, immediately inject 1 part Sodium Citrate aseptically to every 10 parts fluid in the vaccutainer. (Determine the volume of fluid in the vaccutainer by using the graduation marks on the vaccutainer container).

Volume of fluid _____ml x (0.1) = Volume Sodium Citrate Solution to add _____ml

1.2.11. After adding the Sodium Citrate, swirl the vaccutainer bottle vigorously to mix.

1.2.12. Seal the port of the vaccutainer bottle using parafilm tape.

1.2.13. Attach a surgical pre-op label containing the patient name and procurement date to the container. If no pre-op label is available, write the patient name and date of collection on the specimen container

1.2.14. Place the specimen container into a small biohazard bag, add the absorbent strips and seal by tying a knot

1.2.15. Line the insulated shipping container with two large biohazard bags, placing one biohazard bag inside the other in a double-bagging fashion.

1.2.16. Add wet ice to the inner biohazard bag.

1.2.17. Place the vaccutainer container on the ice and pack more wet ice around the container.

1.2.18. Twist and knot the opening of the inner biohazard bag close to the container. Repeat twist and knot with the outer bag.

1.2.19. Seal the insulated shipping container.

1.2.20. The TCRB Specimen Collection Form should be completed and sent with the shipment.

1.2.21. This form should filled in with patient name, demographics, surgery details, date consent signed for proposed research, time and date of tissue collection, person collecting tissue, and details of person submitting the form and package.

1.2.22. If the shipment will be sent overnight, the Form should be faxed to the TCRB site on the day of tissue collection/shipment prior to sending the shipment. The original Form should be enclosed in a document bag and placed outside the insulated shipping container but within the outer shipping box.

1.2.23. The outer shipping box should be adequately sealed with tape and labeled at minimum with the following:
- Shipper / recipient information
- UN 3373 diamond label
- "Biological substance, category B"

1.2.24. The package should be sent on the day of tissue collection as priority overnight.

1.2.25. Upon receipt at the TCRB Bank site, the package needs to be inspected for any visible damage or leakage. The box should then be unpacked to continue the visual inspection to insure no leakage or other damage. Remember to observe Universal Precautions at all times.

1.2.26. Using sterile technique under a BioSafety Cabinet (BSC), remove the contents of the Vaccutainer bottle to sterile centrifuge tubes or bottles using a sterile pipet and pipet-aid and then pellet cells by centrifugation at 250-500 rcf. Combine the cell pellets into a single tube.

1.2.27. It is advisable at this point to remove a small aliquot of the cell slurry and make two smears (akin to blood smears). Allow to air dry and then use a rapid Wright-Geimsa stain (example: CAMCO Quick Stain, Cambridge Diagnostic Products, Inc., Catalog #211) to determine blood cell contamination. The staining process takes about 1-2 minutes to complete. Slides can be coverslipped and preserved for later reference.

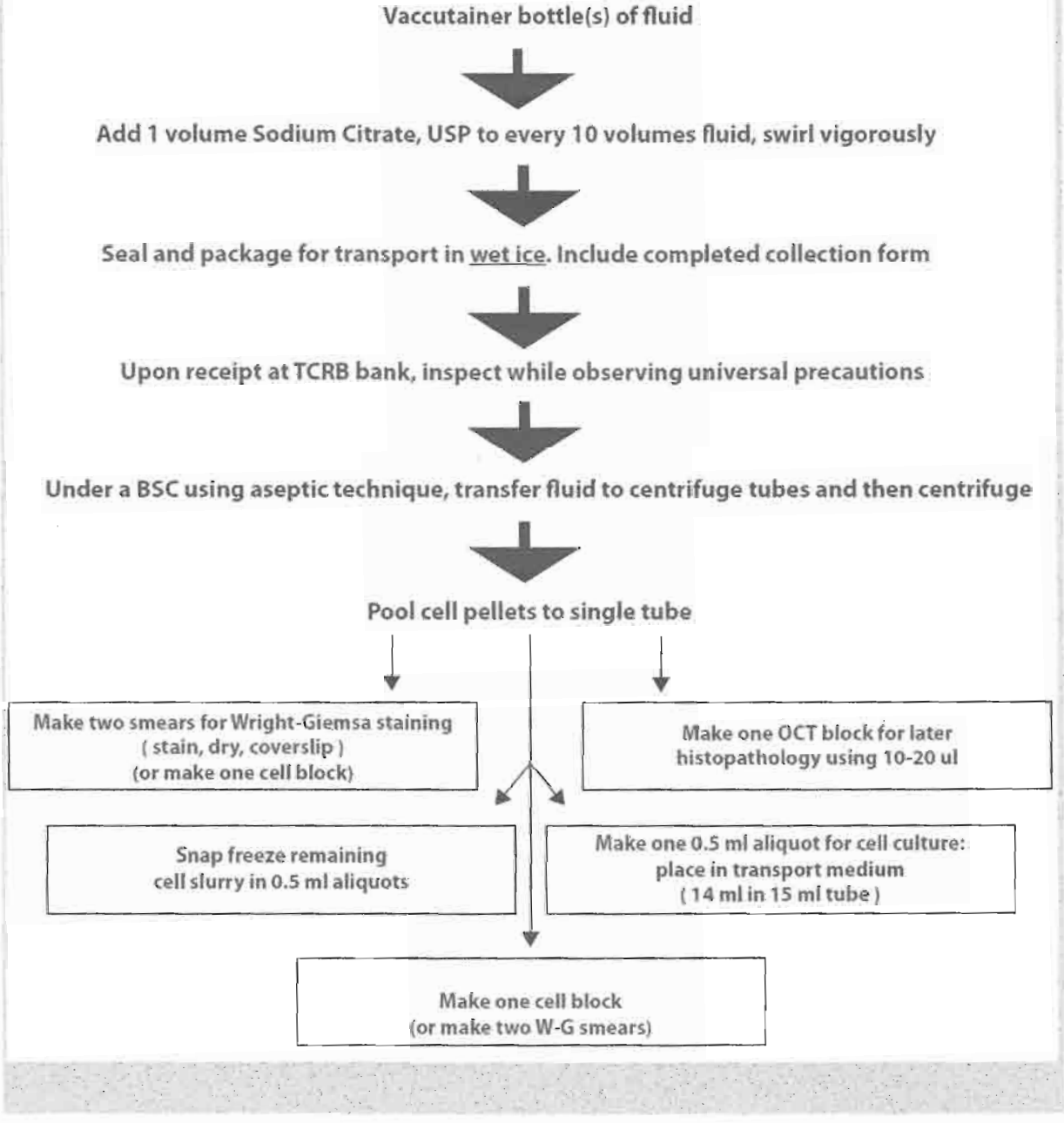
1.2.28. Aseptically remove three 0.5mL aliquots of cell slurry to separate cryotubes for snap freezing in isopentane dry ice or LN2 (DNA, RNA, etc).

1.2.29. Aseptically remove 10-20uL with a sterile pipet and place into OCT that is freezing in cryomold on dry ice for later histology. Freeze completely.

1.2.30. Remove a sterile 0.5mL aliquot for xenograft expansion and place in xenograft transport medium (see next section).

1.2.31. Aseptically dispense remaining aliquots as per sections 1.2.29 and 1.2.31.

Flowchart 3: Collection of Ascities and Pleural Fluid



F. Xenograft Tissue Collection Method

1.1. See Attachment 7 for the Texas Cancer Cell Repository SOP, the most current version of which is available at *www.TXCCR.org*, including any updates.

1.2. **Materials**

MATERIALS REQUIRED	VENDOR	CATALOG #
1. Leibovitz L-15 Culture Medium	Various	Various
2. Pen/Strep	Various	Various
3. 10% FBS	Various	Various
4. Sterile Gloves	Various	Various
5. Sterile Disposable Forceps (or use sterile instruments in OR)	VWR	12576-934
6. Sterile Disposable Scalpels (or use sterile instruments in OR)	VWR	21909-656
7. Sterile container such as a tissue culture Petri dish to receive and cut tissue (or use sterile towel in OR)	VWR	60872-306
8. Sterile 50mL conical centrifuge tubes	Various	Various
9. Small biohazard bag (1)	Various	Various
10. Large biohazard bags (2)	VWR	11217-126
11. Document bag 9"x12" or ziplock bag equivalent	VWR	11217-194
12. Absorbent strips (2)	Various	Various
13. Label	N/A	N/A
14. IATA 650 Compliant Insulated shipping container	Various	Various
15. Wet ice (crushed ice w/ little water added to make a slush)	N/A	N/A
16. Parafilm	Various	Various

1.3. Tissue Procurement of Solid Tumors

1.3.1. Tumor tissue or cells may come from tissue processing in Sections C, D or E above or as detailed below.
1.3.2. Tumor tissue pieces should be aseptically handled at all times.
1.3.3. Tissue pieces should be approximately 1-5mm cubes, having been diced using sterile disposable forceps and sterile disposable scalpels in a small sterile dish or container.
1.3.4. The minced tumor tissue should be transferred into labeled tubes containing sterile Leibovitz L-15 complete media containing 10% Fetal Bovine Serum (FBS) and Pen/Strep antibiotic mixture. This is the preferred media but RPMI-1640 or DMEM or other suitable tissue culture medium may be used (as available).
1.3.5. Viable tumor should total about 50-500mg.
Use sufficient transport to completely cover tissue and fill tube almost full so very little air remains once the lid is secured onto the tube.
1.3.6. Secure the lid of the specimen container tightly.
1.3.7. Seal the lid of the specimen container using parafilm.
1.3.8. Attach a surgical pre-op label (see sample—Attachment 3) containing the patient name and procurement date to the specimen container. If no pre-op label is available, write the patient name and date of collection on the specimen container
1.3.9. Place the specimen container into a document bag or ziplock bag and seal the bag closed.
1.3.10. Place this assembly into the pressure vessel, along with some absorbent strips, and secure the lid of the pressure vessel.
1.3.11. Line the insulated shipping container with two large biohazard bags, placing one biohazard bag inside the other in a double-bagging fashion.
1.3.12. Place the pressure vessel on the ice and pack more wet ice around the vessel up to the lid.
1.3.13. Twist and knot the opening of the inner biohazard bag close to the pressure vessel. Repeat twist and knot with the outer bag.
1.3.14. Seal the insulated shipping container.
1.3.15. The TCRB Specimen Collection Form (Attachment 2) should be completed with the patient, surgery/tissue collection, and protocol details.
1.3.16. It will be useful to compare resulting cell culture/xenograft products to the original tumor: If subsequent access to the frozen tumor procured as described above cannot be ensured, it is advisable to submit at least one OCT-embedded tumor sample (with frozen section slide/imaging) as described above to the same destination as the fresh tumor tissue.
1.3.16.1. If this is not possible, attempt to ensure that enough tumor material is shipped so that a portion may be frozen on receipt.

1.4. Blood or Bone Marrow

1.4.1. This is an acceptable specimen for the TXCCR. See the TXCCR Attachment (Attachment 4) or www.TXCCR.org for detailed protocol.

1.4.2. This includes the following sources:

- 1.4.2.1. Pheresis samples from leukemia patients with high circulating blast counts. These are especially desirable.
- 1.4.2.2. Blood Samples
- 1.4.2.3. Bone Marrow Samples
- 1.4.2.4. Post-Mortem Blood Samples
- 1.4.2.5. Discarded Blood Cell Pellets or Clots

1.4.3. Blood samples are also required on all patients.

- 1.4.3.1. In order to provide a source of non-malignant cells for genomic comparison to the cancer cells we request that 30 mls of heparanized blood be obtained.
- 1.4.3.2. Minimal amount requested is 10 mls but 30 mls is strongly preferred.
- 1.4.3.3. Blood obtained in vaccutainers can be sent in those containers, while blood obtained by syringe should be transferred aseptically to a vaccutainer or similar sealed and robust container for shipping.

1.5. Tissue Shipment for Xenograft / Cell Line Development

1.5.1. Shipping: Samples should be in a sterile inner container and sealed in a light-tight outer container and sent at room temperature.

- 1.5.1.1. Shipment in small Styrofoam containers is preferred, to insulate samples from extreme temperature changes. However, shipment in standard biological specimen envelopes (with inner padding) is a suitable alternative.
- 1.5.1.2. During the summer a cold pack (if available) should be placed in the container, but insulated from the sample, to prevent high temperatures.

1.5.2. If specimens are sent on a Friday, be sure to indicate Saturday delivery on the air-bill and contact Tito Woodburn (information below) for a shipping address (TTUHSC does not accept Saturday deliveries).

1.5.3. Advanced notice of incoming samples is kindly requested by email or phone, especially if shipment of material may arrive on a weekend or holiday.

- 1.5.3.1. Emailing of the tracking information is especially helpful.
- 1.5.3.2. If shipment of material may arrive on a weekend (i.e. when shipping is done on a Friday) or holiday, advance arrangements must be made with the laboratory.

1.5.4. Laboratory Contact: Tito Woodburn

- i. **Lab phone:** 806-743-2707.
- ii. **Email:** TITO.WOODBURN@TTUHSC.EDU
- iii. If it is impossible to contact the lab prior to shipping, it is requested that the tracking number and carrier information for the shipment be emailed to the laboratory when shipping the specimen.

1.6. All samples should be sent directly to:

1.6.1. C Patrick Reynolds, MD PhD
- i. Cancer Center Core Labs STOP 9445
- ii. Texas Tech University Health Sciences Center
- iii. School of Medicine Cancer Center
- iv. 3601 4th Street
- v. Lubbock, Texas 79430-6450
- vi. **Phone:** 806 743-1558
- vii. **Fax:** 806 743-2691
- viii. **Email:** PATRICK.REYNOLDS@TTUHSC.EDU

1.6.2. Contact in lab: Tito Woodburn
- i. **Lab phone**: 806-743-2707
- ii. **Email:** TITO.WOODBURN@TTUHSC.EDU

G. Phlebotomy PaxGene Blood Collection Method

1.1. Please obtain **1** complete PAXgene™ Blood DNA Tube (Qiagen, catalog number 761115, 8 mL) of whole blood for processing. Label each tube with the subject UUID / Bar Code, the date and time of the blood draw.

1.2. For cancer patients, it is preferred that blood is collected prior to, or on the day of procurement. However, blood may be collected after procurement.

1.3. **Surgical PaxGene Blood Collection and Storage**

1.3.1. For patient comfort, venous or arterial whole blood will be collected from participating patients in an 8.5 cc plastic PreAnalytiX PAXgene blood collection tube after administration of general anesthesia. To eliminate potential contamination with non-native leukocytes, patient blood will be collected prior to the administration of any blood products or, in the case of transplants, graft reperfusion.

1.3.2. The blood tube will be labeled using the standard de-identified nomenclature indicating sample source and patient number.

1.3.3. Filled blood tube will be stored at progressively cooler temperatures per manufacturer instructions, for ultimate long-term storage in an ultra low -80°C freezer.

1.4. **Shipment of PaxGene Blood and Normal and Tissue Samples**

1.4.1. Only by advanced notice and agreement, batch ship collected samples on Monday or Tuesday for receipt no later than Tuesday or Wednesday to the attention of:

Baylor College of Medicine
ATTN:Marie- Claude Gingras
Alkek/N1416
One Baylor Plaza
Houston, TX 77030
713-798-1284

1.4.2. Enter the sample into the Acquire / TCRB Database and generate the HGSC Sample Intake form

1.4.3. Pack the sample(s) along with the corresponding Chain of Custody forms and HGSC Sample Intake form

Examples of shippers include:

holds 3 tubes at a time
http://www.thermosafe.com/model/473.html?&Pr10=Science

or

(Catalog No DS500S-B)
http://www.casingcorp.com/TEN%20PAGE%20BOX%20BRO%2096%20DPI%20%20APR%204%20UNIT%20AND%20PER%20CASE%20PRICING.pdf

Before samples are submitted, all relevant data fields are entered in Acquire /TCRB Database including the deidentified histopathology report, percentage necrosis, percentage tumor nuclei, etc. such that there is sufficient qualifying information prior to the nucleic acid extraction steps.

H. General Instructions for Biologicals Shipment

1.1. The outer shipping box should be adequately sealed with tape and labeled at minimum with the following (see sample—Attachment 4):

1.1.1. Shipper / recipient information
1.1.2. UN 3373 diamond label
1.1.3. "Biological substance, category B"

1.2. The TCRB Specimen Collection Form (Attachment 2) should be completed with submission details.

1.3. The package should be sent to the TCRB BioBank site on the day of tissue collection as same-day courier or priority overnight along with the completed TCRB Specimen Collection Form.

1.3.1. If the shipment will be sent overnight, the TCRB Specimen Form should be faxed to the BioBank site on the day of tissue collection/shipment prior to sending the shipment. The original Form should be enclosed in a document bag and placed outside the insulated shipping container but within the outer shipping box.

1.4. **Biologicals Receipt**

1.4.1. When the tumor tissue sample is received personnel should verify that the specimen information of the received sample matches the information provided in the written requisition before accepting the tissue.

1.4.2. If discrepant information is provided, steps should be taken to rectify the information with the Clinical Site immediately to avoid delay in processing.

1.4.3. The sample must be logged in.

1.4.3.1. A unique sample ID is assigned to every tissue received prior to beginning any processing.

1.4.3.2. The ID numbers are assigned with the prefix "GB" followed by the next sequential 4-digit number.

1.4.4. The recipient should complete the TCRB Specimen Collection Form as having received the sample and enter the assigned unique sample ID on the form.

1.4.5. A copy of the completed TCRB Specimen Collection Form should be forwarded to the Clinical Site via email or fax as confirmation of having received the tissue.

7. ATTACHMENTS

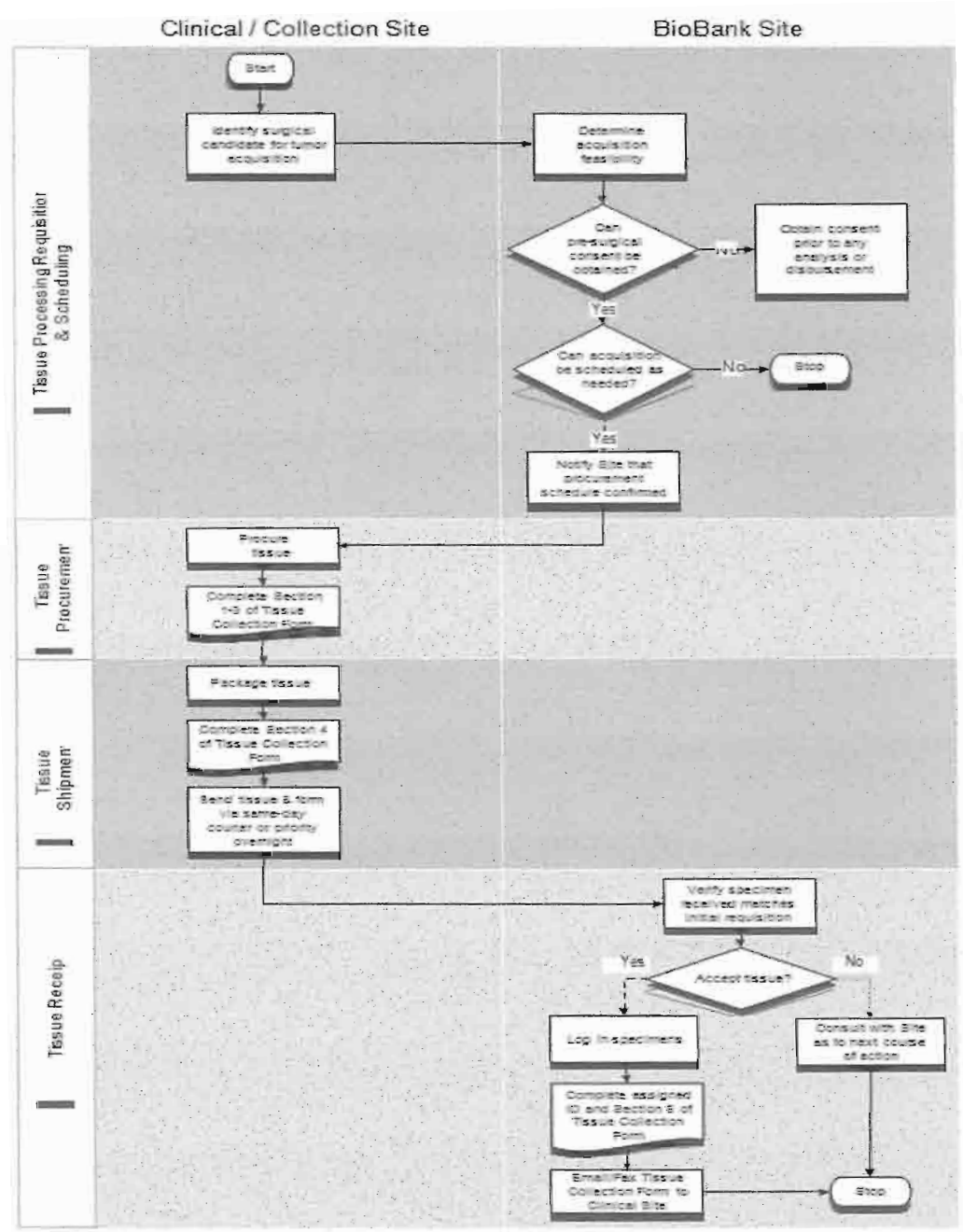
- Attachment 1: Tissue Processing Requisition, Procurement, and Transport (Flowchart)
- Attachment 2: TCRB Specimen Collection Form
- Attachment 3: Specimen Container Label (sample)
- Attachment 4: Shipping Container Label (sample)
- Attachment 5: Step-by-Step Photo Explanation of Specimen Collection/ Processing
- Attachment 6: Pancreatic Cancer Sequencing Consortium: Specimen Collection Flowchart
- Attachment 7: SOP for Collection of Tissue, Blood, or Bone Marrow for Biobanking and for Establishing Continuous Cell Lines and Xenografts from Neoplasia

8. DOCUMENT REVISION HISTORY

Date	Version #	Change Summary
	01	Original

ATTACHMENT 1

Tissue Processing Requisition, Procurement, and Transport (Flowchart)



ATTACHMENT 2

TCRB Specimen Collection Form

TEXAS CANCER RESEARCH BIOBANK

TCRB Specimen Collection Form

Attachment#2: RE-SOP-100 Version: 1.00 Issue Date: 03/29/2012

Part I. Patient Demographics and Specimen Collection

Patient Number: _____
Assigning Hospital or Clinic: _____
Vital Status: _____
Race: ☐ American Indian or Alaska Native ☐ Asian ☐ Black or African American ☐ Native Hawaiian or Pacific Islander ☐ White ☐ Not Reported ☐ Unknown

Bio-Bank Site Name: _____
Collection Personnel: _____
Consent Taken: ☐ Yes ☐ No
Prior Treatment/ Neoadjuvant Therapy: ☐ Yes ☐ No
Gender: ☐ Male ☐ Female ☐ Unknown
Ethnicity: ☐ Hispanic ☐ Non-Hispanic ☐ Unknown

Tumor Specimen	Barcode:	Label:
Tumor Type: ☐ Solid Primary ☐ Metastatic ☐ Hematopoietic ☐ Other: _____		
Clinical Diagnosis:		Collection Date:
Preservation Protocol:		Specimen Amount (circle) mg / ml:
Freeze Type: ☐ LN2 ☐ Isopentane / Dry Ice Bath	Storage Temperature (C):	Warm Ischemia Time (min): (Time from devascularization to freezing)
Comments:		
Pathology Diagnosis (if available)		
Disease Diagnosis (ICDO Morphology Axis):		
Anatomic Site (ICDO Topography Axis):		

Normal Specimen	Barcode:	Label:
Tube Type/ Preservative:		
Clinical Diagnosis:		
Amount in Tube:	(circle) mg / ml	Collection Date:
Anatomic Collection Site: ☐ Skin ☐ Adjacent Normal ☐ Blood ☐ Other:		Normal is from a satisfactory source: ☐ Yes ☐ No
Comments:		

Part II. Tumor Aliquot Processing

Tumor Aliquots

SOP Section 8. Order of Specimen Processing

Parent Barcode:

Parent Label:

1. Intended Aliquot Use: ☐ DNA ☐ RNA ☐ Xenograft and/or Cell Culture ☐ Banked As Is ☐ Other:	Aliquot Barcode:	Preservation Method:	
	Aliquot Label:	Storage Temperature (C):	Specimen Amount (mg ml cell ug):
2. Intended Aliquot Use: ☐ DNA ☐ RNA ☐ Xenograft and/or Cell Culture ☐ Banked As Is ☐ Other:	Aliquot Barcode:	Preservation Method:	
	Aliquot Label:	Storage Temperature (C):	Specimen Amount (mg ml cell ug):
3. Intended Aliquot Use: ☐ DNA ☐ RNA ☐ Xenograft and/or Cell Culture ☐ Banked As Is ☐ Other:	Aliquot Barcode:	Preservation Method:	
	Aliquot Label:	Storage Temperature (C):	Specimen Amount (mg ml cell ug):
4. Intended Aliquot Use: ☐ DNA ☐ RNA ☐ Xenograft and/or Cell Culture ☐ Banked As Is ☐ Other:	Aliquot Barcode:	Preservation Method:	
	Aliquot Label:	Storage Temperature (C):	Specimen Amount (mg ml cell ug):
5. Intended Aliquot Use: ☐ DNA ☐ RNA ☐ Xenograft and/or Cell Culture ☐ Banked As Is ☐ Other:	Aliquot Barcode:	Preservation Method:	
	Aliquot Label:	Storage Temperature (C):	Specimen Amount (mg ml cell ug):

Collection Forms Instructions

Each Part of the *TCRB Specimen Collection Form* represents a time point in the collection protocols. Part I. is to be filled out at time of tissue procurement. Part II. is to be filled out during laboratory processing of the tumor specimen. This data or additional data may also be directly entered into TCRB Acquire, https://tcrbacquire.research.bcm.edu/

Specimen Collection Form Part I Instructions

Tumor or Normal Specimen Barcode and Label Fields:

Sticking a printed copy of the respective specimen label or barcode on the form is an accurate method to complete these fields. The label may also be hand written, but a copy of the printed label is preferred to eliminate transfer errors.

Specimen Collection Form Part II Instructions

Intended Aliquot Use Field:

For this field, select the Aliquot Type that represents the intended designation for each Aliquot. Some Aliquots are sent to HGSC or other labs for derivative creation and the actual aliquot may be completely used during this process, such as DNA extraction. The link from the tumor aliquot and the child derivative is important to record for TCRB banking records and because it is no longer available for future tissue distribution. The descriptions below, explain which value should be toggled in the Intended Aliquot Use field.

Value Descriptions:

- Toggle DNA for aliquots that are to be shipped to HGSC for DNA isolation.
- Toggle RNA for aliquots that are to be shipped to HGSC for RNA isolation.
- Toggle Xenograft and/or Cell Culture, for aliquots that are to be shipped for either cell culture or Xenograft creation.
- Toggle Banked As Is, for any aliquot being reserved at the collection site for TCRB future use. For sites not currently sending specimen for derivative creation, this value can be used and then changed after the aliquots are distributed.
- Toggle Other and indicate the derivative type, for aliquots to be used for the creation of other derivative types not listed.

Preservation Method Field:

For this field, please indicate the method used in preserving the aliquot. Often the aliquot will be preserved with the same method the parent tumor is preserved; others will be specific to the derivative to be created. Please indicate the method, such as FFPE, RNA later, TXCCR transport media, etc. as appropriate in this field.

ATTACHMENT 3

Specimen Container Label (sample)

SPECIMEN CONTAINER LABEL

Sample Pre-op Label →

DOE, JOHN
MR #: A123456789 Surgery: 11/30//09
DOB: MM/DD/YY Surgeon Name
Age/Gender Hospital Name

Sample Handwritten Label →

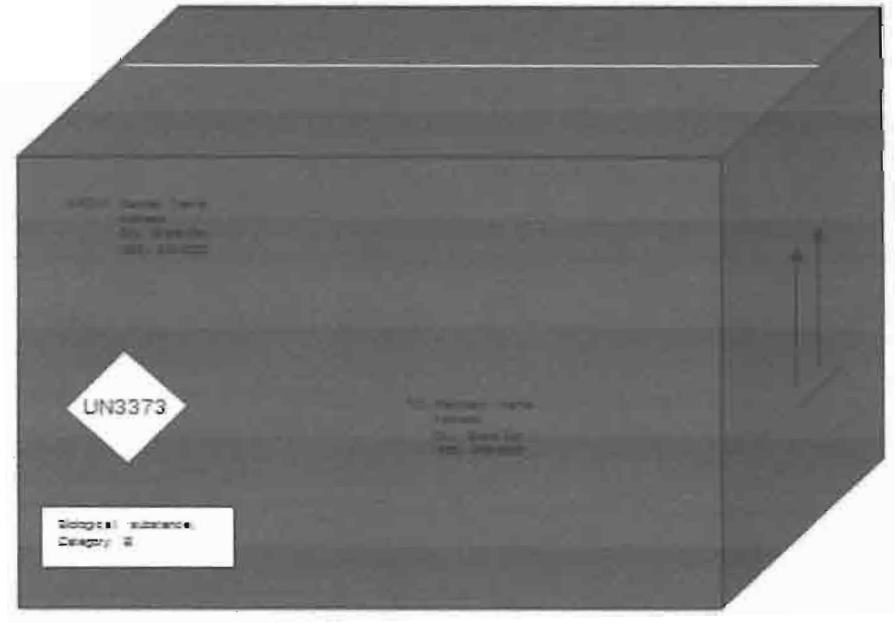
John Doe
Collected 11/30/09

(Encryption of patient identity occurs at the TCRB BioBank Site)

ATTACHMENT 4

Shipping Container Label (sample)

SHIPPING CONTAINER LABELS



ATTACHMENT 5

Step-by-Step Photo Explanation of Specimen Collection/Processing

TEXAS CANCER RESEARCH BIOBANK

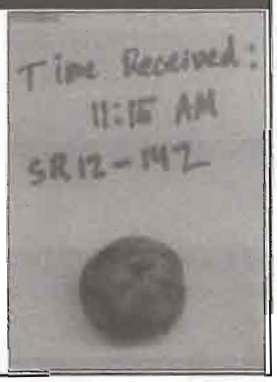
Step 1: Receive specimen

The specimen arrives in Pathology.

Cryovials have already been labeled and the appropriate vials already contain culture media or RNA later.

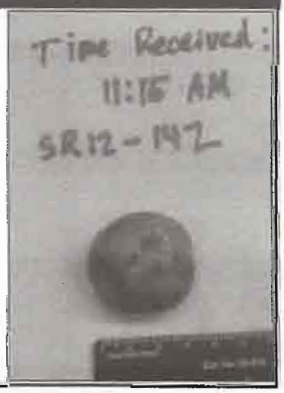
Step 2: Record time of removal

Time of removal from patient (or arrival in Pathology if immediately necessary) is noted.



Step 3: Determine surplus

The attending pathologist completes his/her duties and determines that surplus tissue will be available for research.

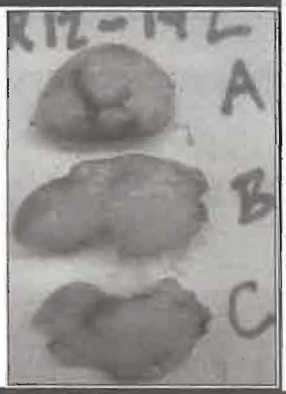


Step 4: Section tissue

The tissue is sectioned by the pathologist or handed over to research personnel for this and all subsequent procedures.

Step 5: Label sections

Each section is specifically labeled. In this case, there are 3 sections: A, B and C.



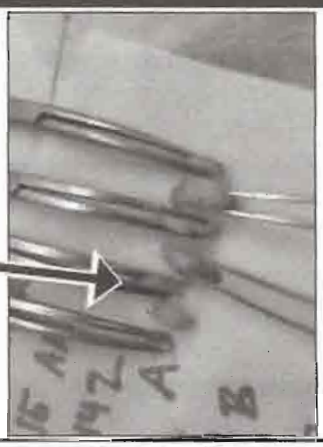
Step 6: Measure sections

Each section is 1 cm thick. Sections are cut at 1 cm intervals. The length may vary, but should be 1 cm or more. In this case, it is about 4 cm.

Step 7: Cut tissue into smaller fragments

Starting with "A," the tissue is sequentially cut into smaller fragments, rather like cutting a loaf of bread, except that the thickness of each slice will alternate between 0.2cm and 0.5cm as described below.

The 0.2cm sections are for histology; the 0.5cm sections are for the biobank.



A piece of tissue 0.2cm long is cut from each end and from between the larger blocks. In this case, these pieces are held by the 4 forceps (2 end sections, 2 interval sections).

These are the histology sections.

Step 8: Size sections

The sections will all be ~1x1 cm; only the thickness will be different.

Step 9: Biobank section size

The biobank specimens (once the end and interval sections have been cut away) will be ~0.5 cm thick.

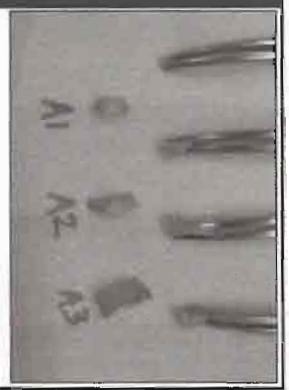
Step 10: Histology section size

The histology (interval and end) sections will be ~0.2 cm thick.

Step 11: Label biobank sections

The 3 biobank sections from "A" will be labeled sequentially.

In this case, they are A1, A2 and A3.

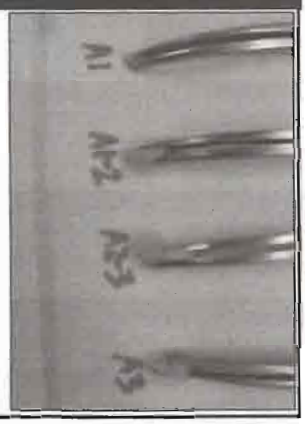


Step 12: Label histology sections

The histology (end and interval) sections from "A" will be labeled sequentially depending on where they occur.

In this case:

- the first end is A1, as it is part of biobank section A1;
- the next interval is A1-2, as it lies between biobank sections A1 and A2;
- the next interval is A2-3, as it lies between biobank sections A2 and A3;
- the final end is A3, as it is part of biobank section A3.

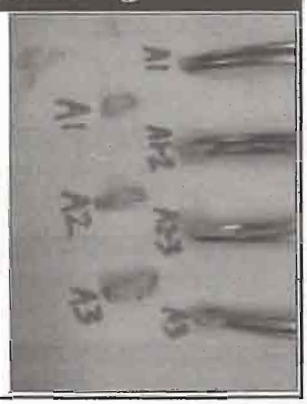


Step 13: Label biobank and histology sections together

Thus, we have cut tumor block A sequentially into 7 pieces; 3 are for biobank submission and 4 represent the histology sections from the end or between the biobank sections.

The labeling allows us to know exactly which histology sections (2 each) represent the opposite ends of any particular biobank section.

Thus, Histology sections A1 and A1-2 are the opposite ends for biobank section A1. Histology sections A1-2 and A2-3 must lie at opposite ends of biobank section A2.

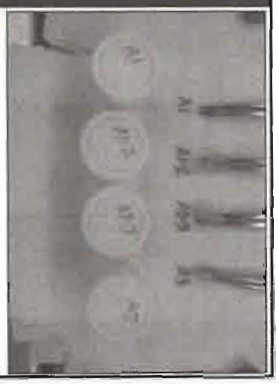


Step 14: Formalin containers

Properly labeled (e.g. A1, A1-2, etc...) formalin containers are used to place the corresponding histology sections into formalin for fixation, paraffin embedding and histologic sectioning (FFPE).

Step 15: Separate into containers

One section is placed into each container. It is necessary that they are not mixed up with one another.



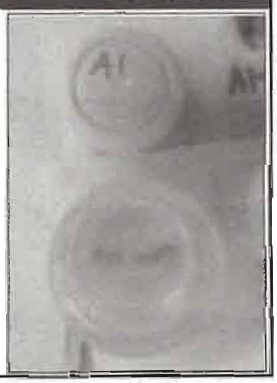
Step 16: Place into each container

The individual histology section is placed into the corresponding container.

Step 17: Fix sections in formalin

Each histology section will fix in formalin.

These are not for diagnostic pathology; these are the histology sections for the TCRB case.



Step 18: Tissue into labeled tubes

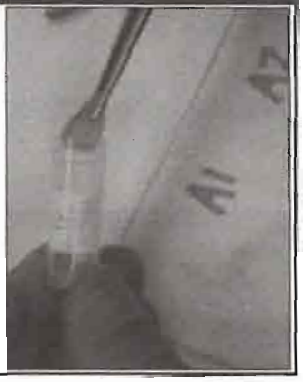
The 3 pieces of biobank tissue will also be placed into individually-labeled tubes.

Step 19: Note case and specimen on tube

Each tube should note the case and specimen. In this example, the date and time and the specimen (A1) are indicated. The TCRB biobank identifier would be placed on the tube later. It is preferable, though, for the tube to be pre-labelled with a unique identifier so that the identity (case and specimen number, A1 here) can be associated with it in the case notes. It is good biobank practice, though, to pencil in the case number and specimen number (A1) as a secondary identifier even if the vials are pre-labeled.

Step 20: Tissue for sequencing

For A1, it is the first biobank section. As the order of submission is to ensure that frozen tissue is available for sequencing, it is placed entirely into the cryovial for rapid freezing.



Step 21: Remaining tissue for sequencing

The same logic holds for A2, so it is placed entirely into a cryovial for rapid freezing.

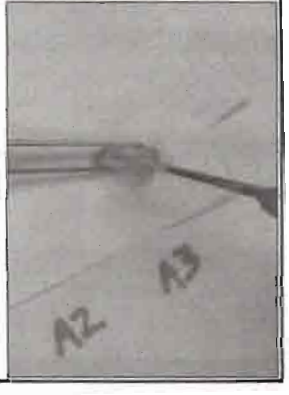
Since A1 and A2 are at least 0.5cm thick, and are about 1cm in the other 2 dimensions, the 2 of them should add up to 1x1x1 cm of frozen tissue for sequencing.

As well, we have already reserved 3 histology sections (one from each end and one from the middle) at 0.5-cm intervals, so it will be possible to precisely know the histology of each of these biobank sections prior to selection for sequencing.

Step 22: Sequencing and cell culture

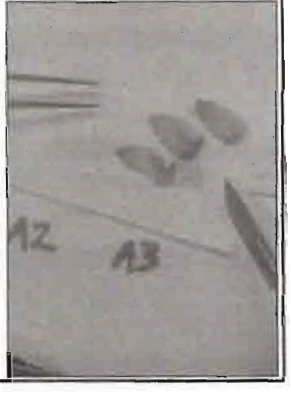
Any biobank tissue beyond A2 may be used for sequencing, but also for the cell culture (TXCCR) and RNA sequencing parts of the TCRB intiative.

So, the next section, A3, is sliced in the same manner as in the original slicing of the larger specimen, A.



Step 23: Make additional sections

A3 (and any further biobank sections) is divided into 3 equal sections. Since it is cut parallel to the same slicing plane as in the beginning, the histology pieces will represent the histology in each of these.



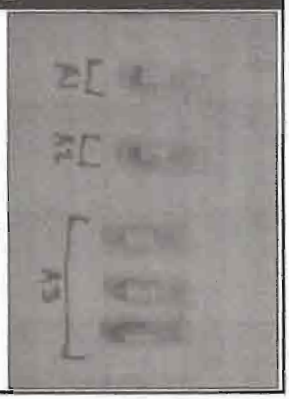
Step 24: RNA, DNA and TXCCR

Each of these sub-sections will go for DNA, RNA (in an RNAlater-filled tube, which appears light pink in this image) or cell culture/TXCCR (in a culture media-filled tube which appears dark pink in this image)

Step 25: Separating into tubes

So, the first 2 biospecimen sections go into a single tube each (A1 and A2), while the third (and any thereafter) is divided and submitted in 3 tubes.

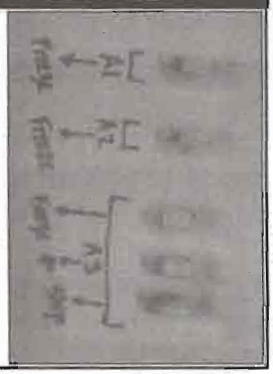
The tubes for A3 are all labeled as A3; the DNA/RNA/TXCCR designation identifies their purpose.



Step 26: Prepare for rapid freezing

The DNA tubes are to be snap-frozen as quickly as possible. This will be A1, A2 and one of each of the sets of 3 tubes that may follow.

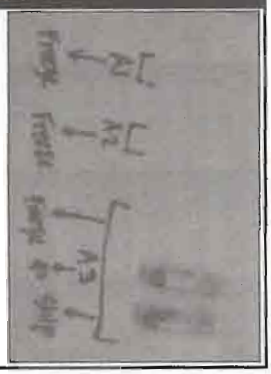
The RNAlater and cell culture media will stabilize those specimens once they have been put in those tubes, but it is necessary to note the time that they were immersed.



Step 27: Rapid freeze

So, why are you wasting time reading this?

Get those DNA tubes rapid-frozen NOW!!!



Step 28: Method of rapid freezing

The method of rapid freezing is not important (call TCRB if you have a question). Anything that will freeze the specimen in 30 seconds or less will do.

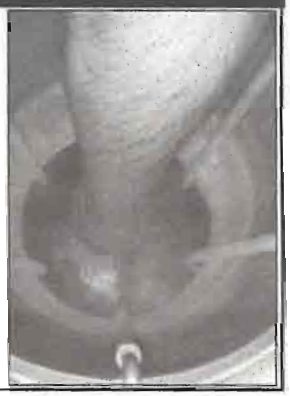
In this example, a container of liquid nitrogen should do the trick, but if it is not available to you immediately, use whatever else you have.

Step 29: Snap-freeze and note time

Working quickly, freeze each vial, noting the time of freezing (it will all be about the same time).

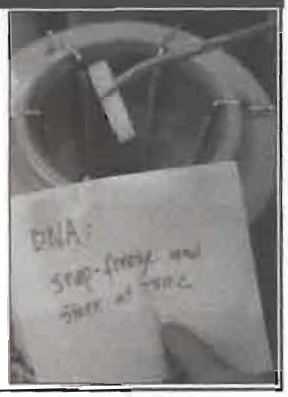
Step 30: Snap-freezing vial

Nothing fancy here. In West Texas, we are strictly old-school (and have no sensory nerve endings).



Step 31: Snap-freeze and store

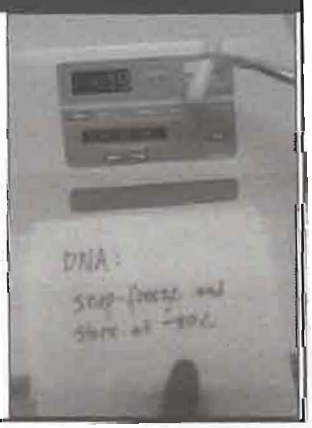
Once frozen, it is ready to be entered into the biobank.



Step 32: Store cryovials

Store the cryovials at -80C (vapor-phase liquid nitrogen is acceptable but not necessary preferable) in accordance with biobank protocol, such that the location and identity of the specimen (by case/specimen and TCRB designation) is readily available.

Ensure that the location is not one in which the specimen might be accessed by persons for reasons other than TCRB retrieval for sequencing.



Step 33: RNA to reside overnight

The RNA specimens will reside overnight at 4C before freezing and storing at -80C.

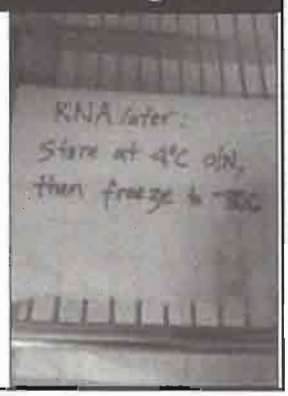
Step 34: Store vials in stable place

Store these vials in a place in the refrigerator where they will not be subject to movement/removal/ manipulation by other persons for non-TCRB reasons.

Step 35: Remove vials to ship

The next day, remove them from the refrigerator and place them with the DNA specimens from the same case at -80C.

It is not necessary, or even useful, to rapid-freeze the RNA specimen beforehand.



Step 36: Ship vials

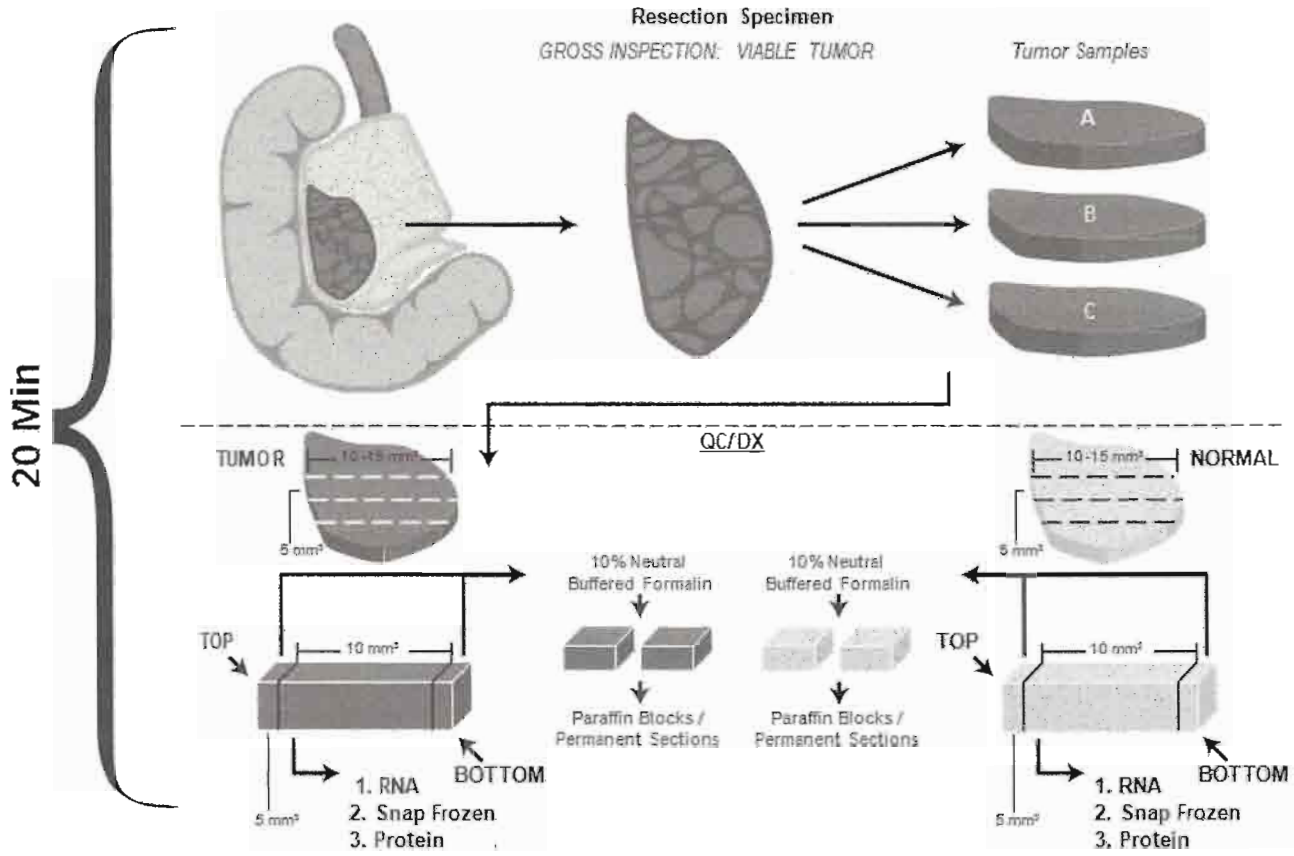
The TXCCR vials will be urgently (overnight) shipped to TTUHSC according to the shipping instructions provided in the detailed TCRB Pathology protocol.

ATTACHMENT 6

Pancreatic Cancer Sequencing Consortium: Specimen Collection Method and Flowchart

Pancreatic Cancer Sequencing Consortium

Specimen Collection Flowchart



Pancreatic Tumor Collection Method (W. Fisher / S. Hodges)

After excision of tissue from the patient, the specimen is placed in a sterile bag on ice and transported to cryostat by our research coordinator (tissue advocate). A realistic time from excision to preservative immersion should be no more than 30-60 minutes and should always be tracked and recorded.

After frozen sections are complete, we work with the pathologist to collect as large a sample as is reasonable. At a minimum, we try to achieve a 1 cm slice of tumor and normal tissue. An effort should be made to harvest the samples away from necrotic, hemorrhagic, or fibrotic capsular tissue since these factors risk the purity and abundance of tumor cells. The slices are divided into three sections and ~1mm ends cut from each section. The specimen for RNA study should be incubated overnight in RNAlater solution at 4°C so that the solution penetrates the tissue and then frozen at -80°C. The specimen for DNA study should be fresh frozen in dry ice and 95% alcohol. The specimen for protein study should be immersed in a proteinase inhibitor solution (Roche) and then frozen in dry ice and 95% alcohol and stored at -80°C. Screw cap cryovials should be used for all specimens because they are leak proof and stable to freezing. We use 2.0 ml cryovials.

The ends are preserved in 10% Formalin and designated which ends are associated with the central sections for histologic examination. This is a critical quality control measure because the pathologist cannot accurately distinguish tumor from surrounding fibrosis or pancreatitis on gross examination at the time of specimen procurement. Specimens should be handled with gloves, and clean instruments changed between tumor and matched normal tissue, to prevent contamination with foreign DNA, RNA etc. When the tissue quantity is ample, extensive study can be achieved using a sample piece for immunohistochemistry after freezing in OCT and tissue microarrays can be constructed from FFPE tissue.

Irrespective of the exact preservation solution, the time that elapses between the procurement of the specimen until fixation or immersion into preservative and freezing is the most important factor. The temperature at which the sample is kept, transported, and processed also needs to be considered and standardized. These parameters need to be standardized since both proteins and nucleotides are subject to enzyme degradation and chemical modification. This is particularly true for a pancreatic cancer tissue resource since pancreatic specimen have an abundance of pancreatic enzymes which may be active during warm ischemia.

We recommend use of PAXgene blood collection tubes, which are commercially available and simplify storage of blood samples without the need to separate blood constituents. These tubes contain a proprietary blend of reagents that can stabilize the cellular constituents of blood for up to 14 days at room temperature, 28 days at 4°C, or indefinitely at -80°C allowing some time until the DNA is extracted.

Patient serum and pancreatic juice are also very valuable and should be collected for future studies that will concern possible clinical correlations with soluble biomarkers. These studies may lead to much needed diagnostic tests for pancreatic cancer. Genetic changes, such as KRAS mutations, are already beginning to be studied in pancreatic juice. Pancreatic juice can be obtained in the operating room at the time of resection by aspiration from the pancreatic duct at the transaction margin using a small syringe and plastic angiocath. The juice is placed in a cryovial, frozen in dry ice and 95% alcohol, and stored at -80°C.

Texas Cancer Cell Repository

Standard Operating Procedure 01

Collection of Tissue, Blood, or Bone Marrow for Biobanking and for Establishing Continuous Cell Lines and Xenografts From Neoplasia

Current Versions of this SOP and all updates are available at:

www.COGcell.org

Version 1.1

June 1, 2011

1.0 GUIDELINES FOR PROCURING, PROCESSING, AND SUBMITTING SAMPLES

Surgeons, pathologists, oncologists, pulmonologists, and radiologists are all important contributors to cancer banking protocols, and it is important that all are provided copies of this SOP. Surgeons should obtain the maximum amount of tumor that is prudent at the time of biopsy or resection. The resected specimen should be placed in a sterile container, covered with saline-soaked gauze, and sent to the pathologist's attention immediately. Pathologists must maintain the sterility of tumor specimens, and allocate as generous an amount as possible for biological studies and tumor banking, while retaining sufficient tissue to determine accurate conventional histology (diagnosis, differentiation, margins, etc.) and (if required) ultrastructural examination or special stains. Blood, marrow, ascites, and pleural effusion samples are all important specimens obtained via oncologists, pulmonologists, and radiologists, but are less time-sensitive than surgically resected tissue.

THE FOLLOWING SAMPLES SHOULD BE OBTAINED AND SHIPPED WHEN APPROPRIATE.

Please include a paper copy of a SPOC or TXCCR Viable Tissue Biology Data Sheet (available at sponc.org and txccr.org) with every sample. Please label all tubes and other primary containers with the appropriate clinical banking protocol, Patient ID Number, patient initials (if allowed by protocol) and the collection date & time. Also, if possible, please send us tissue/blood/bone-marrow specimens no older than 2 days after obtaining from the patient. Unless there are circumstances preventing it, all viable (non-frozen) samples should be shipped via Fed Ex the same day as collection from the patient. Specific information concerning the specimen collections is given below.

1.1 Tumor Tissue

Viable, fresh tumor tissue for cell line and xenograft generation. Fresh, aseptic, viable tumor tissue (total 50 to 500 mg) should be minced with sterile scissors or a scalpel into pieces weighing approximately < 100 mg (i.e. pieces of tumor < 100 µL in volume) and placed into sterile RPMI-1640, DMEM, L-15 or other suitable tissue culture medium (if at all possible, 10% fetal bovine serum should be added to culture medium). It is also preferred that the medium contain 50 to 100 micrograms/ml of gentamicin. Tumor should be placed into a sterile polypropylene or glass tube or vial in tissue culture medium using strict aseptic technique in a, and kept at room temperature. It is preferred that very small samples (~100 mg) be placed in 2 ml tubes; sterile tubes used to cryopreserve cells work well for this purpose. The tubes should be sealed with parafilm and labeled with the patient's name, birth date, BPC number and SPOC patient ID number. Packaging for shipping should be as for any biological specimen. Although samples can be sent in envelopes, submission in small styrofoam boxes (to avoid radical temperature shifts) is strongly encouraged. Containers with transport medium for viable tumor tissue may be requested by emailing Tito Woodburn at TITO.WOODBURN@TTUHSC.EDU or to Dr. Patrick Reynolds at PATRICK.REYNOLDS@TTUHSC.EDU (or) by calling the lab at 806-743-2707 (see www.TXCCR.org for more contact details). Please use the same contact to obtain a Fed Ex number to cover the cost of the shipment.

Snap frozen tumor tissue for genomic studies. In addition to viable samples collected for cell line and xenografts, it is important to also bank snap-frozen tissue for genomic studies and for comparison to cell lines and xenografts established from the patient. As much tissue as can be snap frozen should be provided, after all tissue needed for clinical purposes has been obtained, and once a small amount for cell culture is obtained.

Tissue should be snap-frozen (liquid nitrogen is preferred for this) to -86 deg C or colder. The time from removal of tissue from in vivo blood supply (devascularization) to snap freezing should be < 60 minutes, with 30 minutes or less preferred. Tissue that is handled beyond those time limits should still be processed, but the time from devascularization to snap freezing should be noted for all specimens, especially those beyond 60 minutes.

Frozen section material. Tumor tissue frozen onto a "chuck" with OCT is requested whenever possible. As many Pathology laboratories prefer to process frozen tissue as a "positive control" in a case that may otherwise not have tumor, it is requested that pathologists reserve the frozen section tissue as such while processing the remaining specimens. Once acceptable tumor is demonstrated in subsequent specimens, it is requested that the frozen section tissue be forwarded to us as described below. If confirmatory FFPE histopathology is needed from the block frozen on the chuck, it is requested that a small piece be cut from the block and placed in formalin and retained, and the bulk of the block of tissue frozen for the frozen sections be pried frozen from the chuck, kept frozen and placed in a small plastic container or wrapped in tin foil and included with the snap frozen tissue for submission to TXCCR.

1.2 Pleural Effusion or Ascites Fluid

Pleural effusions and ascites from cancer patients with tumors in lung or the peritoneum can be very rich sources of viable tumor cells for banking and for expansion as cell lines and xenografts. We request any size tap (a few mls from a diagnostic tap to a liter from a therapeutic tap) be provided whenever possible. The fluid should be heparanized with ~ 10 units of sodium heparin per ml of fluid, handled in an aspetic fashion, and send shipped to the laboratory in a Styrofoam container to prevent large temperature ships. During the summer a cold pack (if available) should be placed in the container, but insulated from the sample, to prevent high temperatures. Sending an entire bag or bags of fluid from a therapeutic tap is the most ideal specimen.

1.3 Blood or Bone Marrow

In the case of leukemias with circulating blasts or other hematological cancers, blood and/or bone marrow may be submitted. For leukemia patients with known or suspected marrow disease and minimal circulating blasts, bone marrow is preferred. For patients with enough circulating blasts to provide at least 2 million cells (generally ~ 1000/mm3) blood is preferred. Blood obtained post-mortem from leukemia, lymphoma, or solid tumor patients (exduding CNS tumors) often can yield cell lines and should be obtained whenever possible (see below for details). The pellet of cells from a clot or plasma tube, drawn for other purposes and frozen, is requested for all patients from whom material is submitted for cell culture/xenografts. DNA extracted from these samples will be used to verify the patient origin of established cell lines and xenografts. Blood samples should be sent at ambient temperature using the same procedures as for tumor tissue; frozen blood pellets should be sent on dry ice; batching to send multiple samples is encouraged for frozen shipments.

Pheresis samples from leukemia patients with high circulating blast counts are especially desirable. If at all possible these should be submitted after dilution 1:1 or 1:2 with sterile tissue culture medium, and should be submitted on wet ice or a cold pack, the latter insulated from the phersis bag. The larger the quantity of cells submitted the better.

1.3.1 Blood Samples
Ten to thirty ml of blood anticoagulated with sodium heparin, 100 units/ml of blood, (NOT lithium heparin) or placed into a green top tube. Keep blood samples at room temperature. Label tube with patient Study ID number, patient initials (if allowed by institutional protocol), and date and time obtained.

1.3.2 Bone Marrow Samples
A minimum of 2-3 ml (but no upper limit) should be anticoagulated with sodium heparin, 100 units/ml of bone marrow (NOT lithium heparin), place in sealable tube and seal the outside of tube(s) with parafilm, or placed into a green top tube, and seal the stopper with parafilm. Keep bone marrow samples at room temperature. Label tube with SPOC patient ID number, birth date, and BPC number.

1.3.3 Note
For leukemia specimens, samples submitted should contain a minimum of approximately 5 million leukemic blasts. For example, in the case of a patient with 5000 blasts per mm3, 1 ml of blood would yield the minimum amount of cells (but more is requested if at all possible). In a patient with 500 blasts per mm^3 10 mLs would be required.

1.3.4 Post-Mortem Blood Samples
Post-mortem blood can contain viable solid tumor or leukemia cells for up to 6 hours after death. We request that whenever possible these be submitted, and can be done in the context of a post-mortem exam limited to removing blood. Blood should be drawn as soon as possible post-mortem, ideally via the central line using heparinized syringes. An alternative is to obtain blood by an aseptic cardiac puncture, either percutaneous or during the conduct of a post-mortem. Submit any amount of blood, but 100 to 200 mLs is preferred.

1.3.5 Discarded Blood Cell Pellets or Clots
A 0.5 to 1 ml extra blood sample, or the pellet of cells from a clot or plasma tube drawn for other purposes, is requested for all patients in which material is submitted for cell culture/xenografts. DNA extracted from these samples will be used to verify the patient origin of established cell lines and xenografts. While sending these samples on ice or frozen is preferred, submission at room temperature is acceptable.

1.4. Blood samples required on all patients.
In order to provide a source of non-malignant cells for genomic comparison to the cancer cells we request that 30 mls of heparanized blood be obtained. Minimal amount requested is 10 mls but 30 mls is strongly preferred. Blood obtained in vacutainers can be sent in those containers, while blood obtained by syringe should be transferred aseptically to a vacutainer or similar sealed and robust container for shipping.

2.0 SHIPPING OF TISSUE/BLOOD SAMPLES
Shipping: Samples should be in a sterile inner container and sealed in a light-tight outer container and sent at room temperature. Shipment in small Styrofoam containers is preferred, to insulate samples from extreme temperature changes. However, shipment in standard biological specimen envelopes (with inner padding) is a suitable alternative. During the summer a cold pack (if available) should be placed in the container, but insulated from the sample, to prevent high temperatures.

All viable specimens should be shipped at ambient temperature or cooled with a cold pack (not frozen) via Federal Express PRIORITY OVERNIGHT using the TXCCR Federal Express Account number (contact TXCCR for the number). Arrange for Federal Express pick-up through your usual institutional procedure, but stress that pickup is at your institutional address. If specimens are sent on a Friday, be sure to indicate Saturday delivery on the air-bill and contact Tito Woodburn (information below) for a shipping address (TTUHSC does not accept Saturday deliveries).

Please include a paper copy of the SPOC or TXCCR Viable Tissue Biology Data Sheet with each shipment of specimens.

When possible, collection of separate samples for this protocol (i.e. placed in separate tubes and labeled as for TXCCR), even if submitted via another resource lab, is requested.

Advanced notice of incoming samples is kindly requested by email or phone, especially if shipment of material may arrive on a weekend or holiday. Emailing of the tracking information is especially helpful. If shipment of material may arrive on a weekend (i.e. when shipping is done on a Friday) or holiday, advance arrangements must be made with the laboratory.

Laboratory Contact: Tito Woodburn, Lab phone: 806-743-2707. Email: TITO.WOODBURN@TTUHSC.EDU. If it is impossible to contact the lab prior to shipping, it is requested that the tracking number and carrier information for the shipment be emailed to the laboratory when shipping the specimen.

All samples should be sent directly to:
C Patrick Reynolds, MD PhD
Cancer Center Core Labs STOP 9445
Texas Tech University Health Sciences Center
School of Medicine Cancer Center
3601 4th Street
Lubbock, Texas 79430-6450

Phone: 806 743-1558
Fax: 806 743-2691
Email: PATRICK.REYNOLDS@TTUHSC.EDU

Contact in lab: Tito Woodburn
Lab phone: 806-743-2707
Email: TITO.WOODBURN@TTUHSC.EDU