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.TXCCR.org

Version 1.1

July 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 date, time, and appropriate banking patient ID numbers and patient initials (if allowed by institutional protocol),. 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. Alternate contact is: 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 aseptic 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 (excluding 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

It is highly important to collect blood samples on patients who provide tissue for cell line and xenograft generation. If those samples are not collected at the time of initial tissue collection, and a cell line or xenograft is generated, the TXCCR will contact the submitting center for the initial sample and request that a blood sample be provided, if the patient agrees, next time the patient is seen in clinic.

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 patient ID number and date.

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 Fed Ex 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