Common Blood Collection and Plasma Processing Protocol

Clinical Proteomics Technologies Assessment for Cancer (CPTAC) Biospecimen Working Group

Note: This protocol was developed in order to provide a set of plasma samples for CPTAC-wide experimentation that are collected under similar blood collection and plasma processing conditions, to assure, as much as possible, that differences in molecular profiles of such specimens will not be due primarily to different collection and processing conditions. The common protocol was developed after analysis of many protocols in use and an examination of the available scientific rationales for different steps in these protocols, as well as the reasonable accommodations to a common protocol required by the different program sites. Please note in particular that the use of refrigeration in processing, as prescribed here, can result in platelet activation and thus may result in molecular profiles that are distinct from protocols performed at room temperature. This protocol assumes that informed consent has been obtained and a method for tracking consent is in place.

The document, in its present form, is confidential and not to be distributed without specific approval from CPTAC Program Coordinating Committee. The NCI makes no guarantee of the adequacy of this protocol for any intended use.

MATERIALS:

- Rubber (non-latex) band for tourniquet
- Antiseptic wipes
- 21-23 gauge Butterfly needle with attached tubing and Luer adapter
- 3 ml tube for capturing initial 3 ml whole blood discard (unless the EDTA tube is drawn later in a blood draw of multiple tubes)
- Ice bucket and ice for chilling tubes
- Refrigerated centrifuge capable of spinning samples at 1500-2000 x g
- 10 mL lavender-top K2 EDTA BD Vacutainer® venous blood collection tubes (BD 366643, 10 mL plastic, whole blood EDTA tube with lavender top)
- Sterile disposable 10 cc pipette
- 15 mL polypropylene Falcon tube (BD 352196)
Simport 3 ml cryovial (Sim-T310-3A)

Labeling system for all of the above tubes to keep the biospecimens matched (EDTA tube, Falcon tube, and cryovial). Plan to obtain labels from biorepository and have labels on-site.

PROCEDURES

I. BLOOD COLLECTION
   a. Patient position
      i. Patient must be seated at least 5 minutes before the draw
      ii. The arm should be positioned on a slanting armrest in a straight line from the shoulder to the wrist. The arm should not be bent at the elbow.
   b. Source of blood
      i. Median, cubital, basilic, or cephalic veins (never from a port)
   c. Tourniquet technique
      i. Apply a tourniquet 2 inches above the antecubical fossa or above area to be drawn with enough pressure to provide adequate vein visibility. Have the patient form a fist. Select the site for venipuncture.
      ii. Clean the forearm of the patient with antiseptic wipe in a circular motion beginning at the insertion site. Allow the antiseptic to dry.
      iii. Anchor the vein by placing the thumb 2 inches below the site and pulling the skin taut to prevent the vein from moving. The holding finger is placed below the site, not above, to prevent accidentally sticking the finger with the needle.
      iv. Using the dominant hand, insert either the vacutainer needle or the butterfly needle (if using vacutainer needle, attach hub first). Push the evacuated tube onto the vacutainer hub or the Luer adapter if using a butterfly.
      v. Release the tourniquet once blood flow is established. [The elapsed time for the tourniquet should be less than 1 minute. In the case that additional time is required, the tourniquet must be removed in a fashion that restores both the circulation and normal skin color.]
      vi. Make sure that tube additives do not touch the stopper or the end of the needle during venipuncture.
   d. Drawing blood into tubes
      i. Pre-chill 10 mL lavender-top K2 EDTA BD Vacutainer® venous blood collection tubes (BD 366643, 10 mL plastic, whole blood EDTA tube with lavender top) on ice for at least 5 minutes.
      ii. Aspirate and discard approximately 3 mL of blood prior to collecting the EDTA plasma for CPTAC. [If EDTA tube for CPTAC is in a later order of draw of multiple tubes, there is no need to collect this discard.]
iii. For CPTAC, completely fill the tubes. Carefully remove the tubes when full without dislodging the needle.

e. Inversion of EDTA tubes
   i. Immediately after allowing the lavender-top Vacutainer® tube to completely fill, slowly and gently invert the tube 8-10 times
   ii. Immediately insert the tube into wet ice

f. Immediately place on ice
   i. Sample processing and freezing must be completed within 90 min of collection

II. PLASMA PROCESSING

a. Centrifugation I
   i. Within 30 minutes of collection, centrifuge at 1500 g for 15 min in a refrigerated centrifuge (4 °C).

b. Collection of supernatant I
   i. Transfer plasma (using sterile disposable 10 cc pipette) to centrifugation tubes (BD 352196, 15 mL polypropylene Falcon tube), taking care to not disturb the buffy coat.

c. Centrifugation II
   i. The secondary tubes are then centrifuged at 2000 g at 4º C for 15 minutes to remove all potentially remaining cells.

d. Collection of supernatant II
   i. After second centrifugation, transfer the top 2.5 ml of the supernatant into a 3 ml cryovial (Simport Cryovial Sim-T309-3A; sterile cryovials with silicone washer seal and external threads; self-standing; certified DNase-free, RNase-free, DNA-free and Pyrogen free; available through LABSCO).
   ii. Additional aliquoting and storage to be determined by each site at their discretion.

III. STORAGE AND SHIPMENT

a. Storage at the collection and processing site
   i. Biospecimens should be immediately placed on dry ice or in a -70 to -80°C freezer.
   ii. Biospecimens should be stored at -70 to -80°C before shipment to the biorepository.
   iii. Biospecimens should be shipped to the biorepository. **Periodicity to be determined and tailored to the site.**

b. Transfer and Shipment to Biorepository
   i. Do not permit specimens to thaw
   ii. Ship to biorepository in Frederick on at least 5 lbs of dry ice.
   iii. Specimen data sheets coded with the same label on cryovial: **to be developed**

c. Storage at Biorepository
   i. Cryovials will be immediately transferred to the vapor phase of liquid nitrogen for storage at the biorepository

d. Aliquoting at Biorepository
i. Once the experimental plans have been established for analysis of the CPTAC biospecimens within the program, the biospecimens will be thawed on ice, gently mixed, and aliquoted into cryovials for shipment and/or continued storage at the biorepository. Aliquot size(s): to be determined

ii. Aliquots will be immediately frozen in the vapor phase of liquid nitrogen (?dry ice?): to be determined

e. Shipment of aliquots (on at least 5 lbs of dry ice) to CPTAC laboratories for experimental analysis

f. Plans for storage and distribution of CPTAC biospecimens at the conclusion of the 5-year cooperative agreement: to be determined

1 Procedure adapted from the NCI/EDRN/SPORE Lung Cancer Biomarkers Group SOP for Collection of Serum and Plasma Samples for Proteomic Analysis

2 Procedure adapted from HUPO’s recommended SOP for EDTA-Plasma Specimen Collection (Plasma Proteome Project, 2006)

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