Prospective Biospecimen Collection Protocol
Blood Collection and Processing for Plasma and Whole Cell Components
v2.0

Overview
The Clinical Proteomic Tumor Analysis Consortium (CPTAC) sponsored by the NCI's Office of Cancer Clinical Proteomics Research is a comprehensive and coordinated effort to accelerate the understanding of the molecular basis of cancer through the application of robust, quantitative, proteomic technologies and workflows. The overarching goal of CPTAC is to improve our ability to diagnose, treat and prevent cancer. To achieve this goal in a scientifically rigorous manner, the NCI launched CPTAC to systematically identify proteins that derive from alterations in cancer genomes and related biological processes, and provide this data with accompanying assays and protocols to the public.

CPTAC consists of a network of Proteome Characterizations Centers (PCCs) and a Data Coordinating Center (DCC) serving as a hub and central repository for CPTAC data. CPTAC will be expanded to include 1) a network of Tissue Source Sites (TSS) to obtain clinical specimens for proteomic and genomic analysis, 2) a Biospecimen Core Resource (BCR) to serve as a repository for tissue and associated, de-identified clinical data submitted to the program, and 3) a Genomic Characterization Center (GCC) dedicated to the genomic analysis of CPTAC specimens.

PURPOSE

The purpose of this protocol is to establish a uniform procedure for collecting and processing blood samples obtained as part of the CPTAC prospective tissue procurement project. The blood samples are intended to provide 1) plasma for subsequent biomarker discovery and 2) cellular blood components for germ line genetic analysis of tissue donors. This protocol applies to TSSs collecting tissues from breast, colon, and ovarian cases.

This procedure was originally developed to provide plasma samples for CPTAC-wide experimentation 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 procedure for obtaining plasma 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. 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. The original procedure has been expanded to include the collection and storage of the cellular blood components remaining after fractionation to be used for determining the germ line genetics of patients providing tumor and normal tissue to the CPTAC.

The original procedure was adapted from the NCI/EDRN/SPORE Lung Cancer Biomarkers Group SOP for Collection of Serum and Plasma Samples for Proteomic Analysis HUPO's recommended SOP for EDTA-Plasma Specimen Collection (Plasma Proteome Project, 2006).

Scope

The protocol applies to any samples obtained by a Leidos Biomedical, Inc. CPTAC Tissue Source Site subcontractor for submission to the CPTAC Biospecimen Core Resource (BCR) in conjunction with the submission of tumor and corresponding normal tissue for proteomic analysis.

Procedures

Blood Collection Requirements

1. Blood must be collected before any anesthesia is administered prior to commencing the procurement.
2. Patient consent must have been obtained.
3. All other requirements for the respective CPTAC Tissue Procurement protocol have been satisfied
4. Preferred Method for Collecting Blood
    a. Venipuncture from a readily accessible peripheral vein using a conventional blood collection system and the appropriate Vacutainer® (Product Number BD366643; provided by the BCR).
5. Acceptable Alternative Methods for Collecting Blood
    a. Venipuncture from a readily accessible peripheral vein using a syringe and needle/butterfly with the contents of the syringe immediately transferred to the appropriate Vacutainer® (Product Number BD366643; provided by the BCR).
    b. Withdrawal via syringe from a peripheral intravenous catheter with the contents of the syringe immediately transferred to the appropriate Vacutainer®. (Product Number BD366643; provided by the BCR). If this method is used, the TSS shall employ best practices for obtaining blood samples from a peripheral intravenous catheter (e.g., Heyer N.J. et al. Effectiveness of practices to reduce blood sample hemolysis in EDs: a laboratory medicine best practices systematic review and meta-analysis, Clin Biochem. 45, pp. 1012-32, 2012 [PMID 22968086]).
6. Other Requirements
    a. Regardless of the collection method employed, slowly and gently invert the Vacutainer® 8-10 times after filling.
    b. Keep the Vacutainer® on wet ice after mixing.

Sample Processing Steps (must be completed within 90 minutes of collection)

1. Centrifugation 1
    a. Within 30 minutes of collection, centrifuge at 1500 g for 15 min in a refrigerated centrifuge (4° C).

   b. Transfer plasma using sterile disposable 10 mL pipette to centrifugation tubes (Product Number 352196; 15 mL polypropylene Falcon tube; provided by the BCR), taking care to not disturb the buffy coat.
   c. Recap Vacutainer® tube containing buffy coat and red cell mass and return to wet ice.
2. Centrifugation 2
   a. Centrifuge the 15 ml tube containing the plasma at 2000 g at 4° C for 15 minutes to remove all potentially remaining cells.
   b. Transfer the top 2.5 ml of the supernatant into 2.0 mL cryovials (~1.25 ml per vial; Corning Product Number 430488; provided by the BCR with a blue cap insert).
3. Transfer buffy coat and red cell mass (using sterile disposable 10 mL pipette) into 2.0 mL cryovials (~1.50 mL per vial; Corning Product Number 430488; provided by the BCR with a white cap insert).
4. Immediately freeze tubes containing the processed plasma and buffy coat/red cell mass and freeze in LN2 vapor.
5. Complete the appropriate section of the CPTAC Submission CRF.
6. Store at vapor phase LN2 temperature until shipping to the CPTAC BCR.