

# Exploring the effects of different blood collection and processing protocols on molecular profiles

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Abstract: **The CPTAC Biospecimen** Working Group

There is considerable heterogeneity in the way that human specimens (tissues and biofluids) are collected, processed, stored, and disseminated. There is also limited scientific evidence available relating to how this diversity of specimen handling affects the quality and reproducibility of data from cancer research. The NCI recently established the Office of Biorepositories and Biospecimen Research (OBBR) to quide, coordinate, and develop the NCI's biospecimen resources and capabilities, with the overall goal of increasing access to high-quality biospecimens for research. A division of the OBBR, the Biospecimen Research Network (BRN), was formed to perform analytical studies to inform the development of appropriate data-driven, evidence-based practices and protocols for specific specimen types and molecular analysis platforms. Blood is a readily accessible biofluid for the potential detection of disease associated biomarkers, but it has been suspected that the diversity of methods used for its collection and storage could result in artifacts that affect the presentation of various biomarkers and therefore complicate target validation and reproducibility. As part of its mission, the CPTAC Biospecimen Working Group was tasked with collecting and analyzing the blood collection and processing protocols across the program and designing a single optimized plasma protocol for use within the program. A common protocol was designed and is presented here; however, the process was hampered by the lack of substantial data supporting various steps in the different protocols analyzed by the Working Group. To provide this data, OBBR has initiated a series of research studies in collaboration with SAIC-Frederick. These studies will be designed to identify preanalytic variables which may cause irreproducibility of proteomic profiles and will include testing of the CPTAC protocol. To begin these studies, two plasma processing protocols will be compared systematically to determine whether they result in significant

differences in molecular profiles. Assistance is sought to help choose reproducible proteomics methods for assessing the molecular readout of specimens prepared using these two protocols.

# **CPTAC Working Group on Biospecimens and the Biospecimen Research Network (BRN):**

Developing a Common Plasma Protocol

### Rationale: Different blood collection and processing protocols can result in different molecular profiles

- · Collect and compare blood collection, plasma processing, and storage protocols from the different CPTAC institutions
- Analyze differences and use evidence-based methodology to develop a common protocol
- •BRN: Conduct experiments in areas where the effects of the variability between protocols is not understood

### Plasma Collection and Storage Variables

Plasma collection protocol varied significantly among 5 institutions in CPTAC

Procedure	Variations
Venipuncture (Needle gauge, details of blood collection set)	Needle gauge and priming volumes differed
Phlebotomy (tourniquet technique, patient position, tube order, blood source, volume collected)	Patient position varied from seated to lying down, variable tube orders, variable venipuncture sites
Collection device	Different types of tubes
Blood derivative and processing (anticoagulant type, processing time and protocols)	Different anticoagulants, different temperatures, different centrifugation temperatures and speeds
Amount of elapsed time between collection and storage	Variations between institutions
Storage (temperature, elapsed time for storage, storage duration, storage material, shipping temperature)	Different elapsed times before storage, different storage temperatures

### Preliminary Observations

 Differences in blood collection techniques might result in sample heterogeneity due to ex-vivo activation of signaling pathways, degradation of proteins and key enzymes, activation of platelets, etc.

. There is a lack of substantial data supporting various steps in the different protocols analyzed by the Working Group

### BRN: Transdisciplinary Research Approach for Developing Uniform, Evidence-Based Protocols

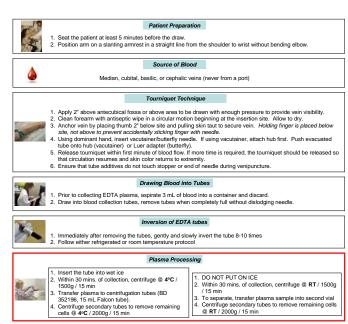
- Plan and execute prospective analytical studies
- Determine how the results of DNA, RNA and protein analysis are affected by defined pre-acquisition and post-acquisition specimen variables
- OBBR, in collaboration with SAIC-Frederick, will perform experiments to test the CPTAC and other blood collection and processing protocols and identify key preanalytical variables that may contribute to differences in molecular profiles

### BRN Studies in Plasma Collection. Handling, and Storage

The experiments described here focus on how temperature may or may not affect the proteomic and genomic profiles of similarly collected blood samples

- Objective: To identify post-acquisition variables that affect the components and properties of blood
- Variable addressed: Does the temperature during plasma processing affect its molecular profile?

## **Experimental Protocol:** Blood Collection and Plasma Processing



Aliquot and Storage

Aliquot into screw-cap cryo-vials Store samples in -80°C tubes

## **Developing an Evidence-Based Protocol:**

### What is the best method/technology for plasma analysis? What Proteomic Analyses should be performed? What molecular markers should be tested?

Focus on Reproducibility: Produce verifiable evidence for specific steps in blood collection and processing

#### Comprehensive Metabolic Profile (CMP):

<ul> <li>Glucose</li> </ul>	
<ul> <li>Calcium</li> </ul>	
<ul> <li>Kidney and Liver Tests</li> </ul>	

· Proteins (Total and Albumin) Electrolytes (Na<sup>+</sup>, K<sup>+</sup>, CO<sub>2</sub>, Cl<sup>-</sup>)

#### Lipid profile:

Total Cholesterol

LDL Cholesterol

 HDL Cholesterol Tryglycerides

### Complete Blood Profile (CBC):

· White blood cell count (WBC, leukocyte) · White blood cell types (WBS differential) Red Blood cell count (RBC)

 Hemoglobin · Red Blood cell indices

· Hematocrit (HCT, packed cell volume, PCV)

#### · Platelet count Endogenous Hormones:

Estrogen (Estradiol)

Testosterone

#### **Biomarkers:**

· Carcinoembryonic anitgen (CEA) · CA 19-9 (male and female)

 PSA (male) brandykinin

- Technologies:
  - Mass spectrometry SELDI MALDI ELDI MS/MS

· Antibody Arrays Protein Arrays

"Metabolomics"

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Increasing access to high quality

biospecimens for research by

guiding, coordinating, and

developing NCI's biospecimen

resources and capabilities

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