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 guide, 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

Patient Preparation

1. Seat the patient at least 5 minutes before the draw.
2. Position arm on a slanting armrest in a straight line from the shoulder to wrist without bending elbow.

Source of Blood

Median, cubital, basilic, or cephalic veins (never from a port)

Tourniquet Technique

1. Apply 2" above antecubical fossa or above area to be drawn with enough pressure to provide vein visibility.
2. Clean forearm with antiseptic wipe in a circular motion beginning at the insertion site. Allow to dry.
3. Anchor vein by placing thumb 2" below site and pulling skin taut to secure vein. *Holding finger is placed below site, not above to prevent accidentally sticking finger with needle.*
4. Using dominant hand, insert vacutainer/butterfly needle. If using vacutainer, attach hub first. Push evacuated tube onto hub (vacutainer) or Luer adapter (butterfly).
5. Release tourniquet within first minute of blood flow. If more time is required, the tourniquet should be released so that circulation resumes and skin color returns to extremity.
6. Ensure that tube additives do not touch stopper or end of needle during venipuncture.

Drawing Blood into Tubes

1. Prior to collecting EDTA plasma, aspirate 3 mL of blood into a container and discard.
2. Draw into blood collection tubes, remove tubes when completely full without dislodging needle.

Inversion of EDTA tubes

1. Immediately after removing the tubes, gently and slowly invert the tube 8-10 times
2. Follow either refrigerated or room temperature protocol

Plasma Processing

1. Insert the tube into wet ice
2. Within 30 mins. of collection, centrifuge @ **4ºC** / 1500g / 15 min
3. Transfer plasma to centrifugation tubes (BD 352196, 15 mL Falcon tube).
4. Centrifuge secondary tubes to remove remaining cells @ **4ºC** / 2000g / 15 min

1. DO NOT PUT ON ICE
2. Within 30 mins. of collection, centrifuge @ **RT** / 1500g / 15 min
3. To separate, transfer plasma sample into second vial
4. Centrifuge secondary tubes to remove remaining cells @ **RT** / 2000g / 15 min

Aliquot and Storage

1. Aliquot into screw-cap cryo-vials
2. Store samples in -80ºC 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):

- Glucose
- Calcium
- Kidney and Liver Tests
- Proteins (Total and Albumin)
- Electrolytes (Na^+, K^+, CO_2, Cl^-)

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)
- Platelet count
- Hematocrit (HCT, packed cell volume, PCV)
- Hemoglobin
- Red Blood cell indices

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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