Assessing the Effects of Preanalytical Variables on Molecular Research: The Biospecimen Research Network

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NCI Best Practices Forum
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NATIONAL CANCER INSTITUTE

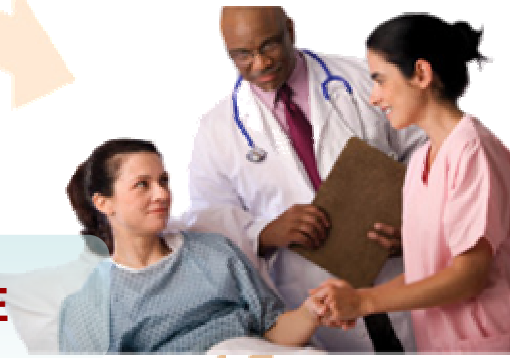
Translational Research Promises to Advance Molecular Medicine for Cancer Patients

OBBR Office of Biorepositories and Biospecimen Research

Molecular Data

Diagnosis / Therapy

Translational Research



PERSONALIZED CANCER CARE

Biospecimen Distribution

Biospecimen Collection

Biospecimen Processing and Banking

Progress in Cancer Treatment and Research are Dependent on High Quality Human Specimens

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Identification of targets for drug development, treatment and prevention

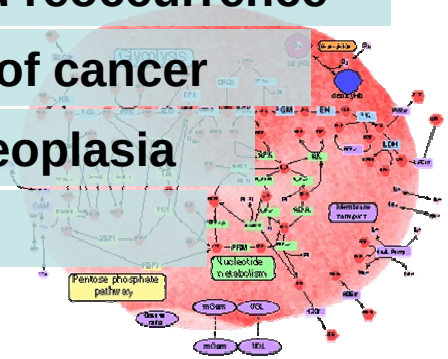
Identify biologic variations that determine drug efficacy and drug toxicity

Defining markers for susceptibility, screening and reoccurrence

Development of molecular based taxonomy of cancer

Elucidation of molecular mechanisms of neoplasia

Validation of new therapeutics



All Depend On High-Quality, Annotated Human Biospecimens

Multiple variables can affect the molecular integrity of the biospecimen

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Variables (examples):

- Antibiotics
- Other drugs
- Type of anesthesia
- Duration of anesthesia
- Arterial clamp time

Time 0

Variables (examples):

- Time at room temperature
- Temperature of room
- Type of fixative
- Time in fixative
- Rate of freezing
- Size of aliquots








Patient | Medical/ Surgical Procedures | Acquisition | Handling/ Processing | Storage | Distribution | Scientific Analysis | Restocking Unused Sample

Pre-acquisition **Post-acquisition**

Potential Effects of Biospecimen Variables

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- **Effects on Clinical Outcomes**
  - **Potential for incorrect diagnosis**
    - Morphological/immunostaining artifact
    - Skewed clinical chemistry results
  - **Potential for incorrect treatment**
    - Therapy linked to a diagnostic test on a biospecimen (e.g., HER2 in breast cancer)
- **Effects on Research Outcomes**
  - **Irreproducible results**
    - Variations in gene expression data
    - Variations in post-translational modification data
  - **Misinterpretation of artifacts as biomarkers**

Pathway to Improving Biospecimen Quality: Systematic, Comprehensive Approach

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- **Supporting the development of Best Practices for collection, annotation, processing, and storage, based on <u>scientific evidence</u>**
  - *What evidence is already available?*
  - *What new research is needed?*
  - *How do we get that research done?*
- **The NCI Biospecimen Research Network**

The NCI Biospecimen Research Network (BRN)

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- **Biology**
  - Provide a forum for research results on how biospecimen variables affect molecular analysis:
    - The Biospecimen Research Database: Make existing and emerging biospecimen research data more accessible
    - Upcoming national conference: Biospecimen Science in the Genomic and Postgenomic Era
  - Generate new research data:
    - OBBR Intramural Biospecimen Research Laboratory
    - New Extramural Programs: approved and coming soon
- **Technology Development**
  - IMAT Program – Innovative technologic solutions for biospecimens (RFA)
- **Strategic Partnerships**
  - The College of American Pathologists (CAP)
    - Data-driven, specimen-specific, platform-appropriate SOPS
    - Implementation and monitoring: Laboratory Accreditation Program

Central themes of BRN research

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- "Bridging the gap" between existing clinical practice for biospecimens and emerging technologies for personalized diagnostics and therapies
  - Tissue preservation variables and their impact on downstream applications (e.g., HER2)
  - Robotic surgery vs. manual surgery for prostate – are tissues harvested from robotic surgery suitable for advanced biomarker detection?
- Defining the most significant variables for prospective collection of tissues, blood, and body fluids
  - Effects of pre-acquisition variables and biomolecule extraction methods on biomolecule analysis results in blood
- Developing evidence-based biospecimen quality indicators for specific analytical platforms
  - How to assess whether a banked specimen is suitable for a specific molecular analysis approach?

Biospecimen Research Case Study: Blood Collection and Plasma Processing Variables

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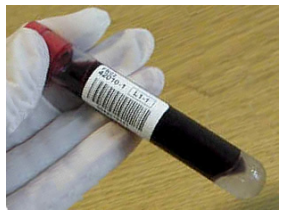


Collection Tubes and Order of draw

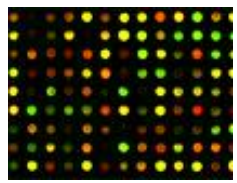
Processing Procedure, Temperature and Time

Blood Draw Procedure

Storage & Distribution

Patient Consent and Preparation

Molecular Analysis



CPTAC and the BRN: Developing and Testing a Common Plasma Protocol

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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 institutions in the Clinical Proteomic Technology Assessment for Cancer Program (CPTAC)**
- ✓ **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 protocol varied significantly among 5 institutions in CPTAC

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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

General Observations

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- **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**

- *Recognizing those caveats* -

- **The CPTAC Working Group came to consensus on a common protocol for blood collection and plasma processing**

BRN Studies in Blood Collection, Processing, and Storage

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- **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 contribute to differences in molecular profiles**

- **First set of experiments:**
  - Does the temperature during plasma processing affect its molecular profile?
  - 4^0 C vs. room temperature processing
  - Other variables kept as constant as possible
  - Aliquots removed at various steps for sample testing

Molecular Analysis: Blood Collection and Plasma Processing

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Focus on Reproducibility:

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

Building Better Biospecimen Resources

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Developing and implementing

state-of-the-science processes that ensure

the molecular integrity and clinical relevance

of human biospecimens

used in cancer research and clinical medicine

Defining the Problems and Refining the Solutions

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We need your input!

http://biospecimens.cancer.gov

biospecimens@mail.nih.gov

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