***Importance of preanalytical factors in biospecimen research***

Biospecimen preanalytical variability can affect the reproducibility of clinical research. Biospecimens are the essential starting materials for the biomarker assays that will enable precision medicine. Clinical assays used for diagnosis and therapeutic decision-making are based on assessment of biological molecules (DNA, RNA, and proteins) from a patient’s biospecimen. Such assays are often based on the detection of one or more biomarkers and must be both accurate and reproducible. False positive or negative results from the evaluation of biomarkers in clinical assays can directly affect patient diagnosis, treatment, and outcomes and can lead to over-treatment, under-treatment, or incorrect treatment.

***An increasing number of reports demonstrate that preanalytical factors such as the handling of a clinical biospecimen prior to analysis can have a significant impact on assay results, which can in turn affect patient care.*** Biospecimen preanalytical factors can directly influence molecular results from assays conducted for basic research, biomarker discovery, biomarker validation, and development of validated clinical assays. In fact, preanalytical processing has been highlighted as a significant impediment in the development of predictive biomarkers for oncology. Preanalytical variability has also been identified as a challenge in the implementation of next-generation technologies in the analysis of tissue biopsies. The methods used to collect, process and store small biopsy biospecimens such as core biopsies, fine needle aspirates (FNAs), and lung aspirates, for example, can vary widely within and across medical institutions and laboratories, introducing variability in such preanalytical factors as biopsy method, needle gauge, vacuum-assistance, biospecimen size/volume, duration and temperature of transport, preservation method, temperature and duration of storage, and number of freeze thaw cycles. A wide variety of preanalytical factors alone and in combination, are known to affect the accurate assessment of biomarkers, adding to the complexity of biospecimen challenges for the clinical laboratory.

New knowledge about preanalytical effects can support evidence-based biospecimen procedures for cancer research activities including clinical trials. Smaller biopsies such as FNAs, core biopsies, and lung aspirates present a unique set of preanalytical factors and associated challenges for reliable biomarker assay results. Such biopsies are clinically valuable now and in the future for assessing current and recurrent disease state as well as for assessing the likely efficacy of therapeutic intervention. Research has shown discordant biomarker assay results when comparing such small biopsies with larger tumor resections.

Detection of biomarkers in blood analytes such as cfDNA (cell-free DNA) and CTCs (circulating tumor cells) is a promising approach for non-invasively tracking tumor behavior over time, potentially enhancing cancer management by assessing tumor burden, detecting recurrence, monitoring early response and identifying drug resistance. Preanalytical considerations are also challenging for such “liquid biopsies,” as inconsistency between sample handling protocols and lack of standardization among analytical techniques has created obstacles for translating cfDNA analysis to clinical practice. CTC analysis, including enumeration and characterization, is also affected by preanalytical factors. Challenges introduced by preanalytical variability also affect the isolation and analysis of exosomes and other extracellular vesicles, underscoring the need for rigorous analytical validation.

Bodily fluids such as urine and cerebrospinal fluid (CSF) have also emerged as biospecimens for potential detection of biomarkers in clinical assays that can directly affect patient diagnosis, treatment, and outcomes. Other biospecimen types such as tissue swabs, tissue secretions, pleural and esophageal aspirates, feces, sweat, breast milk and saliva also have potential application in clinical biomarker research and are included in this FOA.

Differences in assay results can be a result of differences in preanalytical factors such as ischemia times, preservation method, and tumor heterogeneity. Such preanalytical factors may alter biomarker measurements and influence the performance of assay technologies. Variability and biases introduced in the early stages of biomarker assay development, if not addressed and understood, can increase the likelihood of irreproducible data and incorrect conclusions. This is of significant concern for NCI clinical research. Basic and applied biospecimen science investigation will provide valuable insights into how to limit variability in clinical assay results from small biopsies.

***Examples: Effects of preanalytical factors on diagnostic assays***

A classic example of how biospecimen preanalytical factors can have a dramatic influence on assay results is companion diagnostic HER2 assays that guide the therapeutic use of trastuzumab, a breast cancer therapeutic. Recommendations for the reliable assessment of HER2 status in patient biospecimens were established 14 years after the initiation of phase 1 clinical trials in 1992. These measures were developed in response to multiple issues with HER2 reproducibility including preanalytical variability. For example, one study showed that the number of patients reported to overexpress HER2 differed by approximately 20% between specimens processed in local pathology departments and those processed in centralized laboratories.

When investigating whether non- standardized approaches between laboratories directly influenced the clinical assay results, the American Society of Clinical Oncologists (ASCO) and the College of American Pathologists (CAP) concluded that several biospecimen preanalytical factors including fixative, delay time to tissue fixation, method of tissue processing, and time in fixative could influence HER2 assay results. ***A major issue for such guidelines is that there is often a lack of fundamental scientific data on the behavior of clinical biomarker assays in response to biospecimen preanalytical factors.*** Systematic evaluation and increased understanding of the impact of these factors in the context of particular assay platforms and tissue types, and how to mitigate their impact, are necessary to improve current clinical assays and the development of new biomarker assays.

Preanalytical factors also impact the reproducibility of highly sensitive liquid biopsy assays that require optimized conditions for identification of circulating tumor material. For example, blood collection tube type and a time-to-assay delay affect CTC enumeration and characterization of breast cancer patient samples using the high-definition single-cell analysis assay (HD-SCA). CTC recovery, EpCAM and cytokeratin immunostaining, and nuclear content are also affected by storage time and tube type. Concentration and fragmentation of circulating cell free DNA (cfDNA) are also influenced by differences in sample handling procedures. These examples highlight the crucial importance of biospecimen science in effective development and application of clinical diagnostic assays.

***Previous support of Biospecimen Science at the NCI***

To begin to address concerns of preanalytical variability, the NCI established the Biospecimen Research Network (BRN) in 2007. Through an extramural funding program, “Biospecimen Research for Molecular Medicine," an online literature database, “The Biospecimen Research Database” (BRD), and an annual conference, “The BRN Symposium,” the BRN program advanced the field of biospecimen science through systematic investigation of the effects of different biospecimen collection, processing, and storage procedures on downstream molecular analyses. Research contracts were competitively awarded to identify and develop novel approaches to identify key preanalytical factors associated with collection, processing, and storing of blood, plasma and tissue. <https://biospecimens.cancer.gov/about/researchnetwork/projects/default.asp>

*Sponsored studies to date include:*

* Studies demonstrating that preservation methods can impact gene expression of a subset of transcripts differentially;
* Studies on the effects of blood biospecimen collection tubes, processing times, time in freezer, and freeze/thaw cycling on protein integrity;
* The effects of tube type, time on bench and storage temperature on circulating miRNA detection;
* An assessment of the effect of cold ischemia on protein detection in breast cancer tissues;
* The effects of cold and warm ischemia on the detection of gene expression and protein phosphorylation in post resection tissues;
* Studies to evaluate the effects of cold ischemic time, specimen preservation, and storage methods on RNA yield, RNA Integrity Number (RIN) values, transcript integrity and microarray-based measurements of gene signatures.

Results of these studies have provided valuable data on how biopsies and resections should be handled (e.g., minimize cold ischemia time and utilize core biopsies, when possible, for IHC assays; use RNAlater® for biopsies if possible, when gene expression studies are planned) and have built a solid knowledge base for evidence-based practices for biospecimen collection, processing, and storage when conducting blood-based mass spectrometry investigations. Overall, results from the BRN and work summarized in the BRD suggest that biospecimen preanalytical factors can significantly affect research and clinical assay results. However, preanalytical factors of particular importance for small biopsies relating to procurement and alterations in processing and extraction due to reduced biospecimen size remain largely unexplored. Research also demonstrates that the downstream effects of different preanalytical factors can vary with respect to the specific analyte being evaluated and the analytical platforms and/or assays being used. Thus, new and emerging clinical biomarkers and assays could be affected to unknown extents.

**Need for continued research in biospecimen science**

Measurement and validation of clinically relevant biomarkers using diagnostic assays are increasingly challenging as reliance on smaller biospecimens increases and new biomarkers and analysis platforms emerge. Particularly in NCI-sponsored treatment trials such as the NCI Experimental Therapeutics Clinical Trials Network (ETCTN), there is a need for accurate assays for integral markers used for selection of patients, integrated markers for evaluation of the clinical correlation of biomarkers for new agents, and pharmacodynamic markers that indicate whether the target has been engaged.