Advancing Research Through Better Biospecimen Practices

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Addressing Global Health Challenges Through Science Collaboration
Washington, DC
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Biospecimens are Collected for Patient Care and for Research

Biospecimen Collection (Blood, Tissues, Urine, etc.)

Processing in Pathology Lab

Storage

Analysis

Patient Care
Clinical Trials
Research

Clinical Data Collection

Adapted from Peggy Devine
Translational Research Will Lead the Way to Personalized Molecular Medicine

Molecular Data ➔ Translational Research ➔ Diagnosis / Therapy

PERSONALIZED CANCER CARE

Biospecimen Analysis ➔ Biospecimen Collection ➔ Biospecimen Processing and Banking
NCI’s Investments in the Future: Molecular Research Using Human Biospecimens

The Cancer Genome Atlas (TCGA)
National Community Cancer Centers Program (NCCCP)
Clinical Proteomic Technologies Assessment for Cancer (CPTAC)
Innovative Molecular Analysis Technologies (IMAT)
Alliance for Nanotechnology in Cancer
Cancer Genetic Markers of Susceptibility (CGEMS)
Clinical trials correlative science
Molecular epidemiology programs
All Utilize Human Biospecimens
SPORE programs
R01 Research
The lack of high-quality, clinically annotated human specimens is the #1 limiting factor for translational cancer research.
Why Is It Difficult to Acquire High-Quality Specimens and Data?

• Collection, processing, storage procedures differ
• Degree and type of data annotation varies
• Scope and type of patient consent differs
• Access policies are lacking or unknown to potential users
• Materials transfer agreement conditions differ
• Supporting IT structures differ in capacity and functionality

→ WIDE VARIATION IN QUALITY OF SPECIMENS AND DATA
Libraries of Flesh: The Sorry State of Human Tissue Storage

By Steve Silberman  May 24, 2010 | 12:00 pm  | Wired June 2010

Of all the forms of woe that take root in the human genome, the cancer called Glioblastoma multiforme is one of the most merciless. It can infiltrate the brain’s white matter for months before causing any symptoms. By the time memory loss and seizures reveal the presence of an invader, there’s often little to do but minimize the patient’s suffering. Most who are diagnosed with the disease—people like the late senator Edward Kennedy—are dead within two years.

In fall 2005, the National Cancer Institute announced that it was going after glioblastoma and several
OBBR’s Strategic Approach to the Problem

- **STANDARDS:** state-of-the-science guidance for biobanking to harmonize procedures for collection, processing, storage and distribution of biospecimens
  - *The NCI Best Practices for Biospecimen Resources*

- **SCIENCE:** research to better understand how pre-analytical factors affect the molecular integrity of the biospecimen
  - *The NCI Biospecimen Research Network*

- **SYNERGIES:** collaboration to share resources and develop community-wide and international standards
  - *CAP, FDA, National and International Partners*
STANDARDS: NCI Best Practices for Biospecimen Resources

Objectives:

- Unify policies and procedures for NCI-supported biospecimen resources for cancer research
- Provide a baseline for operating standards on which to build as the state of the science evolves
- Updated in 2011

http://biospecimens.cancer.gov
One of the most widely recognized and significant roadblocks to progress in cancer research is the lack of standardized, high-quality biospecimens. The National Cancer Institute (NCI) developed the NCI Best Practices for Biospecimen Resources (NCI Best Practices) based on extensive research and expert input into the state of NCI-funded biospecimen resources and the quality of biospecimens used in cancer research. The NCI Best Practices outline the operational, technical, ethical, legal and policy best practices for NCI-supported biospecimen resources.

**Scope, Applicability, Implementation**

The NCI Best Practices incorporate key principles that:
- define state-of-the-science biospecimen resource practices
- promote biospecimen and data quality
- support adherence to ethical and legal requirements

The NCI Best Practices define principles to guide procedures developed by biospecimen resources. They are intended to be adapted based on the mission and scientific needs of biospecimen resources. While adoption of the NCI Best Practices is voluntary, the NCI believes that these principles optimize biospecimens for cancer research.

**Technical and Operational Best Practices**

Although the specific mission of a biospecimen resource will define its collection and processing procedures, common principles apply to all biospecimen types. The best practices within this section are based on current, published information and will be revised periodically as new information is generated from ongoing research projects.

**Ethical, Legal, and Policy Best Practices**

In addition to technical considerations relating to the physical quality of a biospecimen, multiple ethical, legal, and policy issues relate to biospecimen collection activities. Key ethical issues include:
- respecting the autonomy of research participants
- protecting research participants from breaches of privacy and confidentiality
Workshop on Release of Research Results to Participants in Biospecimen Studies

On July 8-9, 2010 the NCI OBRR held a workshop to provide recommendations concerning the conditions and processes for releasing research results to human research participants who contribute biospecimens. Following state-of-the-science presentations, the workshop engaged participants with general and breakout discussions and generated recommendations in three key areas: (1) the appropriate handling of diagnostic discrepancies or incidental findings that are discovered during the pathology review of biospecimens; (2) the mechanisms by which individual or aggregate research results might be provided to participants in clinical studies; and (3) the conditions that warrant disclosure of aggregate research findings. The goal was to generate guiding principles for the development of NCI Best Practices on the release of research results and policies for the cancer Human Biobank (caHUB), a national biospecimen resource in development by the NCI.

- NCI Return Research Results Summary (PDF Document: 846 KB)
NIH Biospecimens Interest Group

- Forum for new information about the multiple issues that are important to the use of human biospecimens in research
- Meetings held on the NIH campus with a live Videocast.
- Recent presentations on Informed Consent of pediatric donors in research
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Multiple pre-analytical factors can affect the molecular integrity of the biospecimen.

Factors (examples):
- Antibiotics
- Other drugs
- Type of anesthesia
- Duration of anesthesia
- Arterial clamp time

Factors (examples):
- Time at room temperature
- Temperature of room
- Type of fixative
- Time in fixative
- Rate of freezing
- Size of aliquots

The Lifecycle of the Biospecimen
How Can Changes in Molecular Integrity of Biospecimens Affect Molecular Readout?

**Genomics**
- Changes in specific transcript levels based on ischemic time, not disease

**Proteomics**
- Lack of reproducibility of protein biomarkers in discovery research
- Inconsistent IHC results in Research and Clinical Labs

**Metabolomics**
- Inconsistencies in small molecule readouts, yielding results that point to the wrong pathway
Does it Matter? Cold Ischemia and Molecular Assay Results

HER2 IHC and FISH in Breast Cancer: Loss of Biomarker Signal with Time to Fixation

Phosphoprotein pMAPK IHC of Colon Cancer: Gain of Biomarker Signal with Time to Fixation


Hartmut Juhl, Indivumed GmbH, BRN
• 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
  • Annual symposium: “Advancing Cancer Research through Biospecimen Science” [http://brnsymposium.com](http://brnsymposium.com)

• Generate new research data:
  • Extramural Research Programs
  • IMAT Program – “Innovative and Applied Emerging Technologies in Biospecimen Science” (RFA)

• Collaborate with other programs to facilitate biospecimen research and evidence-based biospecimen practices
<table>
<thead>
<tr>
<th>BRN Contract</th>
<th>Scientific Questions and Approach</th>
<th>Relevance</th>
<th>Results</th>
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</table>
| **Title:** R&D on Human Biospecimen Integrity  
**Subcontractor:** Caprion  
**PI:** Daniel Chelsky, PhD | Do different blood collection, processing and storage procedures result in significantly different proteomic profiles?  
Different tube types, varying time and temp on bench, freeze-thaw; prostate and breast cancer patients and age and gender-matched controls.  
Analysis by mass spec and Illumina arrays | Mass Spec Proteomics: Basic research, R&D, diagnostics  
Development of assays to assess plasma stability in biobanks  
Data will be used to develop guidelines for blood collection and storage | • Different tube types isolate different sets of proteins  
• P100 tubes significantly better than EDTA + protease inhibitors  
• Short times on bench OK <24h; 48h a problem; 37°C a problem  
• 1-3 freeze-thaws OK, >4 a problem  
• Proteins identified that can be used as sentinel markers of degradation  
• Developed open source tablet PC software for complex protocol management  
• Multiple scientific publications (2 in preparation) |
| **Title:** Credentialing Plasma and Serum Biospecimen Banks for Proteomics Analyses  
**Subcontractor:** UCSF  
**PI:** Katy Williams, PhD; Susan Fisher, PhD | Do different blood collection, processing and storage procedures result in significantly different proteomic profiles?  
Different plasma prep protocols varying temp, number of spins, time on bench before and after spin, and freeze-thaw.  
Serum and plasma proteomics to measure protein integrity. | Mass Spec Proteomics: Basic research, R&D, diagnostics  
Development of assays to assess plasma stability in biobanks  
Data will be used to develop guidelines for blood collection and storage | • Short times on bench (<24h) OK  
• No effect of pre-chilling collection tubes on proteomic profiles; no effect of second spin on proteomic profiles; no difference in EDRN and CPTAC plasma SOPs on proteomic profiles  
• Identified proteins subject to degradation, developing MRM assay to assess plasma quality  
• Publications in preparation |
| **Title:** Effects of Pre-analytic Variables on Circulating microRNAs  
**Subcontractor:** Roswell Park Cancer Institute  
**PI:** Hua Zhao, PhD | Is miRNA highly stable in whole blood and plasma collected and stored under different conditions?  
Whole blood vs. plasma; varied bench times, storage containers, storage temp, freeze-thaw.  
Exiquon miRNA and MiScript PCR assays. | miRNA detection: Basic research, R&D, diagnostics  
Data will contribute to assay development guidelines for miRNA | • Identified and validated new “housekeeping” miRNAs  
• miRNA is highly stable in PAXgene whole blood; degrades in plasma after 24h on bench; stable in different storage containers and freezer temps  
• Identified new miRNA markers associated with breast cancer  
• Publications in preparation |
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<tr>
<td>Subcontractor: UT M.D. Anderson</td>
<td>Does snap-freezing preserve RNA better than other methods? How does intra-tumoral heterogeneity contribute to gene expression patterns?</td>
<td>mRNA profiling and individual gene expression: Basic research, R&amp;D, diagnostics</td>
<td>•RNAlater significantly better than snap freezing; corroborates unpublished FDA-approved approach (Mammaprint)</td>
</tr>
<tr>
<td>PI: W. Fraser Symmans, MD, Christos Hatzis, PhD</td>
<td>Post-op ischemia; tissue preservation method; intra-tumoral heterogeneity; host organ dilution; fresh and frozen breast cancer tissues.</td>
<td>Data will contribute to guidelines for tissue collection for RNA analysis</td>
<td>•Ischemic times &lt;2h OK</td>
</tr>
<tr>
<td></td>
<td>Gene expression profiling by microarray and PCR</td>
<td></td>
<td>•Major gene expression pattern in metastatic cancer is reflective of tissue of origin</td>
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<td></td>
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<td>•Published manuscript in JNCI, more in preparation</td>
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<th>Title: Intrinsic Controls for FFPE Tissue</th>
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<td>Subcontractor: Yale University</td>
<td>Does delay to fixation affect reproducibility of molecular tests in tissue sections?</td>
<td>Immunostaining of FFPE tissues: Basic research, R&amp;D, diagnostics</td>
<td>&lt;2h ischemia time generally OK for proteins commonly used in diagnostics (ER, PgR, HER2, Ki67)</td>
</tr>
<tr>
<td>PI: David Rimm, MD</td>
<td>Post-op ischemia; needle biopsy vs. surgical excision; inter-individual variation; fresh and frozen breast cancer tissues.</td>
<td>Data will contribute to clinical guidelines for tissue fixation</td>
<td>•Intra-individual and intra-tumoral differences more dramatic than variations in ischemic times &lt;2h</td>
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<td></td>
<td>FFPE/quantitative immunofluorescence assessed by immunohistochemistry.</td>
<td></td>
<td>•23 proteins evaluated; changes noted in phosphoproteins and hypoxia markers</td>
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<td></td>
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<td>•Publications (1 submitted)</td>
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<th>Title: Research Studies on the Effects of Intraoperative Ischemia Time on Protein Expression Patterns in Liver and Colon Tissue</th>
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<td>Subcontractor: Indivumed GmbH</td>
<td>Are proteins detected in post-surgical tissue reflective of in vivo biology?</td>
<td>Protein detection in post-surgical tissues (FFPE/IHC; frozen tissues/Ab arrays): Basic research, R&amp;D, diagnostics</td>
<td>•Novel data on effects of surgical clamp time on specific proteins and genes; major changes in phosphoproteins</td>
</tr>
<tr>
<td>PI: Hartmut Juhl, MD</td>
<td>Intra-op and post-op ischemia; colon and liver tissues; effects of anesthetics agents on blood.</td>
<td></td>
<td>•Markers of tissue stability and instability after anesthesia and surgery</td>
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<td>IHC and Meso Scale Discovery detection of phosphoproteins; follow up studies on gene expression.</td>
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Collaborations to Assure Implementation of Data-Driven Procedures for Human Specimens

- **College of American Pathologists**
  - Collaboratively develop evidence-based standard operating procedures for fit-for-purpose specimens
  - Encourage implementation of those SOPs through CAP
  - Support CAP’s development of a biorepository accreditation program

- **Food and Drug Association**
  - Active harmonization with FDA around standards for biomarkers identification and assay development

- **SPIDIA Consortium**
  - Collaborate with European Union program SPIDIA* to share results from biospecimen research and develop standards

*Standardisation and improvement of generic Pre-analytical tools and procedures for In-vitro DIAgnostics
BRISQ: International Collaborative Project

- BRISQ (Biospecimen Reporting for Improved Study Quality) complements existing reporting recommendations (STARD, STROBE, REMARK)

- Help authors, reviewers, editors, and regulatory officials evaluate whether sufficient information about the biospecimens was provided to enable assessment of the influence of pre-analytical factors on study results.

- Help readers evaluate, interpret, compare, and or reproduce results.

- Sensitize the biobanking and research communities to the importance of documenting biospecimen pre-analytical data.

- Manuscript now published in three journals, additional commentaries in progress
Advancing Research and Molecular Medicine Through Improved Biospecimen Practices

- Build a stronger research community in Biospecimen Science
- Sponsor new biospecimen research and improve access to related literature
- Develop documented Best Practices for the entire biospecimen lifecycle (*ethics through storage*)
- Develop evidence-based SOPs for biospecimen collection, processing and storage
- Support biorepository training and accreditation
- Develop Publication standards for biospecimens (BRISQ)
- Significant cost savings could result from harmonized biospecimen practices
  - Get more value from Research, from R&D, from better medical tests and thus better medical decisions
Our Goal

Garbage in…

…Garbage out
Advancing Research Through Better Biospecimen Practices

Helen M. Moore, PhD
Addressing Global Health Challenges Through Science Collaboration
Washington, DC
June 6, 2012