National Cancer Institute

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Moving Toward Evidence-Based Biobanking

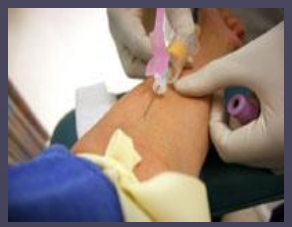
Helen M. Moore

National Day – Germany's Way Towards Excellence in Biobanking

ISBER 2016, Berlin

5 April, 2016

Improving Biospecimen Processes is Essential to Enable Better Research, Clinical Trials, and Molecular Medicine



Biospecimen Collection

(Blood, Tissues, Urine, etc.)

Processing in Pathology Lab

Storage

Analysis

Patient Care

Clinical Trials

Research

Clinical Data Collection

Adapted from Peggy Devine

Vision for Improving Biobanking

- **Build Standards**
  - Enable more reproducible research and better diagnostic tests.
- **Build the scientific knowledge base for evidence-based standards**
  - For engagement of research participants, biospecimen processes, biobanking economics.
- **Develop and utilize evidence-based standards**
  - In partnership with the research and medical communities.

U.S. National Cancer Institute Biorepositories and Biospecimen Research Branch

- Created to address specific concerns:
  - How variability in biospecimen collection, processing, and storage procedures may affect reproducibility of basic and clinical research results
  - How this variability may affect the progress and effectiveness of precision or “personalized” medicine

Sources of Variability in Biospecimens

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

→ **WIDE VARIATION IN BIOSPECIMENS**

How BBRB Addresses the Issues

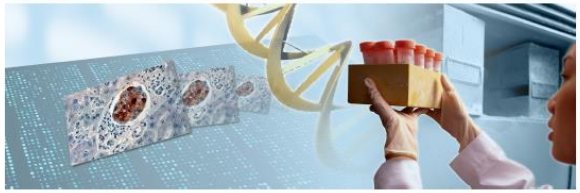
- **BIOSPECIMEN STANDARDS**
  - Creating and Facilitating Biospecimen Standards Development and Adoption.
- **BIOSPECIMEN RESEARCH**
  - Developing scientific evidence to understand biospecimen stability and drive harmonization of biospecimen practices.
- **TOOLS AND PUBLIC PRODUCTS**
  - Public resources for the research community.
- **TECHNICAL ASSISTANCE**
  - Provision of expert assistance to NCI, NIH, the research community at large.

BIOSPECIMEN 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

NCI Best Practices



National Cancer Institute
Best Practices for
Biospecimen Resources

June 2007

Prepared by:
National Cancer Institute
National Institutes of Health
U.S. Department of Health and Human Services

http://biospecimens.cancer.gov

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**

Collaborative Process:

- **Trans-NCI team "Biorepository Coordinating Committee"**
- **Extensive NIH review**
- **Federal Register comment period**
- **Updated 2011, 2016**

NCI Best Practices for Biospecimen Resources

Biorepositories and Biospecimen Research Branch

National Cancer Institute

National Institutes of Health

U.S. Department of Health and Human Services

March 2016

http://biospecimens.cancer.gov

NCI Best Practices

- Released yesterday: 2016 updates to the Best Practices
  - New technical material on biospecimen science, electronic document control for SOPs, newer specimen processing methods such as PaxGene tissue.
  - Informatics sections updated to be more relevant to current standards; removal of outdated material related to caBIG and caGRID.
  - Ethical/regulatory sections updated and rewritten to include new and updated references, recent developments in informed consent and return of research results, changes in NIH Genomic Data Sharing policy.
  - References, web links, glossary and appendices reviewed and updated/corrected.

BIOREPOSITORY ACCREDITATION PROGRAM

The College of American Pathologists (CAP) Biorepository Accreditation program is designed to improve the quality and consistency of facilities that collect, process, store, and distribute biospecimens for research.

APPLY NOW

BIOSPECIMEN STANDARDS

- ***BRISQ****:* publication standards for studies utilizing biospecimens
  - Goal: improve research reproducibility
  - Integration continuing (e.g. inclusion in *Nature* and *Science Translational Medicine* instructions to authors)

Original Article

Biospecimen Reporting for Improved Study Quality (BRISQ)

Helen M. Moore, PhD[1]; Andrea B. Kelly, PhD[2]; Scott D. Jewell, PhD[3]; Lisa M. McShane, PhD[4]; Douglas P. Clark, MD[5]; Renata Greenspan, MD[6]; Daniel F. Hayes, MD[7]; Pierre Hainaut, PhD, MS[8]; Paula Kim[9]; Elizabeth A. Mansfield, PhD[10]; Olga Potapova, PhD[11]; Peter Riegman, PhD[12]; Yaffa Rubinstein, PhD[13]; Edward Seijo, MS[14]; Stella Somiari, PhD[15]; Peter Watson, MB, BChir[16]; Heinz-Ulrich Weier, PhD[17]; Claire Zhu, PhD[18]; and Jim Vaught, PhD[1]

Human biospecimens are subjected to collection, processing, and storage that can significantly alter their molecular composition and consistency. These biospecimen preanalytical factors, in turn, influence experimental outcomes and the ability to reproduce scientific results. Currently, the extent and type of information specific to the biospecimen preanalytical conditions reported in scientific publications and regulatory submissions varies widely. To improve the quality of research that uses human tissues, it is crucial that information on the handling of biospecimens be reported in a thorough, accurate, and standardized manner. The Biospecimen Reporting for Improved Study Quality (BRISQ) recommendations outlined herein are intended to apply to any study in which human biospecimens are used. The purpose of reporting these details is to supply others, from researchers to regulators, with more consistent and standardized information to better evaluate, interpret, compare, and reproduce the experimental results. The BRISQ guidelines are proposed as an important and timely resource tool to strengthen communication and publications on biospecimen-related research and to help reassure patient contributors and the advocacy community that their contributions are valued and respected. ***Cancer (Cancer Cytopathol)* 2011;119:92–101.** *Published 2011 by the American Cancer Society.**

KEY WORDS: BRISQ, best practices, biobank, biospecimen, human, quality, research, guidelines.

BIOSPECIMEN RESEARCH

Developing scientific evidence to understand biospecimen stability and drive harmonization of biospecimen practices.

- ***The Biospecimen Research Network (BRN):*** Research to better understand how pre-analytical factors affect molecular integrity of biospecimens
- ***ELSI research****:* Ethical, Legal and Social Implications of Biobanking
- ***Biorepository economics research***

TOOLS AND PUBLIC PRODUCTS

Public resources for the research community

- ***Biospecimen Research Database*** (online literature and SOPs; >2000 articles; published meta-analyses)
- ***Biobank economic modeling tool***
- ***White papers and publications***
- ***Patient brochures***

BBRB Patient Brochure

- Comprehensive patient education brochure on the importance of specimen donation
- Compared all available materials, addressed gaps in brochures available at the time from NCI and External Organizations
- Text reviewed by patient advocates
- Readability and Comprehension testing
  - Brought down to 7th grade reading level
- English and Spanish versions available

TECHNICAL ASSISTANCE

Provision of expert assistance to NCI, NIH, the research community at large.

- Best Practices
- Biospecimen Science collaboration
- SOP development, sharing
- ELSI issues
- Ongoing communications through web site, Twitter, LinkedIn
- GTEx biospecimen collection and management
- Comprehensive Data Resource (CDR) adoption
- Biobanking data elements and ontologies

Genotype-Tissue Expression (GTEx)

NIH Common Fund Program

- Team science project intended to:
  - Understand how genomic variation affects differential gene expression in various normal tissues
  - Produce benchmark genomic data for human biology
- Tissue acquisition challenge:
  - Pilot: collect 20-30 different normal tissues from 190 individual postmortem donors
  - High quality RNA needed for comprehensive genomics analysis pipeline at Broad Institute
- Program met pilot goals, scale up to 965 postmortem donors is complete
  - Data being regularly released on dbGaP; SOPs available
  - Biospecimen access policy posted on GTEx web site
  - Proposals for management of legacy collection under review

Genotype-Tissue Expression (GTEx) Operations

Biospecimen Source Sites

- **National Disease Research Interchange**
  - *Organ Procurement Organizations*
    - LifeNet Health
    - Gift of Life Donor Program
  - *Surgery & autopsy*
    - Drexel University College of Medicine
    - Albert Einstein College of Medicine
    - Virginia Commonwealth University
  - **ELSI Study**
    - Virginia Commonwealth University
- **Roswell Park Cancer Institute**
  - *Organ Procurement Organization*
    - Upstate New York Transplant Service

Van Andel Research Institute
Comprehensive Biospecimen Resource (CBR)
Biorepository Operations & Pathology Review

Leidos Biomedical, Inc.
Comprehensive Data Resource (CDR)
BBRB Data Coordinating Center

Laboratory, Data Analysis & Coordinating Center

BROAD INSTITUTE

- *Project coordination*
- *Nucleic acid extractions*
- *Genotyping*
- *Gene expression (array & RNA-Seq)*
- *Statistical analysis*

Brain Bank
Receive & dissect whole brains
MIAMI

GTEx Database
http://www.ncbi.nlm.nih.gov/gtex/
NCBI
- *eQTL display*
- *Controlled access to individual-level data*

Statistical Methods Development (R01s) [Cox, Dermitzakis, Liu, Pritchard, Rusyn]

cer Institute

Science : A representation of how variation in the human genome affects gene expression among individuals and tissues. Colors and shapes show variations between people and within individuals. The Genotype-Tissue Expression (GTEx) Consortium examined postmortem tissue to document how genetic variants confer differences in gene expression across the human body.
See pages 618, 640, 648, 660, and 666.

National Cancer Institute

GTEx

Genotype–Tissue Expression Project

The GTEx Symposium:

All things considered — biospecimens, ’omics data, and ethical issues

May 20–21, 2015

Ruth L. Kirschstein Auditorium

National Institutes of Health

Comprehensive Data Resource (CDR)

- Supports two ongoing biospecimen programs:
  - The Genotype-Tissue Expression Program (GTEx) – a NIH Common Fund study of genomic variation and tissue-specific expression, analyzing up to 30 tissues per donor in 900 deceased donors.
  - The Biospecimen Preanalytical Variables program (BPV) – a study of preanalytical variation in tissue processing and storage (FFPE and frozen tissues) and the effects of such variation on downstream molecular analysis.

CDR: Now Available to the Biobanking Community

- Management software for biobanking community
- Facilitates use of Best Practices and annotation of biospecimen collection and processing steps
- In use for other NCI programs including the CPTAC program (Clinical Proteomic Tumor Analysis).
- CDR code was posted last year – a new “CDR Lite” code is now available.
- Collaborative Announcement:

https://ttc.nci.nih.gov/opportunities/opportunity.php?opp_id=748093754466223

Biospecimen Science Research: Building the Knowledge Base for Evidence-Based Collection, Processing, and Storage

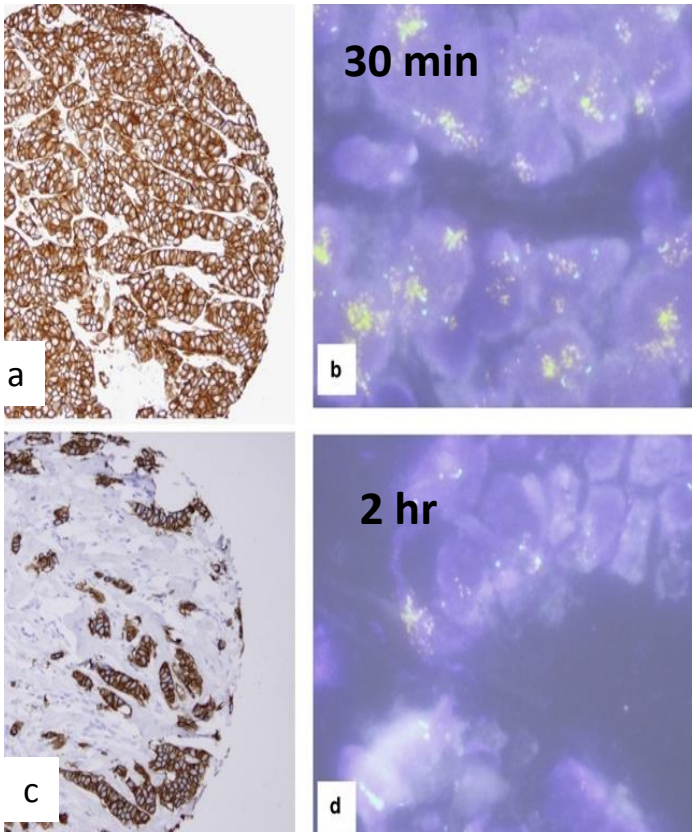
- What do we want to know?
- Does it matter for research and patient care?
- Building biospecimen evidence-based practices
  - Biospecimen Research Database
  - New research in Biospecimen Science
  - Building annotated procedural guidelines
- New directions in Biospecimen Science

Biospecimen Science Research – What Do We Want to Know?

- How is biospecimen molecular integrity affected by different pre-analytical factors?
  - Systematic studies of specific pre-analytical factors; e.g., studies of ischemic time and time in fixative for FFPE tissues
- How should biospecimens be collected, processed, and stored for specific downstream analyses?
  - Development and application of research data for different technology applications, e.g., effects of variation in blood collection, processing, and storage on proteomic analysis
- Once the biospecimens are in storage – how do you know if they are any good for your research purpose?
  - Development of molecular stability assessment markers

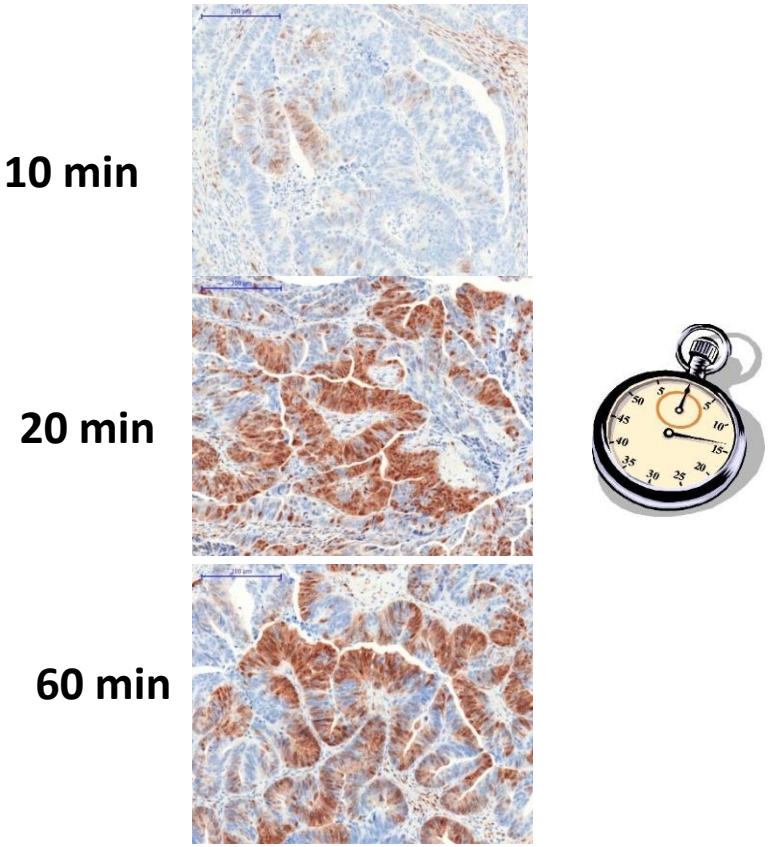
Does it Matter?
Cold Ischemia and Molecular Assay Results

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



Khoury T, et al., Mod Pathol. 2009 Nov;22(11):1457-67

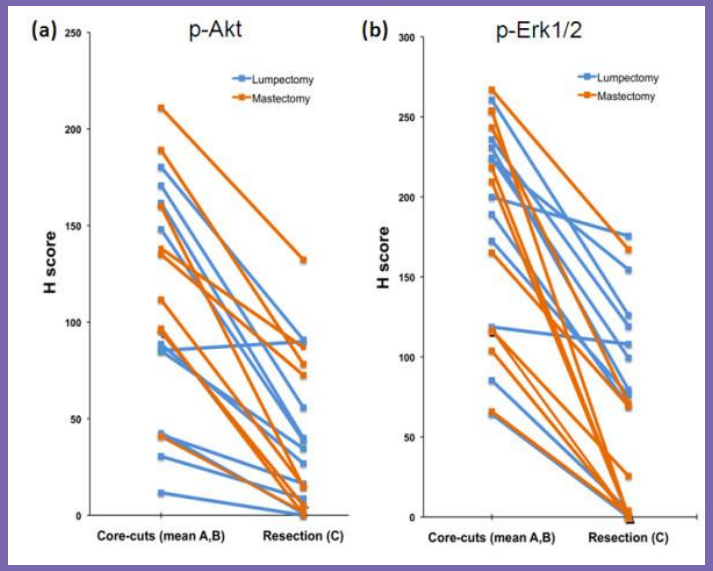
Phosphoprotein pMAPK IHC of Colon Cancer: Gain of Biomarker Signal with Time to Fixation



Hartmut Juhl, Indivumed GmbH, BRN

Examples of Studies Examining Effects of Preanalytic Factors

IHC breast cores vs. resections



Expression of p-AKT and p-ERK was markedly lower in resections (30 min delay) than in cores

Pinhel et al, Breast Cancer Research, 2010

RNA in breast biopsies, BRN

- RNAlater-preserved breast biopsies yielded higher quality RNA than flash frozen.
- Expression level of 3% of transcripts is affected by the preservation method.
- Prolonged ischemic time induces significant transcriptional changes in a small subset of transcripts.

Hatzis et al, J Natl Cancer Inst, 2011

Aktas et al, Mol Oncol, 2014

"BPV" study of FFPE tissues, BRN

- Compared to frozen, FFPE samples have half as many reads mapping to the transcriptome and more than twice the number of reads mapping to the intragenic regions.
- Extended time in fixative (72 h) significantly reduces RNA quality.

Global proteome vs. phosphoproteins, tissues, NCI CPTAC

- Ovarian tumors and patient-derived breast cancer xenograft tissues
- Global proteome unchanged up to 1 hour of cold ischemia time; phosphoproteome 24% affected

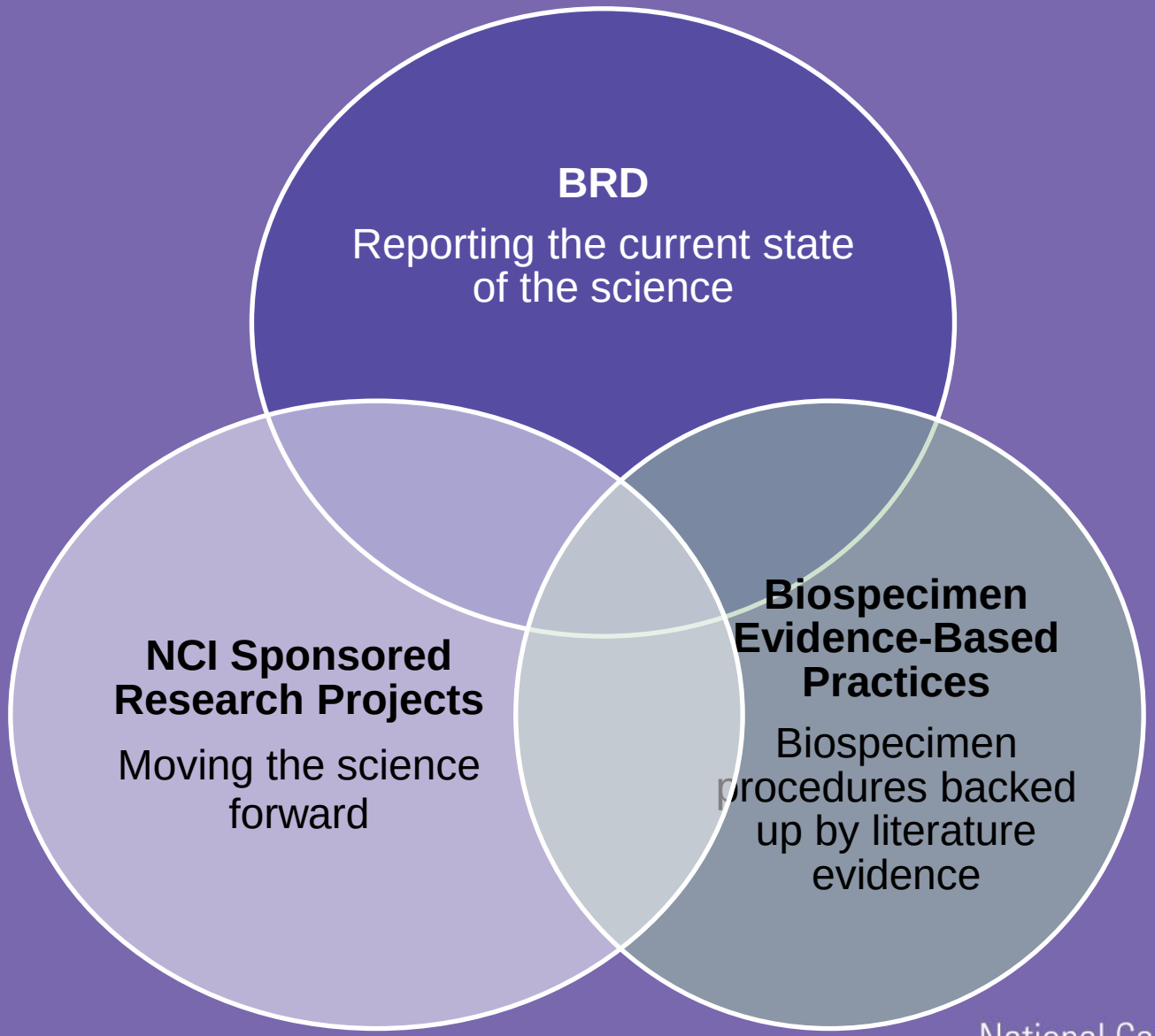
Plasma proteomics, BRN

- Different blood tube types stabilize different peptides/proteins
- Time on bench ok at rt for 24h; one spin ok; 3x freeze-thaw ok
- Degradation after 6 months at -80 C

Building Biospecimen Evidence-Based Practices

- Identifying biospecimen science studies and results
- Conducting targeted biospecimen science research
- Building annotated procedural guidelines: *Biospecimen Evidence-Based Practices (BEBPs)*

Building Evidence-Based Practices



BRD – Literature and SOP resources

- Free, publicly accessible database with peer-reviewed primary and review articles – and – new! – SOPs
- Articles are identified, reviewed, and curated by scientists.
- SOPs organized in hierarchy tiers and related SOP documents are assembled in Compendiums; searchable by keyword or by curated fields.
- Vehicle for literature review, evaluation of "in use" biospecimen handling protocols, development of new protocols, and identification of analytes that are susceptible or impervious to handling variability.

The Biospecimen Research Database

Welcome to the newest version of the Biospecimen Research Database (BRD), which accommodates Standard Operating Procedures (SOP). We encourage your contributions to our new SOP library!

The BRD is a free and publicly accessible database that contains peer-reviewed primary and review articles as well as SOPs in the field of human Biospecimen Science.

Each literature curation has been created by a Ph.D.-level scientist to capture the following: (1) relevant parameters that include the biospecimen investigated (type and location, patient diagnosis), preservation method, analyte(s) of interest and technology platform(s) used for analysis; (2) the pre-analytical factors investigated, including those relating to pre-acquisition, acquisition, preservation, processing, storage, and analysis; and (3) an original summary of relevant results. Browse literature curations or submit specific queries using the **Advanced Search** page with keyword search for specific biomakers or genes, PubMed ID, or pre-analytical factor values (anticoagulant, fixative, reagent, etc).

SOPs are organized in a hierarchy system consisting of two tiers: (1) SOPs, established protocols; and (2) Biospecimen Evidence-based Practices (BEBP), procedural guidelines developed using literature evidence. SOP-tiered documents are a product of the Source organization specified. SOPs shared by external organizations are done so only with their consent, and have not been vetted by BBRB. SOP documents are searchable by keyword, or by curated fields (source organization, tier, applicable biospecimens, and topic) on the **Search SOPs** page. Related SOP documents are assembled in Compendiums, which are viewable on the **SOP Compendiums** page. You can also create your own Compendium and download SOPs together rather than individually.

We encourage you to submit SOPs from your lab or institution for inclusion in the BRD by clicking on the **Submit an SOP** tab or at **biospecimens@mail.nih.gov**. Individuals and organizations that suggest articles for inclusion in the BRD will receive acknowledgement on the paper's curation page. Articles may be submitted by clicking on the **Suggest a New Paper** tab or via the email above. Feedback is also welcome.

The BRD is an initiative of the NCI Biorepositories and Biospecimen Research Branch (BBRB).

Featured Paper

Influence of storage conditions and extraction methods on the quantity and quality of circulating cell-free DNA (ccfDNA): the SPIDIA-DNAplas External Quality Assessment experience.

Author(s): Malentacchi F, Pizzamiglio S, Verderio P, Pazzagli M, Orlando C, Ciniselli CM, Günther K, Gelmini S

Publication: *Clin Chem Lab Med*, 2015, Vol. , Page

PubMed

1 study(s)

1. *The purpose of this study was to determine the effect of DNA extraction method and delayed isolation on the yield of cell-free DNA. Inter-laboratory variability was also examined. Plasma from a single healthy donor was obtained by double centrifugation, aliquoted, stored at 4°C and shipped at 2-8°C. After arrival, specimens were stored for <3 days at 4°C before DNA extraction. Extracted DNA was then shipped back at 4°C. DNA integrity was determined by the*

News and Announcements

2015-05-04

Did you know that PubMed links out to the BRD?

Look for a link to the BRD on the bottom of the PubMed abstract page, under "LinkOut-more resources".

2015-04-06

New Review from BBRB. Accuracy of Molecular Data Generated with FFPE Biospecimens: Lessons from the Literature

The review appears in Cancer Research's OnlineFirst section, and both summarizes the challenges associated with molecular analysis of FFPE biospecimens as well as highlights analytical techniques and parameters that result in strong concordance with a fresh or frozen cohort.

Click here to read the article.

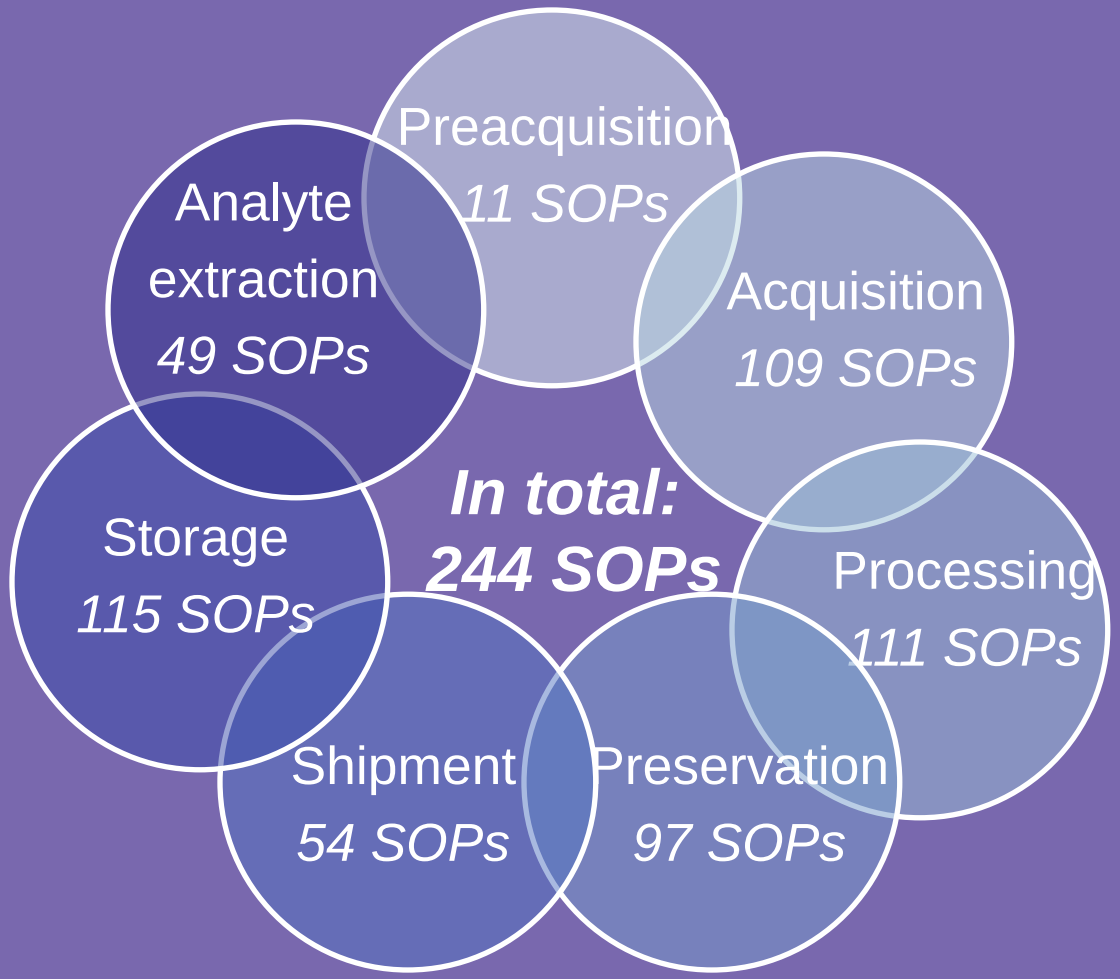
More...

Recently Added

- Diversity of Gene Expression in Hepatocellular Carcinoma Cells.
- Reflex Repeat HER2 Testing of Grade 3 Breast Carcinoma at Excision Using Immunohistochemistry and In Situ Analysis: Frequency of HER2 Discordance and Utility of Core Needle Biopsy Parameters to Refine Case Selection.
- Biopsy sampling of breast lesions: comparison of core needle- and vacuum-assisted breast biopsies.
- Examination of breast needle core biopsy specimens performed for screen-detected microcalcification.
- When have mammographic calcifications been adequately sampled at needle core biopsy?

More...

SOP Topics in the BRD



Published Review Articles

- Greytak SR, Engel KB, Bass PG, Moore HM. **Accuracy of molecular data generated with FFPE specimens: lessons from the literature**. *Cancer Res.* 2015; 75(8):1541-7.
- Bass BP, Engel KB, Greytak SR, Moore HM. **A review of preanalytical factors affecting molecular, protein, and morphological analysis of formalin-fixed, paraffin-embedded (FFPE) tissue: how well do you know your FFPE specimen?** *Arch Pathol Lab Med*. 2014; 138(11): 1520-30.

  Accompanying Editorial
- Engel KB, Vaught J, Moore HM. **National Cancer Institute Biospecimen Evidence-Based Practices: a novel approach to pre-analytical standardization.** *Biopreserv Biobank*. 2014; 12(2): 148-150.

  Accompanying Editorial
- Engel KB, Moore HM. **Effects of preanalytical variables on the detection of proteins by immunohistochemistry in formalin-fixed, paraffin-embedded tissue.** *Arch Pathol Lab Med.* 2011;135(5):537-543.

NCI Biospecimen Science Research Programs

PI-Driven Research in Biospecimen Molecular Integrity

BRN Contracts	Scientific Questions and Approach	Relevance	Results
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; 48 h a problem; 37˚C a problem • 1-3 F/T OK, >4 a problem, P100 tubes most protective against F/T • 6 months storage at -80◦C causes degradation of selected soluble, secreted and membrane proteins • More changes at -20^{0}C than -80^{0}C • Proteins identified that can be used as sentinel markers of degradation and establish utility of stored specimens in clinical research • Developed open source tablet PC software for complex protocol management • Manuscript in preparation: "**Impact of Human Blood Specimen Collection, Processing and Storage on Protein Integrity and Implications for Use in Clinical Research"**
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 (<24 h) OK; 96 h and elevated temperatures a problem • 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 • Developed custom software for comparison of iTRAQ MS data • Hassis et al., **Evaluating the effects of preanalytical variables on the stability of the human plasma proteome,** Anal. Biochem., 478(1), June 2015; 14-22
Title: Effects of Pre-analytic Variables on Circulating microRNAs **Subcontractor:** Roswell Park Cancer Institute/ UT M.D. Anderson **PI:** Dr. Hua Zhao, UT M.D. Anderson	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 and improved "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 • Zhao et al., **Effects of Preanalytic Variables on Circulating MicroRNAs in Whole Blood**, Cancer Epidemiol. Biomarkers Prev.; 23(12); 2643–8

BRN Contract	Scientific Questions and Approach	Relevance	Results
Title: R&D on Human Biospecimen Integrity **Subcontractor:** UT M.D. Anderson **PI:** W. Fraser Symmans, MD, Christos Hatzis, PhD	Does snap-freezing preserve RNA better than other methods? How does intra-tumoral heterogeneity contribute to gene expression patterns? Post-op ischemia; tissue preservation method; intra-tumoral heterogeneity; host organ dilution; fresh and frozen breast cancer tissues; interlaboratory reproducibility Gene expression profiling by microarray and PCR	mRNA profiling and individual gene expression: Basic research, R&D, diagnostics Data will contribute to guidelines for tissue collection for RNA analysis	• RNAlater significantly better than snap freezing; corroborates unpublished FDA-approved approach (Mammaprint) • Ischemic times <2h OK • Major gene expression pattern in metastatic cancer is reflective of tissue of origin; developed assay for measuring contamination of metastatic breast cancer by liver • Inter-tumoral heterogeneity greater than intra-tumoral heterogeneity • Aktas et al., **Global gene expression changes induced by prolonged cold ischemic stress and preservation method of breast cancer tissue**, Molecular Oncology,8, 2014;717-27 • Hatzis et al., **Effects of Tissue Handling on RNA Integrity and Microarray** • **Measurements From Resected Breast Cancers, J** Natl Cancer Inst 2011;103:1871–1883
Title: Intrinsic Controls for FFPE Tissue **Subcontractor:** Yale University **PI:** David Rimm, MD	Does delay to fixation affect reproducibility of molecular tests in tissue sections? Post-op ischemia; needle biopsy vs. surgical excision; inter-individual variation; fresh and frozen breast cancer tissues. FFPE/quantitative immunofluorescence assessed by immunohistochemistry.	Immunostaining of FFPE tissues: Basic research, R&D, diagnostics Data will contribute to clinical guidelines for tissue fixation	• <2h ischemia time generally OK for proteins commonly used in diagnostics (ER, PgR, HER2, Ki67) • Intra-individual and intra-tumoral differences more dramatic than variations in ischemic times <2h • 23 proteins evaluated; miRNAs evaluated; changes noted in phosphoproteins and hypoxia markers and some miRNAs • Quality index developed • Vassilakopoulou et al.,**Preanalytical variables and phosphoepitope expression in FFPE tissue: quantitative epitope assessment after variable cold ischemic time,** Lab Invest. 95(3), 2015;334-41 • Neumeister et al., **A tissue quality index; an intrinsic control for measurement of effects of preanalytical variables on FFPE tissue**, Lab. Investigation, 94, 2014;467-474 • Neumeister et al., **Quantitative Assessment of effect of Preanalytic cold ischemic time on Protein expression in Breast cancer tissues,** J Natl Cancer Inst 2012:104;1815–1824
Title: Research Studies on the Effects of Intraoperative Ischemia Time on Protein Expression Patterns in Liver and Colon Tissue **Subcontractor:** Indivumed GmbH **PI:** Hartmut Juhl, MD	Are proteins detected in post-surgical tissue reflective of in vivo biology? Intra-op and post-op ischemia; colon and liver tissues; effects of anesthetic agents on blood. IHC and Meso Scale Discovery detection of phosphoproteins; follow up studies on gene expression.	Protein detection in post-surgical tissues (FFPE/IHC; frozen tissues/Ab arrays): Basic research, R&D, diagnostics	• Novel data on effects of surgical clamp time on specific proteins and genes; major changes in phosphoproteins • Markers of tissue stability and instability after anesthesia and surgery • David et al., **Surgical procedures and postsurgical tissue processing significantly affect expression of genes and EGFR-pathway proteins in colorectal cancer tissue**, Oncotarget, 5(22), 2014;11017-28

BRN Contract (Extensions)	Scientific Questions and Approach	Relevance	Results
Title: Proteomic changes observed by differential profiling of paired tumor versus normal colon FFPE samples **Subcontractor:** Caprion **PI:** Daniel Chelsky, PhD	Is there a differential protein biomarker expression in tumor tissues obtained from CRC subjects, when compared to adjacent normal tissue? Can a blood-based biomarker panel be developed to diagnose CRC as well as determine prognosis and predict the response to drug therapy? Paired normal and tumor tissues; CRC patients; FFPE sections Label-free intensity based discovery using high resolution tandem mass spectrometry and targeted quantitation using multiple reaction monitoring	Mass Spec Proteomics: Basic research, R&D, diagnostics A blood-based biomarker assay for colorectal cancer could ultimately reduce the number of colonoscopies required, reducing cost and discomfort Data will be used to create a candidate panel of secreted proteins for a diagnostic assay	• Significant changes in secreted proteins were observed in tumor FFPE specimens as compared to adjacent normal specimens. • Some of these candidates include CA2, CEACAM5, CHGA, GDF15, MAOA, MMP2 and MMP9. • Several proteins distributed at multiple points in the serotonin degradation pathway were downregulated in CRC tumor tissues. • Manuscript in preparation: "**Common proteomic changes observed in 40 patients by label-free differential profiling of paired tumor versus normal colon FFPE samples"**
Title: Effect of General Anesthesia on Human Plasma and Peripheral Blood Mononuclear Cells: A proteomics approach **Subcontractor:** Caprion **PI:** Daniel Chelsky, PhD	Does general anesthesia affect the plasma proteome and proteins present in peripheral blood mononuclear cells (PBMCs)? Plasma and PBMCs from CRC patients High resolution Mass Spectrometry	Mass Spec Proteomics: Basic research, R&D, diagnostics Data could be used to monitor effects of anesthesia when studying specific proteins of prognostic significance	• 4382 proteins quantified in human PBMCs; 621 proteins in plasma • Only nine differentially expressed proteins in PBMCs (TPOR, MYADM, DNJA3, UBE4B, APOC2, TTPA, PUR1, CLC4A & TXN4B) and one differentially expressed protein in plasma (Prolactin) were found to be changing after induction of anesthesia. • The nine PBMC proteins were found to be previously linked to known biological effects of anesthesia • Overall the results suggest that general anesthesia does not cause significant protein change in either human plasma or PBMCs • Manuscript in preparation: "**Proteomics Study on the Effect of General Anesthesia on Human Plasma and Peripheral Blood Mononuclear Cells"**

BRN Contract (Extensions)	Scientific Questions and Approach	Relevance	Results
Title: Nanoproteomic analysis of ischemia-dependent changes of signaling protein phosphorylation in colon tissue **Subcontractor:** Indivumed GmbH **PI:** Hartmut Juhl, MD	Is there a overall change in phosphorylation in response to ischemia in normal and tumor tissue? How is expression of phosphoprotein isoforms and stability affected in cancer and adjacent normal tissues with respect to tissue processing and surgical manipulation? CRC patients; paired normal and tumor cancer tissues; cold ischemia NanoPro1000 (Nananofluidic proteomic immunoassay)	NanoPro1000; basic research; differential quantification of protein isoforms and their phosphorylation status The study provides insight into the influence of post-operative ischemia on tissue sample biology, which may inform the future development of markers of tissue quality and assist in the development of diagnostic tests Data will provide basis for the identification of molecular markers sensitive to ischemia	• Changes in overall phosphorylation of the selected proteins in response to ischemia revealed minor variations in both normal and tumor tissue • Significant changes were identified in the phosphorylation of individual isoforms • In normal tissue of post-operative ischemia, phosphorylation was increased in two AKT isoforms, two ERK1/2 isoforms, and one MEK1/2 isoform and decreased in one MEK1/2 isoform and one c-MET isoform • In tumor tissue, one AKT isoform showed decreased phosphorylation and there was an overall increase in unphosphorylated ERK, whereas an increase in the phosphorylation of two MEK1/2 isoforms was observed with no changes in c-MET phosphorylation • Unger et al., **Nanoproteomic analysis of ischemia dependent changes of signaling protein phosphorylation in colon tissue**. J Translational Med 2016, 14:6
Title: Identification and validation of a potential marker of tissue quality using gene expression analysis of human colorectal tissue **Subcontractor:** Indivumed GmbH **PI:** Hartmut Juhl, MD	How does post-operative ischemia affect normal and tumor colorectal tissue samples from patients with colorectal cancer (CRC) using a set of target genes previously identified using microarray? These genes include: (*CYR61*, *RGS1*, *DUSP1*, *DUOX2* and *SLC6A14*) Paired normal and tumor tissue; CRC patients; post-op ischemia Microarray: qPCR	The study provides a potential marker of colorectal tissue quality and a potential reference gene of post-operative tissue quality Data will help find accurate biomarkers instead of ischemic artifacts; *RGS1* and *EEF1A1* may help evaluate surgical samples for integration into research studies.	• *RGS1* gene expression was found to be up-regulated both in normal and colorectal tumor tissue upon resection • *EEF1A1* is stably expressed in normal and tumor colorectal tissue irrespective of the ischemic time • Both microarray analysis and qPCR revealed regulator of G-protein signaling 1 (*RGS1)* as a potential marker of colorectal tissue quality and eukaryotic translation elongation factor 1 alpha 1 (*EEF1A1)* as a potential reference gene of post-operative tissue quality • Manuscript in preparation: **"Identification and validation of a potential marker of tissue quality using gene expression analysis of human colorectal tissue"**
Title: Metabolomics profiling of pre-and post-anesthesia plasma samples obtained via Ficoll separation **Subcontractor:** Indivumed GmbH **PI:** Hartmut Juhl, MD	Do plasma metabolites represent a valuable tool to monitor the effect of different sedation agents and/or the individual metabolic response to anesthesia? Plasma samples from CRC and Liver cancer patients 1H Nuclear Magnetic Resonance (NMR) spectroscopy	Data will lead to understanding of metabolic consequences of general anesthesia in the plasma of patients with non-metastatic/metastatic colorectal cancer and patients with liver-metastasis.	• The comparison of the NMR metabolomic profiles of patients treated with etomidate or propofol at equipotent dose ranges demonstrated that both agents significantly decrease the plasma levels of several NMR-detectable metabolites • The metabolomic signatures were slightly different in patients anesthetized with etomidate versus propofol, with significantly higher concentrations of endogenous metabolites corresponding to the branched amino acids leucine, isoleucine and valine in the latter case • Manuscript in preparation: **"Metabolomics profiling of pre-and post-anesthesia plasma samples obtained via Ficoll separation"**

NCI Biospecimen Science Research Programs

Biospecimen Preanalytical Variables (BPV) Program: Multi-Center Biospecimen Science

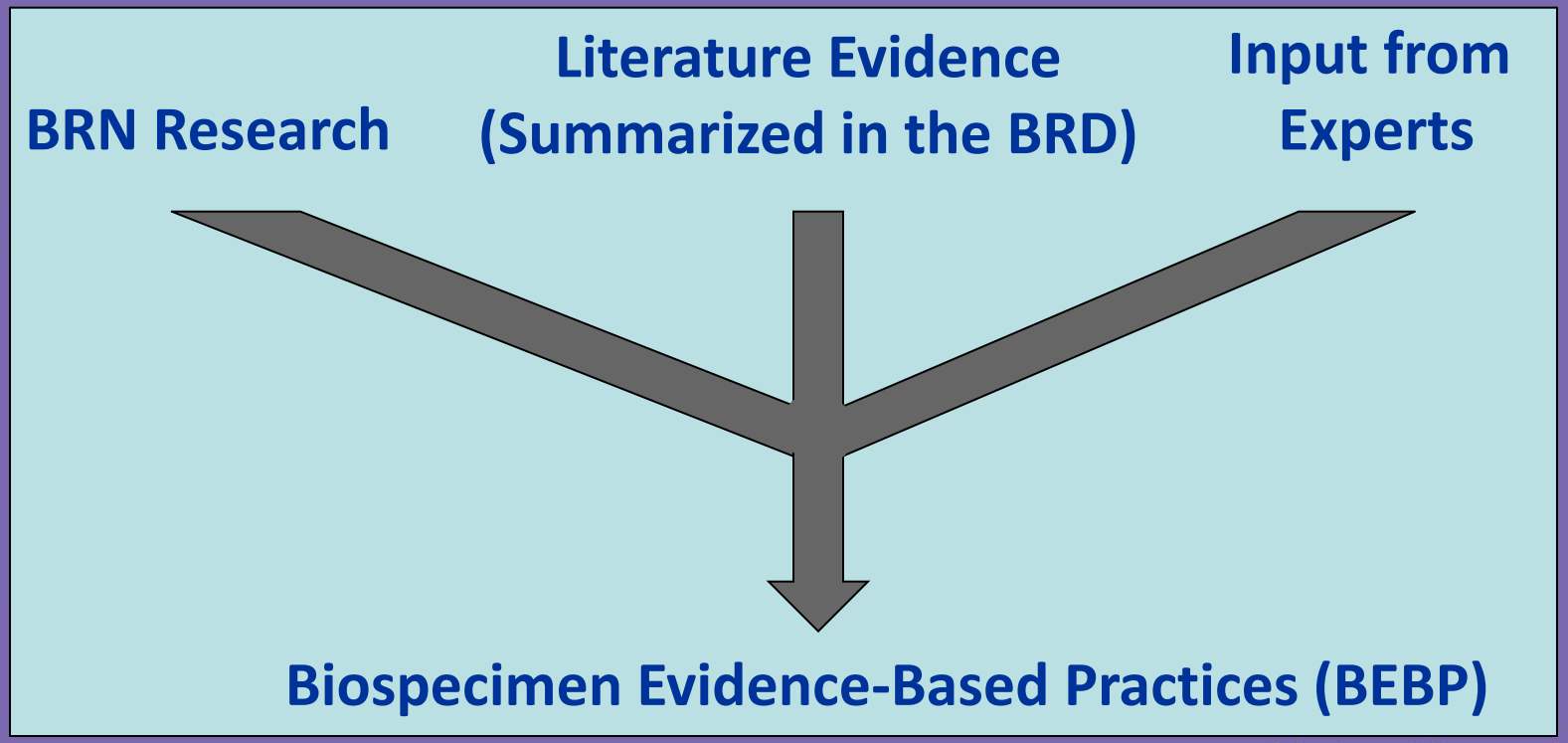
- Designed to systematically investigate the effects of individual pre-analytical factors on the molecular profiles of biospecimens using a wide variety of analysis platforms.
  - FFPE: Time in formalin (6, 12, 23 and 72 hours)
  - FFPE: Delay to fixation (1, 2, 3 and 12 hours)
  - Snap freezing method (LN2 vs. dry ice)
  - Storage temperature (LN2 vs. -80C)
  - Duration of storage (0, 6, 18 months)
- Colon, kidney, lung and ovarian tumor specimens with matching blood samples being collected at four medical institutions under experimental protocols

BPV program – Analytes and Analytical Platforms

- Protein based analysis:
  - Targeted cancer biomarkers evaluation by IHC
  - Profiling of phosphoproteins through MS-based technologies
- Gene expression analysis:
  - mRNA and miRNA analyses using targeted RT-PCR assays, microarrays and *in situ* mRNA detection
  - Genome-wide expression profiling by Next generation sequencing
- DNA Based Analysis:
  - Tumor-specific DNA methylation using PCR-specific assays
  - DNA mutation: SNP, CNV by array-based comparative genome hybridization
- Metabolite profiling
  - Global metabolomics profiling and targeted assays using MS-based technologies

Biospecimen Evidence-Based Practices (BEBPs)

Evidence-based procedural guidelines to support the development of evidence-based SOPs



BEBPs

Available

- Snap-Freezing of Post-Surgical Tissue Biospecimens

In preparation

- Formalin-Fixation and Paraffin Processing and Embedding of Tissue Biospecimens
- Blood Collection and Plasma Processing for Proteomic Analysis by Mass Spectrometry
- Blood Collection and Plasma Processing for Analysis of Circulating Cell Free DNA

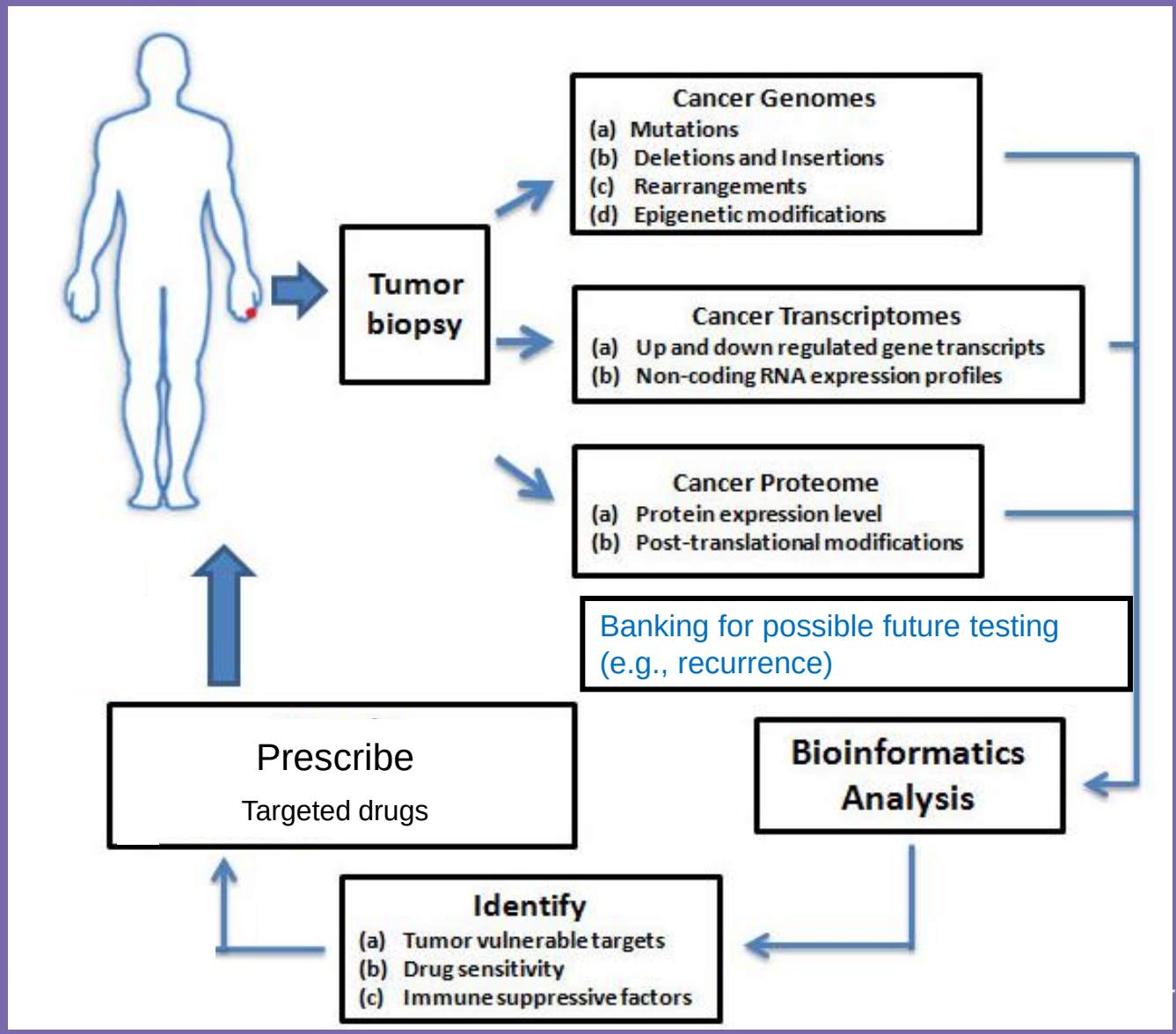
New Directions in Biospecimen Science

- Integrating biospecimen science approaches into clinical biomarker assay development
- Developing quality metrics and methods to assess stored biospecimens for fit-for-purpose research use

Integrating biospecimen science approaches into clinical assay development

- Need applied biospecimen science research to address the convergence of:
  - Emerging clinical biomarker assays in clinical trials
  - New diagnostics challenges
    - FNA, Core Biopsies, Lung Aspirates for Molecular Testing
    - New analysis technologies applied to clinical samples (RNAseq, WES, multiplex IHC, proteomics)
- *Biopsy material of sufficient quality is critical for iterative testing to support therapeutic regimens and testing for recurrence of disease*

Biopsies and Personalized Cancer Therapeutics



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Biospecimen Science Questions for Biomarker Assays Utilizing Biopsy Material

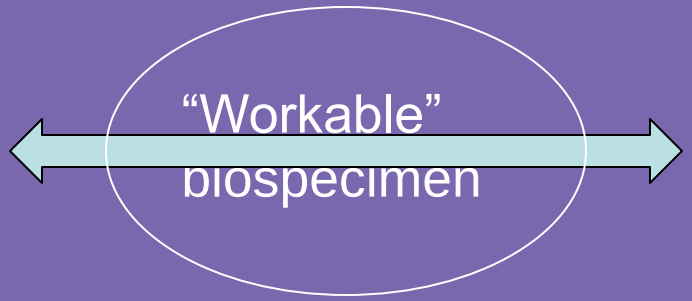
- What preanalytical conditions can a biomarker assay “tolerate?” (*often no information available*)
- Will the assay work on biopsy material? (*on what material was the biomarker identified and the assay developed*?)
- Will the biomarker be stably detected upon storage and retrieval of the biospecimen? (*future testing*)
- Is the preservation method suited for the analytical method? (*fit-for-purpose*)
  - IHC/IF, Whole genome/exome sequencing, RNAseq, mass spec proteomic tests, Nanostring

Preanalytical issues to address

- Biopsy methodology and medical setting
- Tumor heterogeneity
- Preservative type (formalin, RNAlater, frozen, other)
- Timing and temperature considerations
- Tradeoffs morphology vs. molecular preservation
- Shipping and storage considerations

Not Looking for "Perfect" Procedures

Is there a "right" way to preserve a biospecimen for a particular assay?



BBRB Team

- BBRB Shady Grove: Ping Guan, Abhi Rao, Lokesh Agrawal, Phil Branton, Hana Odeh, Merlyn Rodrigues, Jim Vaught, Deborah Robinson
- Biospecimen Research Database (BRD) curators: Kelly Engel, Sarah Greytak, Lori Campbell
- Leidos Team, led by Nancy Roche
- Sponsored investigators

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