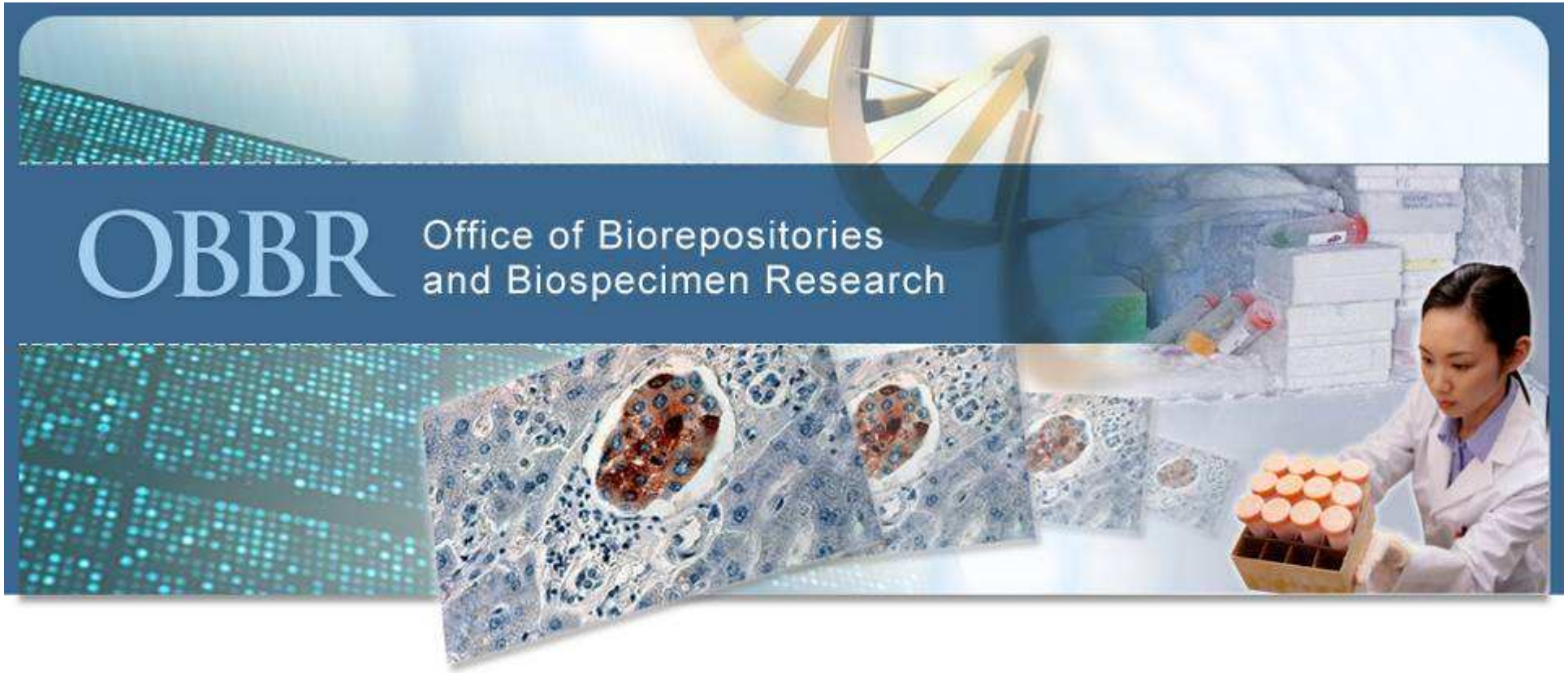


The NCI Biospecimen Research Network and Literature Database

Helen M. Moore, Ph.D.

NCI Biospecimen Research Network Symposium
"Advancing Cancer Research Through Biospecimen Science"
March 13, 2008

NATIONAL CANCER INSTITUTE

Biospecimen Science: What Are the Issues for Cancer Research?

OBBR Office of Biorepositories and Biospecimen Research

- **How do I know if the biospecimen in hand is suitable for my research?**
- **What data do I have about how a biospecimen was collected, processed, and stored?**
- **Will differing ways of collecting, processing, and storing biospecimens affect my ability to obtain reproducible research results using those biospecimens?**

Biospecimen Science: What Are the Issues for Cancer Research?

OBBR Office of Biorepositories and Biospecimen Research

- **How do I prospectively collect good biospecimens for my research purpose?**
- **Will today's biospecimen SOPs, different across different hospitals, allow for advanced molecular testing tomorrow?**
- **What is the scientific basis of a good biospecimen SOP?**

Multiple pre-analytical variables can affect the molecular integrity of the biospecimen

OBBR Office of Biorepositories and Biospecimen Research

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

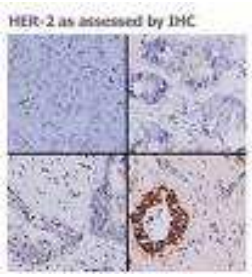
Patient | Medical/ Surgical Procedures | Acquisition | Handling/ Processing | Storage | Distribution | Scientific Analysis | Restocking Unused Sample

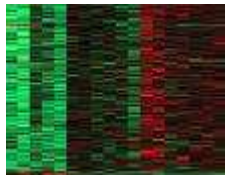
Pre-acquisition **Post-acquisition**

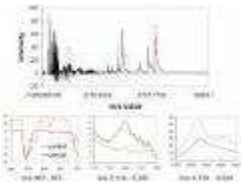
Pre- and Post- Acquisition Variables Impact Clinical and Research Outcomes

OBBR Office of Biorepositories and Biospecimen Research

- **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

OBBR Office of Biorepositories and Biospecimen Research

- **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 accomplish that research?*
- **The NCI Biospecimen Research Network (BRN)**

The BRN: Supporting Collaborative Research

OBBR Office of Biorepositories and Biospecimen Research

- **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
  - This symposium: "Advancing Cancer Research through Biospecimen Science"
- **Generate new research data:**
  - OBBR Intramural Biospecimen Research Laboratory
  - New Extramural Programs: approved and coming soon
  - IMAT Program – Innovative technologic solutions for biospecimens (RFA)
- **Collaborate with other programs, e.g.:**
  - Clinical Proteomics Technologies Assessment for Cancer (CPTAC)
  - The Cancer Genome Atlas (TCGA)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Funding Opportunities
In Focus:
Advancing Cancer Research Through Biospecimen Science
The NCI and the NIH Office of Rare Diseases is pleased to announce the Biospecimen Research Network (BRN) Symposium, "Advancing Cancer Research Through Biospecimen Science", March 13-14, 2008, Washington, DC. The primary goal of the symposium is to address the significant impact of pre-analytical biospecimen variability on cancer research and molecular medicine. For more information, visit www.brnsymposium.com.
Response Deadline Extended: Request for Information (RFI): Tissue Acquisition and Processing Variables
The response deadline has been extended to February 29, 2008, for OBBR's RFI on cancer and normal tissue acquisition and processing variables from medical institutions involved in the collection of surgically resected human specimens. For more information, please visit http://grants.nih.gov/grants/guide/notice-files/NOT-CA-08-002.html
OBBR's Mission:
The NCI established the Office of Biorepositories and Biospecimen Research (OBBR) in 2005 to guide, coordinate, and develop the Institute's biospecimen resources and capabilities. The OBBR's mission is to ensure that human specimens available for cancer research are of the highest quality. more
Quick Links
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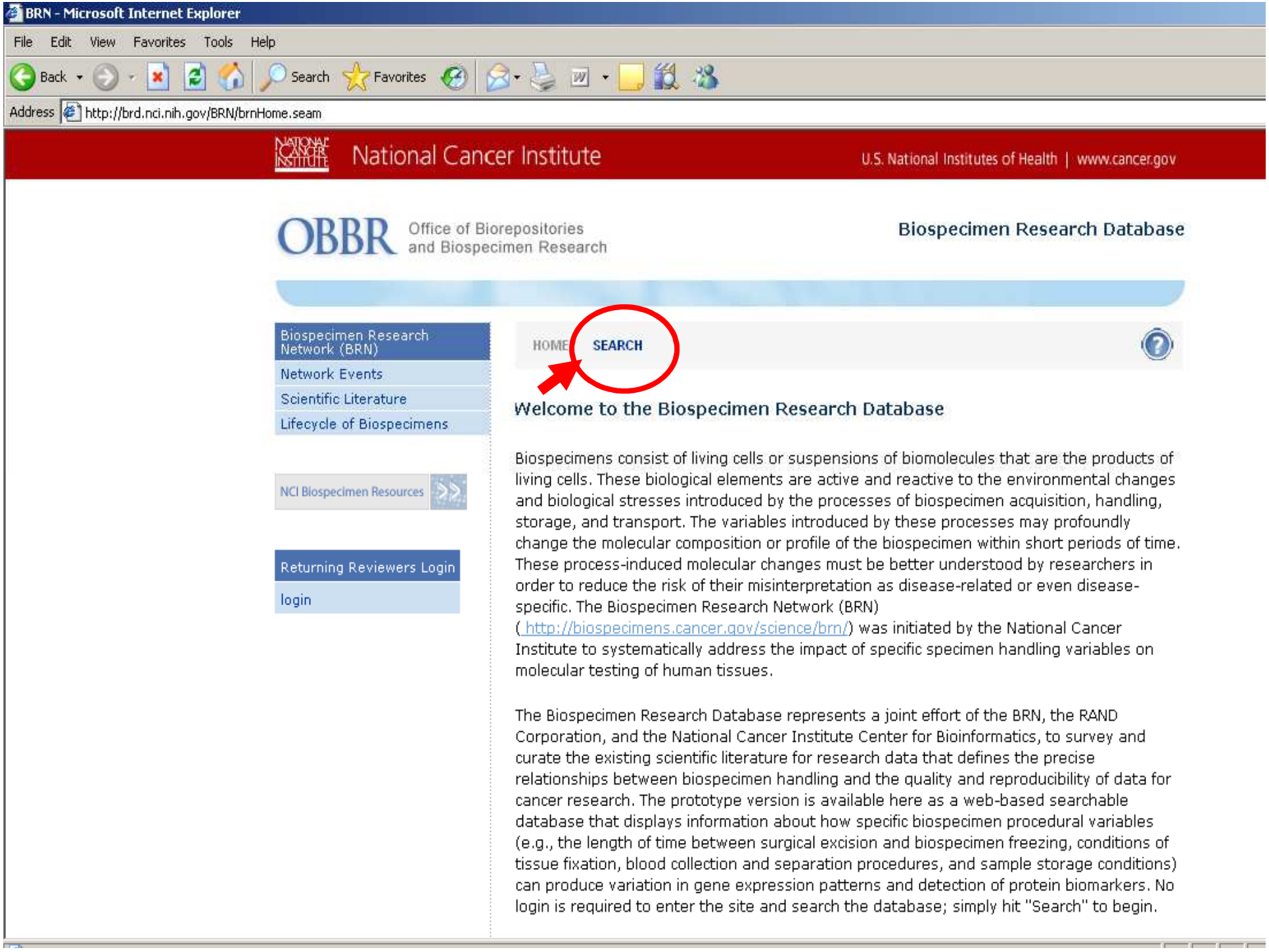
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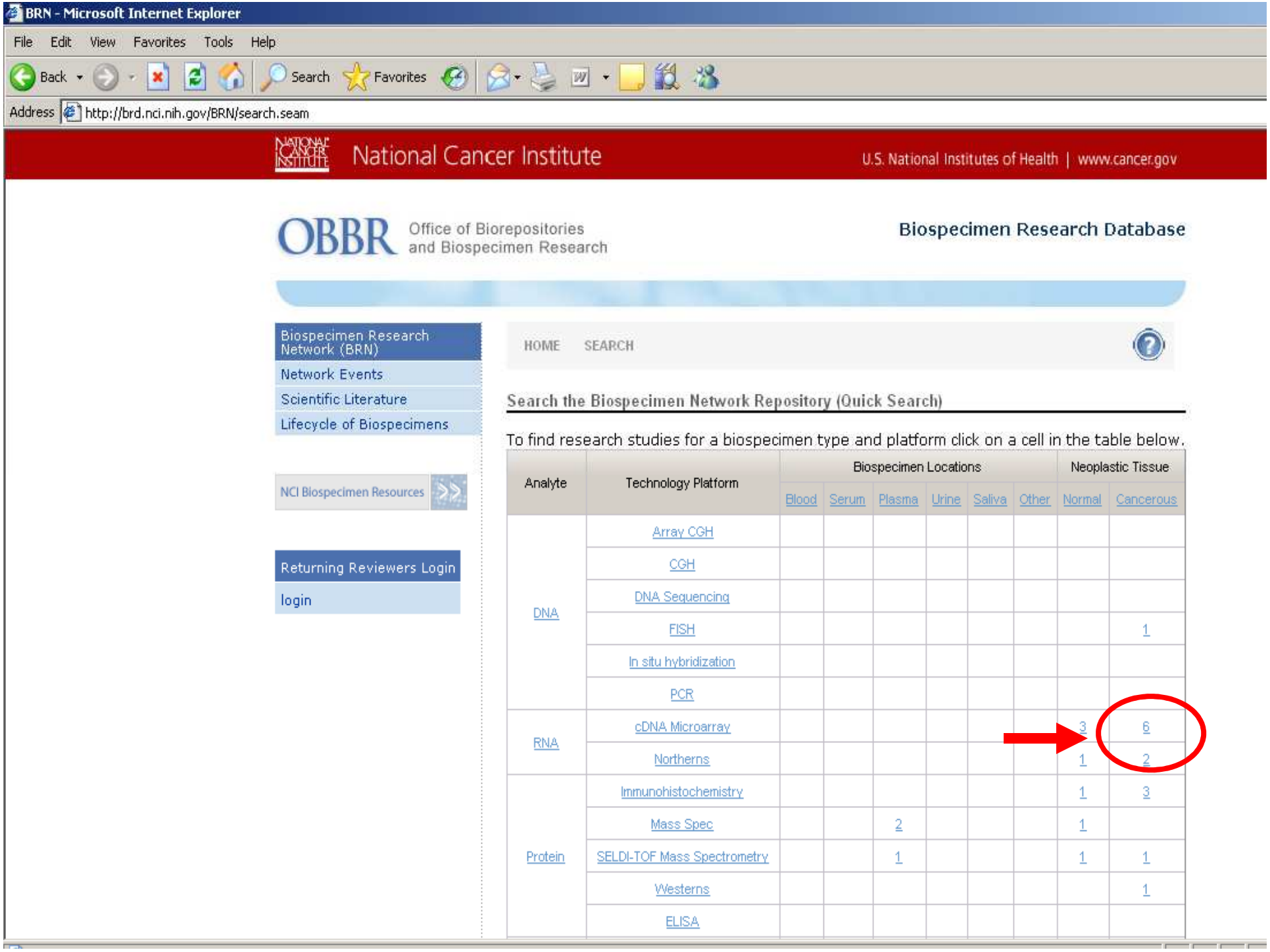
Welcome to the Biospecimen Research Database
Biospecimens consist of living cells or suspensions of biomolecules that are the products of living cells. These biological elements are active and reactive to the environmental changes and biological stresses introduced by the processes of biospecimen acquisition, handling, storage, and transport. The variables introduced by these processes may profoundly change the molecular composition or profile of the biospecimen within short periods of time. These process-induced molecular changes must be better understood by researchers in order to reduce the risk of their misinterpretation as disease-related or even disease-specific. The Biospecimen Research Network (BRN) (http://biospecimens.cancer.gov/science/brn/) was initiated by the National Cancer Institute to systematically address the impact of specific specimen handling variables on molecular testing of human tissues.
The Biospecimen Research Database represents a joint effort of the BRN, the RAND Corporation, and the National Cancer Institute Center for Bioinformatics, to survey and curate the existing scientific literature for research data that defines the precise relationships between biospecimen handling and the quality and reproducibility of data for cancer research. The prototype version is available here as a web-based searchable database that displays information about how specific biospecimen procedural variables (e.g., the length of time between surgical excision and biospecimen freezing, conditions of tissue fixation, blood collection and separation procedures, and sample storage conditions) can produce variation in gene expression patterns and detection of protein biomarkers. No login is required to enter the site and search the database; simply hit "Search" to begin.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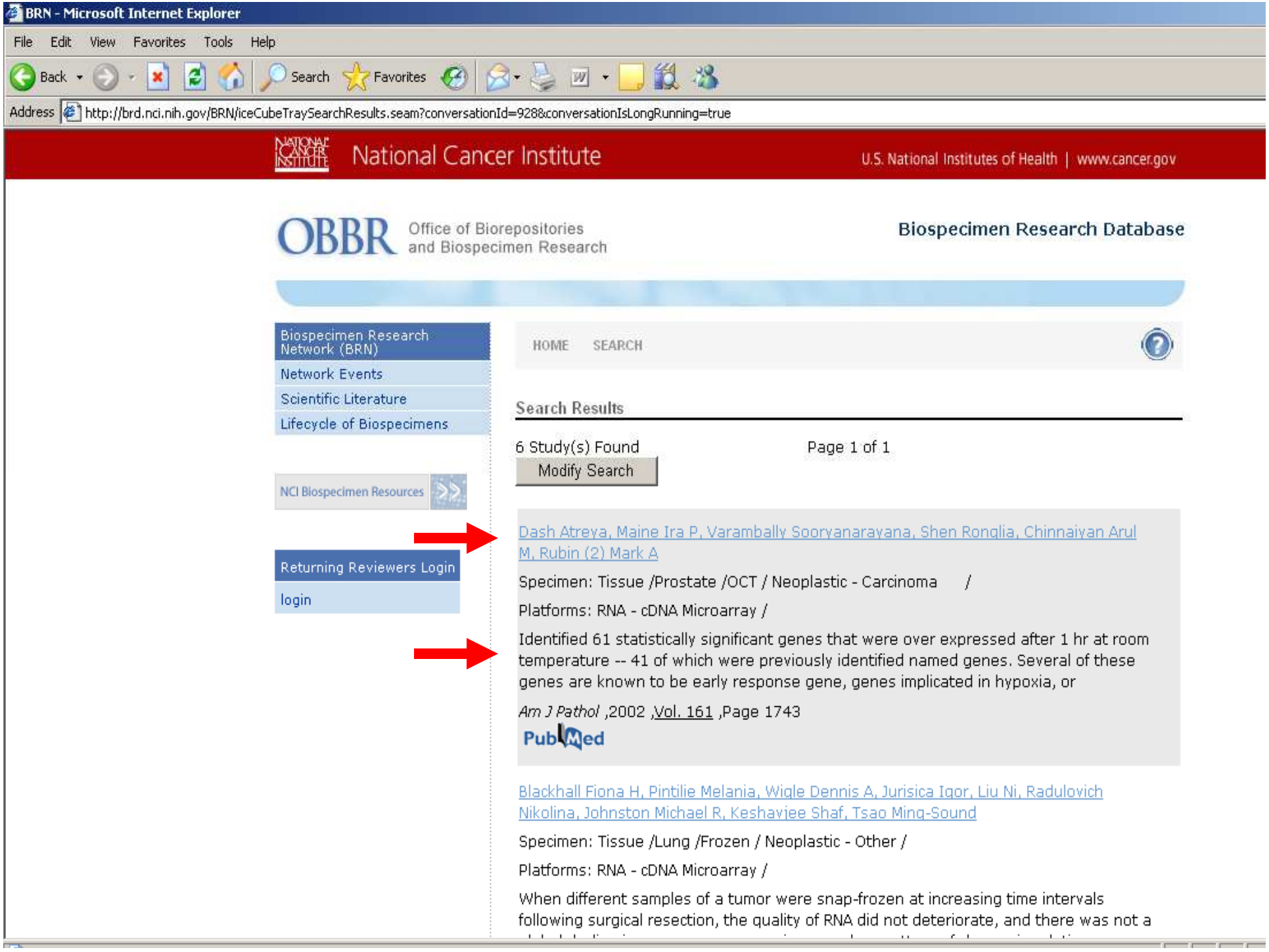
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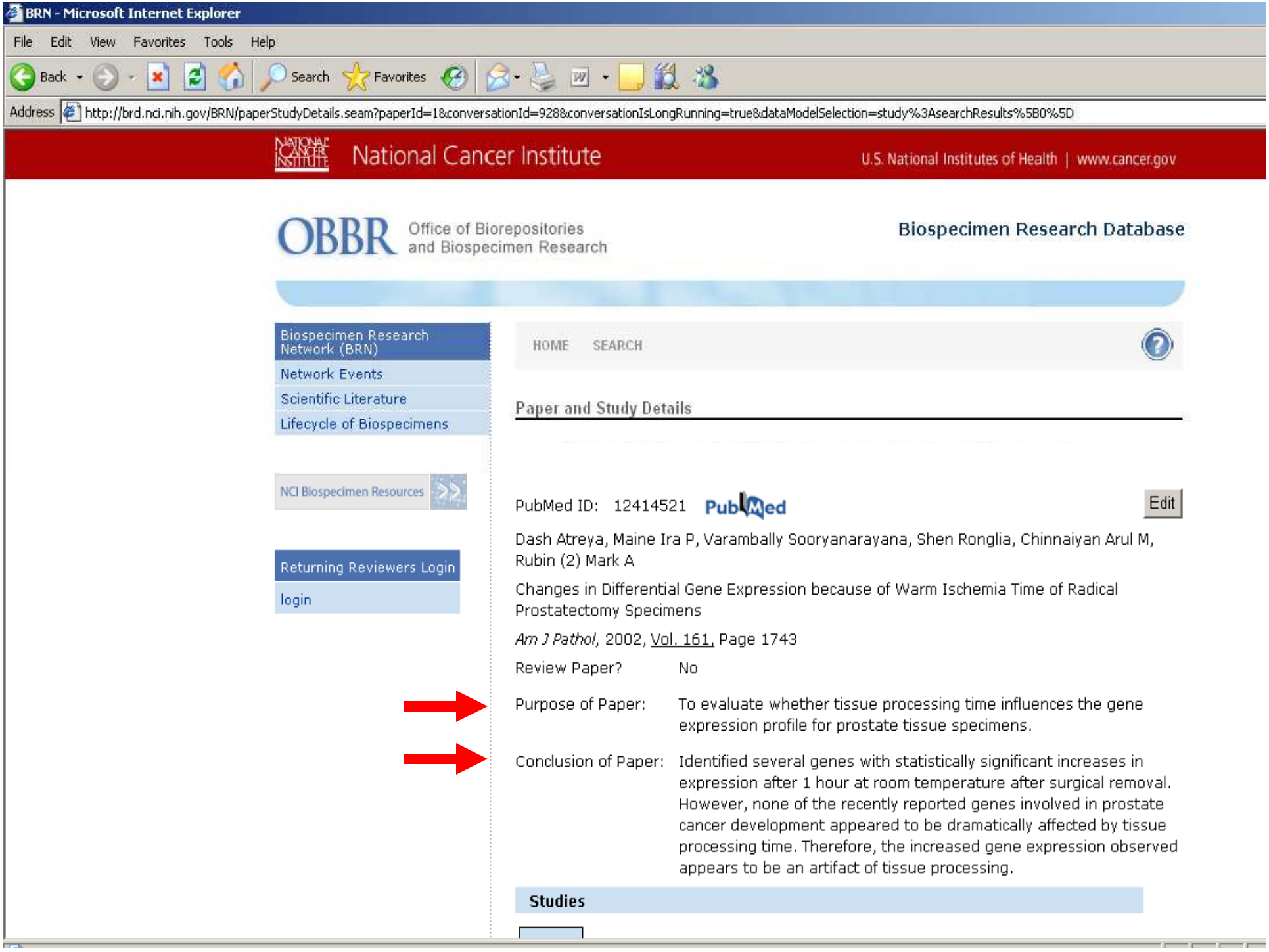
Dash Atreya, Maine Ira P, Varambally Sooryanarayana, Shen Ronglia, Chinnaiyan Arul M, Rubin (2) Mark A
Specimen: Tissue /Prostate /OCT / Neoplastic - Carcinoma /
Platforms: RNA - cDNA Microarray /
Identified 61 statistically significant genes that were over expressed after 1 hr at room temperature -- 41 of which were previously identified named genes. Several of these genes are known to be early response gene, genes implicated in hypoxia, or
Am J Pathol ,2002 ,Vol. 161 ,Page 1743
PubMed
Blackhall Fiona H, Pintilie Melania, Wigle Dennis A, Jurisica Igor, Liu Ni, Radulovich Nikolina, Johnston Michael R, Keshavjee Shaf, Tsao Ming-Sound
Specimen: Tissue /Lung /Frozen / Neoplastic - Other /
Platforms: RNA - cDNA Microarray /
When different samples of a tumor were snap-frozen at increasing time intervals following surgical resection, the quality of RNA did not deteriorate, and there was not a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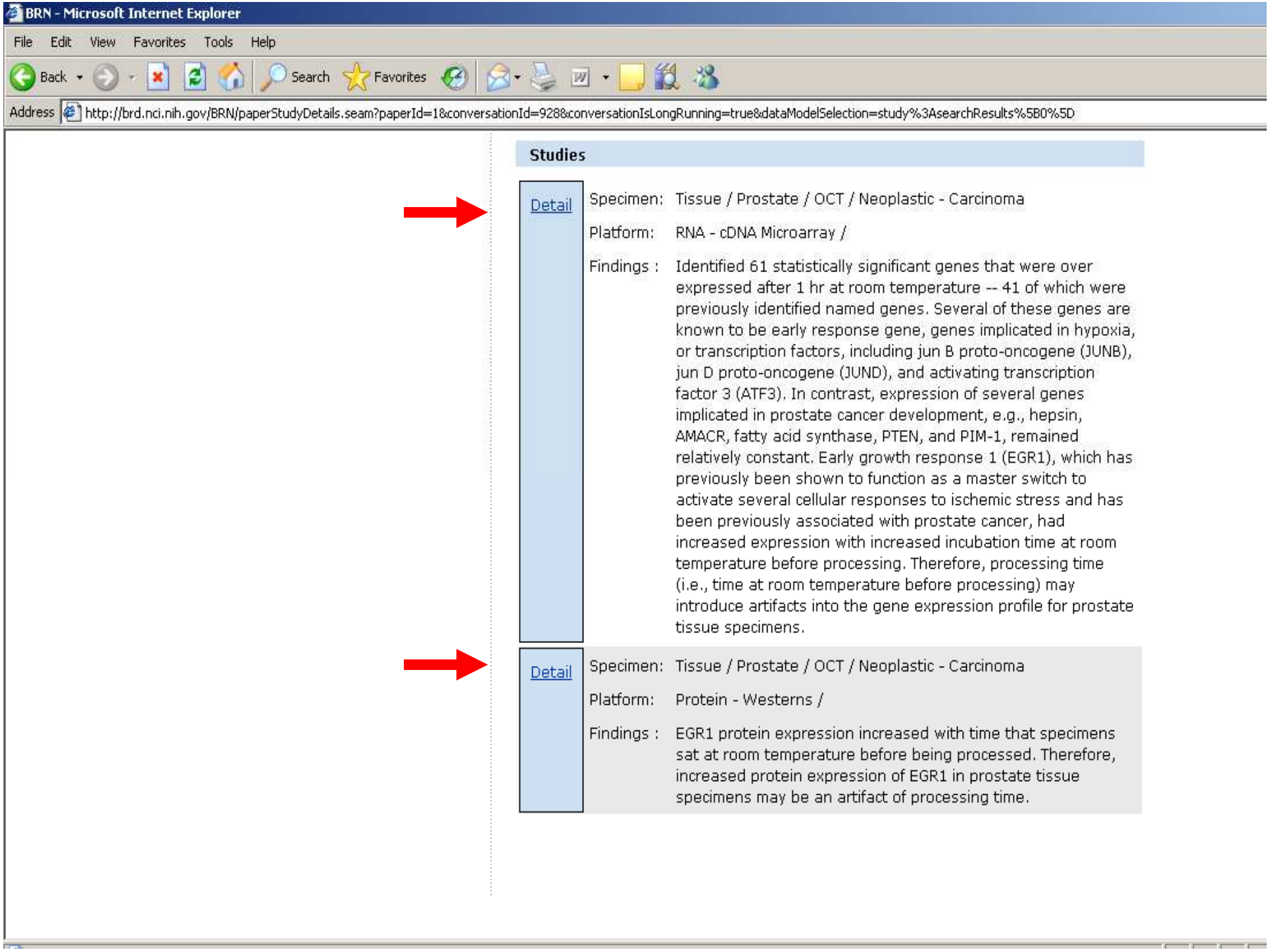
Dash Atreya, Maine Ira P, Varambally Sooryanarayana, Shen Ronglia, Chinnaiyan Arul M, Rubin (2) Mark A
Changes in Differential Gene Expression because of Warm Ischemia Time of Radical Prostatectomy Specimens
Am J Pathol, 2002, Vol. 161, Page 1743
Review Paper? No
Purpose of Paper: To evaluate whether tissue processing time influences the gene expression profile for prostate tissue specimens.
Conclusion of Paper: Identified several genes with statistically significant increases in expression after 1 hour at room temperature after surgical removal. However, none of the recently reported genes involved in prostate cancer development appeared to be dramatically affected by tissue processing time. Therefore, the increased gene expression observed appears to be an artifact of tissue processing.
Studies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Studies
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Specimen: Tissue / Prostate / OCT / Neoplastic - Carcinoma
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Findings : Identified 61 statistically significant genes that were over expressed after 1 hr at room temperature -- 41 of which were previously identified named genes. Several of these genes are known to be early response gene, genes implicated in hypoxia, or transcription factors, including jun B proto-oncogene (JUNB), jun D proto-oncogene (JUND), and activating transcription factor 3 (ATF3). In contrast, expression of several genes implicated in prostate cancer development, e.g., hepsin, AMACR, fatty acid synthase, PTEN, and PIM-1, remained relatively constant. Early growth response 1 (EGR1), which has previously been shown to function as a master switch to activate several cellular responses to ischemic stress and has been previously associated with prostate cancer, had increased expression with increased incubation time at room temperature before processing. Therefore, processing time (i.e., time at room temperature before processing) may introduce artifacts into the gene expression profile for prostate tissue specimens.
Detail
Specimen: Tissue / Prostate / OCT / Neoplastic - Carcinoma
Platform: Protein - Westerns /
Findings : EGR1 protein expression increased with time that specimens sat at room temperature before being processed. Therefore, increased protein expression of EGR1 in prostate tissue specimens may be an artifact of processing time.

What's next for the Database?

OBBR Office of Biorepositories and Biospecimen Research

- **Increase the number of published papers curated and displayed in the database**
  - How? Community participation?
- **Post biospecimen protocols**
  - Can we tap into existing protocols databases?
- **Please see our poster!**
  - Sign up to help!

The BRN: Supporting Collaborative Research

OBBR Office of Biorepositories and Biospecimen Research

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  - This symposium: "Advancing Cancer Research through Biospecimen Science"
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  - New Extramural Programs: approved and coming soon
  - IMAT Program – Innovative technologic solutions for biospecimens (RFA)
- **Collaborate with other programs, e.g.:**
  - Clinical Proteomics Technologies Assessment for Cancer (CPTAC)
  - The Cancer Genome Atlas (TCGA)

Priorities for BRN Research

OBBR Office of Biorepositories and Biospecimen Research

- "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?

New Extramural Research Program

OBBR Office of Biorepositories and Biospecimen Research

An ordered approach to filling the knowledge gaps: RFP

- Studies designed to assess effects of pre-analytical variables in human specimens on the results of genomic, epigenomic, and proteomic analyses
- Model of variable-controlled and/or variable-annotated biospecimen acquisition and invariable molecular analysis
- Trans-disciplinary and highly collaborative design
  - Addresses the many operational factors that influence specimen variation

A creative approach to meeting existing challenges: BAA

- Solicitation of solutions to unmet needs and difficult issues

Biospecimen Research Case Study: Blood Collection and Plasma Processing Variables

OBBR Office of Biorepositories and Biospecimen Research

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

OBBR Office of Biorepositories and Biospecimen Research

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

OBBR Office of Biorepositories and Biospecimen Research

- **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 five different protocols analyzed**

- *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

OBBR Office of Biorepositories and Biospecimen Research

- **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

- **What do we know from the literature?**

Examples of pre-analytical variations in biomarker discovery and validation

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Storage conditions and handling:

- McLerran, D.*, et al.* Analytical Validation of Serum Proteomic Profiling for Diagnosis of Prostate Cancer: Sources of Sample Bias. *Clin Chem* 54, 44-52 (2008).
- Rouy, D., Ernens, I., Jeanty, C. & Wagner, D.R. Plasma storage at-80 degrees C does not protect matrix metalloproteinase-9 from degradation. *Analytical Biochemistry* 338, 294-298 (2005).

Blood collection site

- Yang, Z.W.*, et al.* Comparison of blood counts in venous, fingertip and arterial blood and their measurement variation. *Clinical and Laboratory Haematology* 23, 155-159 (2001).

Patient posture during blood collection:

- Miller, M., Bachorik, P.S. & Cloey, T.A. Normal Variation of Plasma-Lipoproteins - Postural Effects on Plasma-Concentrations of Lipids, Lipoproteins, and Apolipoproteins. *Clinical Chemistry* 38, 569-574 (1992)

Tube type:

- Drake, S.K., Bowen, R.A.R., Remaley, A.T. & Hortin, G.L. Potential interferences from blood collection tubes in mass spectrometric analyses of serum polypeptides. *Clinical Chemistry* 50, 2398-2401 (2004)
- Yucel, A., Karakus, R. & Aybay, C. Effect of blood collection tube types on the measurement of human epidermal growth factor. *Journal of Immunoassay & Immunochemistry* 28, 47-60 (2007)

In-vitro sources of platelet activation in blood specimens

OBBR Office of Biorepositories and Biospecimen Research

Type of material of tubing/syringe

- Gorbet, M.B. & Sefton, M.V. Biomaterial-associated thrombosis: roles of coagulation factors, complement, platelets and leukocytes. *Biomaterials* 25, 5681-5703 (2004).

Tourniquet Time

- Lippi, G., Salvagno, G.L., Montagnana, M., Franchini, M. & Guidi, G.C. Venous stasis and routine hematologic testing. *Clinical and Laboratory Haematology* 28, 332-337 (2006).

Blood collection technique

- Lippi, G., et. al. Preanalytical variability in laboratory testing: influence of the blood drawing technique. *Clinical Chemistry and Laboratory Medicine* 43, 319-325 (2005).

Storage conditions

- Rock, G. & Figueredo, A. METABOLIC CHANGES DURING PLATELET STORAGE. *Transfusion* 16, 571-579 (1976).Zhang, J.N., et al. Effects of low temperature on shear-induced platelet aggregation and activation. *Journal of Trauma-Injury Infection and Critical Care* 57, 216-223 (2004)
- Josefsson, E.C., Hartwig, J.H. & Hoffmeister, K.M. Platelet storage temperature - How low can we go? *Transfusion Medicine and Hemotherapy* 34, 253-261 (2007).

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

OBBR Office of Biorepositories and Biospecimen Research

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

Acknowledgments

OBBR Office of Biorepositories and Biospecimen Research

Research Database: NCI

- Helen Moore
- Ian Fore
- Jim Vaught
- Asha Collins
- NCI-CBIIT Web team
  - Jerry Eads
  - Charles Yaghmour
  - Jyothsna Chilukuri
  - Stephen Hunter
  - Paul Morris

Research Database: RAND

- Elisa Eiseman
- Asha Pathak
- John Zambrano
- Anant Patal

CPTAC Biospecimens Working Group

- Steve Skates and Helen Moore, co-chairs
- Mark Lim, OBBR

We need your input!

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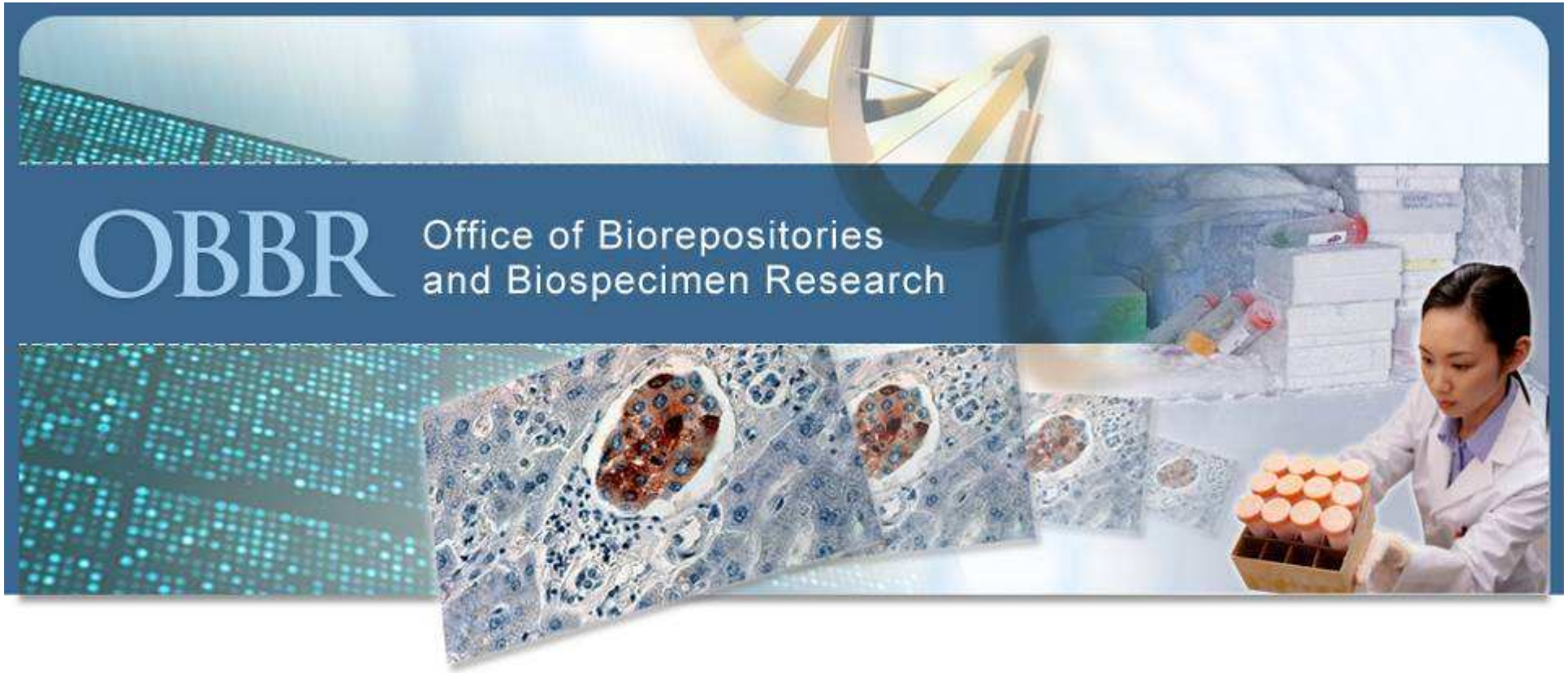
- **Website:**

  http://biospecimens.cancer.gov

- **Email:**

  biospecimens@mail.nih.gov



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Expected Program Outcomes

OBBR Office of Biorepositories and Biospecimen Research

- ✓ **Publications and presentations from the program** on the effect of human specimen pre- and post-acquisition variables on downstream molecular analysis
- ✓ **Publications from members of the scientific community** at large in response to raised awareness of the importance of such studies
- ✓ **Increased attention to QA/QC important to downstream molecular analysis by manufacturers of consumables, reagents, and robotics** (e.g., vacutainers used for blood collection, tissue preservatives, tissue processors)
- ✓ **CAP guidelines based on new data with implementation in the clinical arena**: Greater attention to QA/QC of hospital tissue preservation procedures and equipment, resulting in higher quality preserved tissues for patient molecular diagnosis and research
- ✓ **Implementation of data-driven standards for specimen handling in new venues**: Inclusion of biospecimen handling parameters in clinical trials and in research, development, and regulation of cancer biomarkers
- ✓ **GREATER REPRODUCIBILITY OF RESEARCH AND CLINICAL RESULTS**