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AND ENVIRONMENT

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

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This presentation is based on RAND Corporation research and author's opinions. Portions of this presentation describe work-in-progress that has not undergone RAND quality assurance procedures.

Purpose of Project

Maximize quality and utility of human biospecimens for cancer research by identifying and analyzing existing data on how biospecimens are affected by environmental and biological variables introduced by acquisition, processing, storage, and distribution (i.e., preanalytical variables)

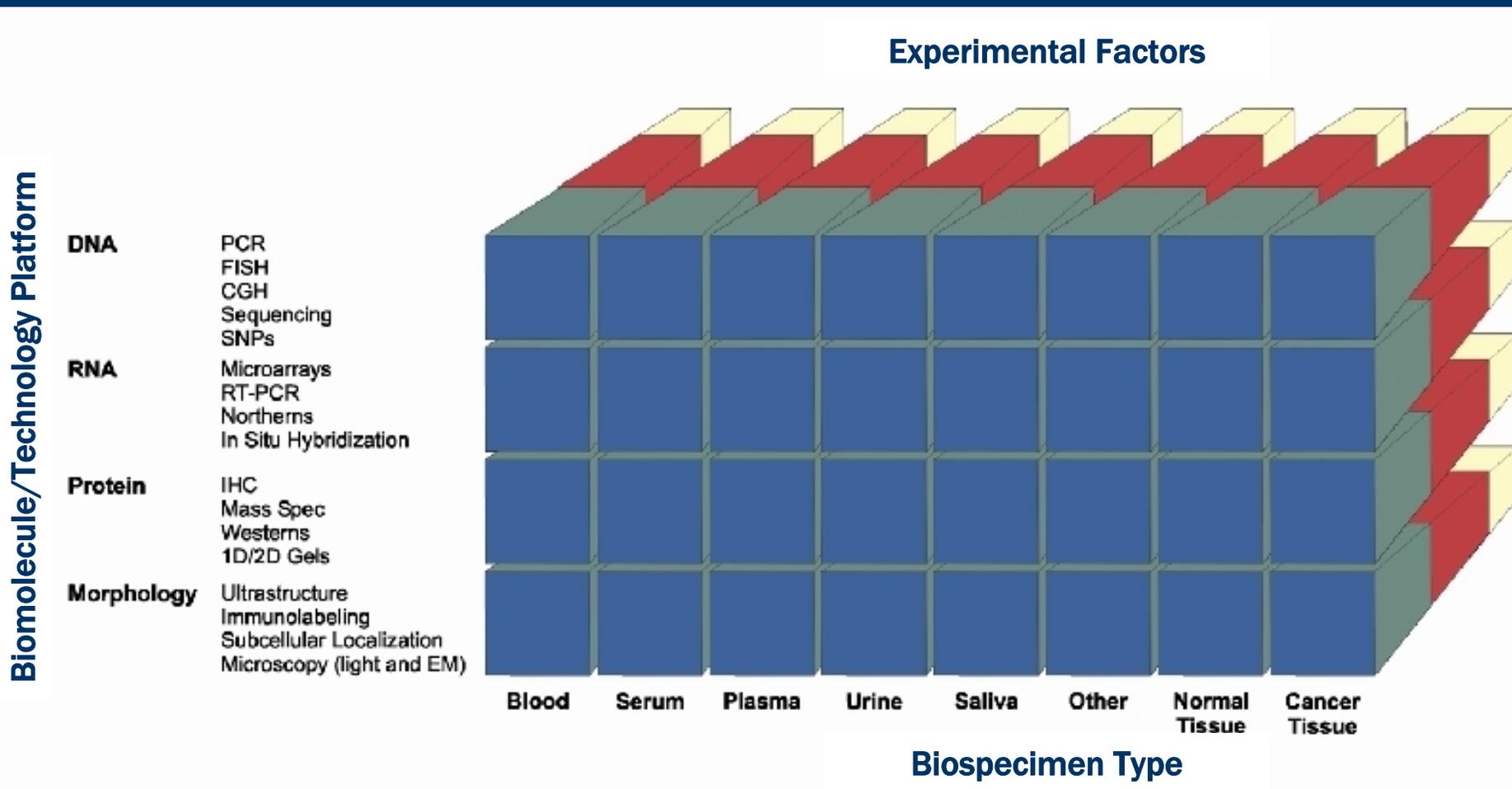
Approach

- Conduct comprehensive search of scientific literature for studies conducted specifically to determine effects of preanalytical variables on quality of biospecimens used to study genetic changes in cancer
- Design data curation tool and populate database with data from literature
- Collaborate with OBBR to develop a searchable, Web-based database
- Analyze data for information about effects of preanalytical variables on analytic methods used

Data Collection Fields

- **Biospecimen Type and Diagnosis**
- **Biomolecule Type/ Technology Platform**
- **Experimental Factors**
(i.e., Preanalytical Variables)

Framework for Analysis of Effects of Preanalytical Variables on Biospecimens



Experimental Factors

Pre-acquisition variables:

- Antibiotics
- Other Drugs
- Type of anesthesia
- Duration of anesthesia
- Arterial clamp time
- Blood pressure variations
- Intra-op blood loss
- Intra-op blood administration
- Intra-op fluid administration
- Type of surgical/medical procedure
- Pre-existing medical condition

Post-acquisition variables:

- Time at room temperature
- Temperature of room
- Type of fixative
- Temperature of fixative
- Time in fixative
- Freezing method
- Rate of freezing
- Size of aliquots
- Type of collection container
- Biomolecule extraction method
- Storage temperature
- Storage duration

Data Curation Tool

Biospecimen Research
Network (BRN)

Network Events

Scientific Literature

Lifecycle of Biospecimens

NCI Biospecimen Resources >>

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STUDIES

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PAPERS

PubMed ID Paper Title *

Authors

Last Name	First Name	MI
Dash	Atreya	
Maine	Ira	P
Varambally	Sooryanarayana	

Journal Publication Year Volume Page Number Check if this is a review paper Check if this is a published paperUnpublished Paper Date 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.

1st Reviewer

2nd Reviewer

Papers

Record: of 65

- Biospecimen Research Network (BRN)
- Network Events
- Scientific Literature
- Lifecycle of Biospecimens**

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STUDIES

Purpose *

To study the increase in gene expression using cDNA microarray technology to identify individual genes that may be artifacts of processing of prostate tissue obtained from radical prostatectomy specimens removed as treatment for localized prostate cancer.

BioSpecimen Type

Tissue

Tissue/Fluid/Cell Type

Prostate

**Diagnosis /
Diagnosis Subcategory**

▶ Neoplastic	▼ Carcinoma	▼ ▲
* []	▼ []	▼ []

Preservative

OCT

**Biomolecule /
Platform**

▶ RNA / cDNA Microarray	▼ ▲
* []	▼ []

Summary Of 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 early growth response 1 (EGR1), 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.

EXPERIMENTAL FACTORS

Experimental Factor * Time at room temperature/pre-fixation time

Select a Value from the List Below

OR Enter a Value

	▼
	▼
	▼

0 hrs
0.5 hrs
1 hr

Experimental Factors

Record:      of 1

Studies

Record:      of 2

PAPER

Title Changes in Differential Gene Expression because of Warm Ischemia Time of Radical Prostatectomy Specimens

PubMedID 12414521

Publication Am J Pathol

Year 2002

Page 1743

Results of Study (Year 1)

- 65 papers reviewed
- 145 studies – Range was 1 – 8 studies per paper
- Biomolecule
 - DNA = 45 studies
 - RNA = 46 studies
 - Protein = 53 studies
 - Morphology = 10 studies
- Technology Platform
- Preanalytical Variable

Technology Platform

Biomolecule	Technology Platform	# of Studies
DNA	Array CGH	5
	CGH	2
	DNA Sequencing	5
	Electrophoresis	8
	FISH	2
	In situ hybridization	1
	PCR	13
	SNP Assay	2
	Tissue Microarray	3
RNA	cDNA Microarray	11
	RT-PCR	24
	Electrophoresis	4
	In situ hybridization	2
	Northerns	4
Protein	SELDI-TOF Mass Spectrometry	5
	Mass Spec	5
	Antibody microarray	2
	Immunohistochemistry	19
	Westerns	8
	Tissue Microarray	3
	1D/2D gels	11
Morphology	Standard H&E microscopy	10

Preanalytical Variables

Experimental Factor	# of Studies
Time at room temperature /pre-fixation time	15
Type of fixative	59
Time in fixative	13
Biomolecule extraction method	37
Storage temperature	8
Storage duration	11

Storage Duration

- **Biomolecule = DNA**
- **Technology Platform = PCR**
- **Experimental Factor = Storage Duration**
 - **0-1 month**
 - **4 weeks**
 - **3-6 months**
 - **6-12 months**
 - **>12 months**
 - **12-24 months**
 - **36-41 months**

Biospecimen Research Database



Biospecimen Research Network (BRN)

Network Events

Scientific Literature

Lifecycle of Biospecimens

NCI Biospecimen Resources



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HOME SEARCH



Search the Biospecimen Network Repository (Quick Search)

To find research studies for a biospecimen type and platform click on a cell in the table below.

Analyte	Technology Platform	Biospecimen Locations						Neoplastic Tissue	
		Blood	Serum	Plasma	Urine	Saliva	Other	Normal	Cancerous
DNA	Array CGH								
	CGH								
	DNA Sequencing								
	FISH								1
	In situ hybridization								
RNA	PCR								
	cDNA Microarray							3	6
	Northern							1	2
Protein	Immunohistochemistry							1	3
	Mass Spec					2		1	
	SELDI-TOF Mass Spectrometry					1		1	1
	Westerns								1
	ELISA								
Morphology	Standard H-n-E microscopy								
	Subcellular localization								
	Ultrastructure								

[Simple Search](#) [Advanced Search](#)



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Search Results

3 Study(s) Found Page 1 of 1

[Modify Search](#)

[Spruessel Annika, Steimann Garnet, Jung Mira, Lee Sung A, Carr Theresa, Fentz Anne-Kristin, Spangenberg Joerg, Zornig Carsten, Juhl Hartmut H, David Kerstin A](#)

Specimen: Tissue /Colorectal /Frozen / Neoplastic - Normal Adjacent /

Platforms: RNA - cDNA Microarray /

No differences of RNA quality were observed over a period of 30 minutes. Changes in gene expression profiles were already observed 5-8 minutes after colon resection. 15 minutes after surgery, 10-15% of all genes differed significantly (>2-fold) from the *BioTechniques* ,2004 ,Vol. 36 ,Page 1030



[Huang J, Qi R, Quackenbush J, Dauway E, Lazaridis E, Yeatman T](#)

Specimen: Tissue /Colorectal /Frozen / Neoplastic - Normal Adjacent /

Platforms: RNA - cDNA Microarray /

Significant changes in gene expression levels occur in normal adjacent colon tissue as early as 20 minutes after surgical removal. Increases in expression of some genes and decreases in expression of others were observed.

Journal of Surgical Research ,2001 ,Vol. 99 ,Page 222



[Lin Daniel W, Coleman Ilsa M, Hawley Sarah, Dumpit Ruth, Gifford David, Kezele Philip, Hung Hau, Knudsen Beatrice S, Kristal Alan R, Nelson Peter S](#)

Specimen: Tissue /Prostate /OCT / Neoplastic - Normal Adjacent /

Platforms: RNA - cDNA Microarray /

Examination of 5,753 cDNAs by microarray hybridization showed 62 unique genes that had higher expression in postsurgical specimens as compared to presurgical specimens with false-discovery rates of 10% or lower. These include several genes

Journal of Clinical Oncology ,2006 ,Vol. 24 ,Page 3763





Biospecimen Research Network (BRN)

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Paper and Study Details

PubMed ID: 11469890

Huang J, Qi R, Quackenbush J, Dauway E, Lazaridis E, Yeatman T

Effects of Ischemia on Gene Expression

Journal of Surgical Research, 2001, Vol. 99, Page 222

Review Paper? No

Purpose of Paper: To determine the effects of time at room temperature after surgical removal on gene expression profiles in normal adjacent tissue from a human colon cancer specimen.

Conclusion of Paper: Time at room temperature after surgical removal of normal colon mucosa from a human colon cancer specimen has significant effects on gene expression in as little as 20 minutes.

Studies

[Detail](#)

Specimen: Tissue / Colorectal / Frozen / Neoplastic - Normal Adjacent

Platform: RNA - Northern /

Findings : No differences in RNA quality were detected by ethidium bromide staining of 18S and 28S ribosomal RNA even after 60 minutes at room temperature after surgical removal in colon cancer and normal adjacent tissue. In addition, there was no noticeable effect on the expression of GAPDH as measured by Northern blot.

[Detail](#)

Specimen: Tissue / Colorectal / Frozen / Neoplastic - Normal Adjacent

Platform: RNA - cDNA Microarray /

Findings : Significant changes in gene expression levels occur in normal adjacent colon tissue as early as 20 minutes after surgical removal. Increases in expression of some genes and decreases in expression of others were observed.



- Biospecimen Research Network (BRN)
- Network Events
- Scientific Literature
- Lifecycle of Biospecimens

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Search the Biospecimen Network Repository (Simple Search)

Select one or more options below to find research studies for a biospecimen type and analytical platform then click the "Search" button.

Specimen

Biospecimen Type	Biospecimen Location
All <input type="button" value="v"/>	All <input type="button" value="v"/>
Diagnosis	
All <input type="button" value="v"/>	
Preservative Type	
All <input type="button" value="v"/>	

Analytical Platform

Technology Platform
All <input type="button" value="v"/>

Search

Clear

Cancel

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Search the Biospecimen Network Repository (Advanced Search)

Specimen

<p>Biospecimen Type</p> <div style="border: 1px solid #ccc; padding: 5px;"> <p>Cell</p> <p>Fluid</p> <p>Tissue</p> </div>	<p>Biospecimen Location</p> <div style="border: 1px solid #ccc; padding: 5px;"> <p>Adipose</p> <p>Adrenal Gland</p> <p>Amniotic Fluid</p> <p>Aorta</p> <p>Appendix</p> </div>
<p>Diagnosis</p> <div style="border: 1px solid #ccc; padding: 5px;"> <p>AIDS/HIV-related</p> <p>Alzheimer's Disease</p> <p>Amyotrophic Lateral Sclerosis</p> <p>Arteriosclerosis</p> <p>Arthritis</p> </div>	<p>Diagnosis Subcategory</p> <div style="border: 1px solid #ccc; padding: 5px;"> <p>Benign</p> <p>Carcinoma</p> <p>Germ Cell</p> <p>Leukemia</p> <p>Lymphoma</p> </div>
<p>Preservative Type</p> <div style="border: 1px solid #ccc; padding: 5px;"> <p>Ethanol</p> <p>Formalin</p> <p>Frozen</p> <p>None (Fresh)</p> <p>OCT</p> </div>	

Platform

<p>Analyte</p> <div style="border: 1px solid #ccc; padding: 5px;"> <p>DNA</p> <p>Morphology</p> <p>Protein</p> <p>RNA</p> </div>	<p>Technology Platform</p> <div style="border: 1px solid #ccc; padding: 5px;"> <p>1D/2D gels</p> <p>Antibody microarray</p> <p>Array CGH</p> <p>CGH</p> <p>DNA Sequencing</p> </div>
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Author(s)

Enter the author's name(s) in the format of last name followed by first initial (first initial is optional). Separate authors' names by a comma. Use "*" as wildcard. Examples: Smith J, Doe L

Next Steps

- Expand information in database with:
 - data from existing studies that focus directly on the effects of preanalytical variables on biospecimens
 - procedures for clinical laboratory testing relevant to research on genetic changes in cancer
 - other potential sources of data (e.g., FDA submissions, unpublished data)
- Meta-analysis of data:
 - to inform development and prioritization of Biospecimen Research Network “wet” laboratory studies
 - to inform development of evidence-based standard operating procedures (SOPs)

Acknowledgments

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