

# Developing Biomarkers for the National Children's Study

Jane Gallagher, PhD<sup>1</sup>, John Rockett, PhD<sup>1</sup>, Suzanne Fenton, PhD<sup>1</sup>, Pauline Mendola, PhD<sup>1</sup>, Danelle Lobdell, PhD<sup>1</sup>, Suzanne McMaster, PhD<sup>1</sup>, David Dix, PhD<sup>1</sup>, Kary Thompson, PhD<sup>1</sup>, Robert MacPhail, PhD<sup>1</sup>, Stan Barone, PhD<sup>2</sup>, and Tara Lyons-Darden, PhD<sup>1</sup>

<sup>1</sup> National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC

<sup>2</sup> National Center for Environmental Assessment, U.S. Environmental Protection Agency, Washington, DC

## Research Goals

- Develop biomarkers that can be incorporated in the National Children's Study
- Maximize human-animal extrapolation by identifying and validating common biomarkers from observational human studies and experimental animal studies
- Focus on samples that could be obtained with minimal invasiveness from human subjects, including children, infants, and pregnant women

## Biomarkers Database

### Goals:

Develop a searchable database of information on biomarkers, emerging or potential markers, sample collection, handling, storage issues

### Coverage:

- General biomarkers
  - 1999-6/2004 review
- Exposure
  - 1999-6/2004 review
  - 10/2001 to 6/2004 all
- Asthma
  - 1999-6/2004 review
  - 10/2001 to 6/2004 all
- Cancer
  - 1999-6/2004 review children
  - 10/2001 to 7/2003 all
- Neurodevelopmental disorders
  - 1996-6/2004 review autism
  - 1999-6/2004 review
  - 10/2001 to 6/2004 all
- Injury
  - 1996-6/2004 review
- Miscellaneous
  - 2000-6/2004



Database:  
<http://cfpub2.epa.gov/ncea/cfm/recorddisplay.cfm?id=85844>

Final report:  
[www.nationalchildrensstudy.gov/research/analytic\\_reports](http://www.nationalchildrensstudy.gov/research/analytic_reports)

## Methods Advancement for Milk Analysis MAMA

### Goals:

- Define collection, preservation, and storage recommendations for human milk samples
- Confirm lack of contamination from pumps and storage vessels
- Provide reliable assays to measure defined endogenous and exogenous constituents of fresh and frozen human milk
- Evaluate blood, saliva, and urine as surrogate media for the analysis of milk constituents

### Current Status:

- Labcorp completed validation analyses
- Sample collection completed

## Blood for Gene Expression Profiling

Two methods of preparing RNA from whole blood were examined: Paxgene and ZR

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Cost: Paxgene - \$12/sample;  
ZR - \$4/sample

Volume of blood required:  
Paxgene - <2.5ml;  
ZR - <200ul

Time to process:  
Paxgene - 2-3 h; ZR - 15 min

Average RNA yield:  
Paxgene - 15 ng/μl of blood;  
ZR - 23 ng/μl of blood

ZR kit has less variability in yield than Paxgene kit

However, the RNA appears less stable in ZR buffer than Paxgene buffer (degradation occurs)

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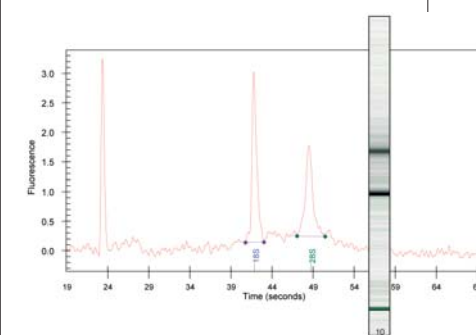
Agilent Bioanalyzer output demonstrating good quality RNA isolated from whole blood by Paxgene and ZR kits

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## Hair Follicles for Gene Expression Profiling

Up to 5 hairs aggressively plucked from subject scalp ("Trichogram")

- Transferred/stored
- RNA extracted using Trizol
- 130-1300ng of RNA/follicle



Agilent Bioanalyzer output demonstrating good quality RNA

Affymetrix gene expression profiling has been conducted on 10 samples to identify genes expressed in hair follicles and determine interindividual variation. Data analysis is underway.

## Results and Conclusions

- Whole blood and hair follicles yield sufficient quantity and quality of RNA to conduct microarray analysis, suggesting that hair samples may be used as a surrogate tissue for gene expression profiling studies.
- Human sperm contain a common set of RNAs reflective of spermatogenesis and male fertility, with variation in gene expression suggesting the utility of sperm RNA as a biomarker of reproductive exposures.
- Uroepithelial cells are of insufficient quality (and in some cases quantity) to be a useful source of RNA for gene expression profiling studies.
- Studies on fingernails in infants suggest that they may be useful for exposures occurring *in utero* that are otherwise difficult to measure.

## Impact

Evaluating the utility of non-invasive sample matrices is important for the long-term success of the National Children's Study, especially for methods in developing fields of science such as gene expression. Understanding data needs relevant to risk assessment implementation, such as comparable animal and human biological markers, as well as their validity relevant to exposures and outcomes.

## Semen for Gene Expression Profiling

Computer-Assisted Sperm Analysis (count, concentration, morphology, motility)

- Sperm extracted from semen
- RNA extracted from sperm
- 1-2ug RNA per ejaculate

Gene expression in 17/21 samples analyzed on Affymetrix U133+2.0 chips:

Genes with the greatest abundance included spermatogenic genes (e.g., testis specific protein 1, protamine 1, outer dense fiber of sperm tails 1), and many others that have been previously identified in ejaculated human spermatozoa.

These data suggest that there is a core set of approximately 5,000 genes expressed across individuals.

Thus, human sperm contain a common set of RNAs reflective of spermatogenesis and male fertility, with variation in gene expression suggesting the utility of sperm RNA as a potential biomarker for reproductive exposures.