National Children's Study

Biological Sample Analytic Laboratory Results Datasets

Version: 2.0

August 2018

THE NATIONAL CHILDREN'S STUDY

HEALTH GROWTH ENVIRONMENT

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I. Introduction

These NCS biospecimen analytical laboratory results data files are SAS datasets of laboratory test results from biological samples collected for the NCS Vanguard Study during all four phases as shown in Table 1. Various types of biological samples were collected from participants during the NCS Vanguard Study and periodically shipped to and analyzed at three laboratories. The laboratories were: (1) Fisher BioServices (FBS) (NCS repository) in Rockville, MD; (2) Quest Diagnostics in San Juan Capistrano, CA; and (3) National Center for Environmental Health (NCEH), Centers for Disease Control and Prevention (CDC) in Atlanta, GA. Laboratory results were submitted to the NCS Program Office (PO) in Excel spreadsheets, and subsequently sent to Westat for data cleaning, quality review, and conversion into SAS datasets for analysis. The information in these datasets will be available to future researchers as part of the NCS Archive. This dataset delivery represents 124,184 assay results from 2,602 unique NCS participants.

Table 1: NCS Vanguard Study Phases

NCS Vanguard Study Phases	Data Collection Period
Initial Vanguard Study (IVS)	2009–2010
Alternate Recruitment Substudy (ARS)	2011–2012; Minimal and Expanded Visits
Provider-Based Sampling Substudy (PBS)	2012–2014
Post-Recruitment Follow-Up (PRF)	2014

The SAS datasets contain analyte results identified by Sample and Participant IDs (PID). If we could not find or confirm the PID or biospecimen consent, the sample result was not included in the dataset. The datasets contain many other variables in addition to the results and IDs; a list of the included variables can be found in Appendix A. Among these variables are analyte reference ranges and the limits of detection (LODs) of the respective assays. Reference ranges and LODs were provided by the reference laboratory or taken from other sources as described below. Descriptions of assay methods can be found in Appendix B.

These SAS datasets include information about the sample analyzed as well as limited participant information. Variables in the datasets are meant to be useful to future investigators for exploring the available laboratory results to answer research questions or to select NCS additional samples for analyses. The variables for Participant ID (PID) and Sample ID are the primary keys, or linkages, to other data found in NCS datasets such as demographic, questionnaire, instrument, or existing inventory files. Some associated data may require the primary keys plus additional variables (i.e. an Event or Participant Type) from the dataset to access specific records of interest.

A good understanding of the Sample ID, also referred to as Specimen ID in some NCS documentation, will assist users in their data queries. Sample IDs were provided centrally by the NCS PO and are not related to the PID, i.e. samples collected from the same participant at different times do not have the same Sample ID nor are they linked in any way by the Sample ID.

The Sample ID Number consists of two components, a **Root ID** (two alpha characters followed by seven numbers) and a **Sequence ID** (four alpha numeric characters). The Sequence ID identifies the type of sample or sample collection vessel. The Root ID and Sequence ID are linked together by a dash to create a unique **Sample ID**.

- **Root ID**: BA2345671
- **Sequence ID**: LV10
- **Sample ID**: BA2345671-LV10

Additional information regarding labeling and other procedural details can be found in the Biospecimen Overviews, SOPs and the NCS Repository Inventory Dataset Delivery Summary.

II. Dataset Descriptions

We divided the data into four categories (hematology, blood, urine, and other sample types) and created four respective SAS datasets and codebooks as accompanying documentation. The filenames and numbers of respective records and variables are shown in Table 2. Descriptions of the dataset variables can be found in Appendix A. Assay methodology information with sample type and laboratory identification is located in Appendix B.

Table 2: NCS Vanguard Biospecimen Laboratory Analytic Data Delivery Files

Dataset	File Name	Codebook	Number of Records	Number of Variables
Hematology	vs_hematology_results_v2.sas7bdat	vs_hematology_results_v2_Codebook.pdf	18,304	20
Blood	vs_bloodlab_results_v2.sas7bdat	vs_bloodlab_results_v2_Codebook.pdf	44,947	20
Urine	vs_urinelab_results_v2.sas7bdat	vs_urinelab_results_v2_Codebook.pdf	55,377	22
Other Sample Types	vs_other_sample_results_v2.sas7bdat	vs_other_sample_results_v2_Codebook.pdf	5,556	20
TOTAL NUMBER OF RECORDS	…	…	124,184	...

Reference Ranges

Adult reference ranges provided in the Hematology dataset for CBCs and cell differentials were from the respective analyzer (Coulter AcT 10 and Beckman Coulter DxH 800 hematology analyzers) user manuals. However, instrument-specific reference ranges from newborns, children and certain adult ranges were not provided by manufacturers and were from other sources. These include well-known hematology text books such as Saunders (Nathan DG, and Oski, FA (1981) Hematology of Infancy and Childhood, 2nd ed, WB Saunders, Elsevier Health Sciences, Atlanta, GA) and Soldin (Soldin SJ, Brugnara C, Wong EC (2005) Pediatric Reference Intervals, 5th ed, AACC Press, Washington, DC). While matching results to these reference ranges was not ideal, we believe that they provide a starting point for comparison - NCS data users should decide how relevant the matches are for their own research purposes.

Reference ranges and LODs for those analytes measured at NCEH were either provided by NCEH along with the submission of the results or were extracted from two national Centers for Disease reports:

- 2009 Fourth National Report on Human Exposures to Environmental Chemicals and table updates from February 2012, March 2013, and August 2014;
- Second National Report on Biochemical Indicators of Diet and Nutrition in the U.S. Population 2012.

Data in these reports are from analysis of samples collected during various rounds of the National Health and Nutrition Examination Surveys (NHANES). The same NCEH laboratory that analyzed NHANES samples also analyzed the NCS samples using the same methods and instrumentation. For reference ranges for biological analytes measured on NCS participants, we selected the 5th and 95th percentile of the best-matched NHANES participant group. For reference ranges for toxicological analytes, we selected the 50th and 95th percentiles of the best-matched NHANES participant group. Matching was done based on sex and closest age. However, matching was not ideal in many cases. For example, these reports contained no data (percentiles) for pregnant women, newborns, and in some cases from children. For matching examples, we selected data from NHANES women closest to pregnancy age (20 – 39 years, ≥ 20 years) to match with NCS pregnant women. We selected data from NHANES children 1-5 years old or 6-11 years old to match with NCS newborns, 1- or 3-year-olds. However, while matching was not ideal, we believe that NHANES data provide a starting point for comparison - NCS data users should decide how relevant the matches are for their own research purposes. We also selected percentiles from the NHANES round that was closest in date to the date of analysis of the NCS samples.

Reference ranges and LODs for those analytes measured at Quest Diagnostics were provided by Quest Diagnostics. If Quest did not provide reference for certain biological matrices or close age groups, the reference range was marked as “not reported” in the delivery datasets.

A. Hematology (Complete Blood Count (CBC) and Differential) Results

The NCS repository contractor, Fisher BioServices (FBS), received and analyzed fresh blood samples shipped within 24 hours of collection from study participants. Results on CBCs from three phases of the study, ARS, PBS and PRF are included in the Hematology dataset delivery. Table 3 presents the number of hematology results by Study Phase, Participant Type and Instrument.

Table 3: NCS Vanguard Hematology Data by Study Phase, Participant, and Analyzer

Study Phase	Participant Type	Instrument	Number of Results
ARS	Child	AcT 10	3696
ARS	Adult	AcT 10	5940
PBS	Child	AcT 10	187
PBS	Adult	AcT 10	383
PRF	Child	DXH800	3114
PRF	Adult	DXH800	4984
TOTAL	…	…	18,304

FBS used the Coulter AcT 10 (AcT 10) hematology analyzer to analyze blood from the ARS and PBS phases, and then upgraded to the Beckman Coulter UniCel DxH 800 (DxH 800) to analyze blood from the PRF Phase. While both are Coulter instruments, there are significant differences between the two analyzers. The AcT 10 analyzer is based on the Coulter principle and measures the impedance of cells as they pass through an orifice. Impedance (electrical resistance) is directly proportional to the cell size or volume. The AcT 10 does not provide any information about reticulocytes, nucleated RBCs, and only limited information on WBC types (lymphocytes only). The DxH 800 hematology analyzer is a much more sophisticated instrument and provides more information including additional red blood cell indices and comprehensive WBC (and other cell) differentials. The DxH 800 uses a combination of three technologies: (1) individual cell volume (impedance); (2) low- and high-frequency conductivity; and (3) and laser light scatter. Table 4 provides parameters for each of the hematology analyzers.

All results received from FBS were numeric so Result_Raw is blank for all items in the hematology dataset and thus has not been included in the hematology dataset. Table 4 provides a description of the test names for each analyzer.

Table 4: Parameters for Hematology Analyzers

Parameter Number	AcT 10 Analyzer	Beckman Coulter UniCel DxH 800
1	WBC – White Blood Cell or leukocyte count	WBC – corrected White Blood Cell or leukocyte count
2	LY# – Lymphocyte number	UWBC – uncorrected White Blood Cell or leukocyte count
3	LY% – Lymphocyte percent	RBC – Red Blood Cell or erythrocyte count
4	RBC – Red Blood Cell or erythrocyte count	HGB – Hemoglobin concentration
5	HGB – Hemoglobin concentration	HCT – Hematocrit (relative volume of RBCs)
6	HCT – Hematocrit (relative volume of RBCs)	MCV – Mean Corpuscular (RBC) Volume
7	MCV – Mean Corpuscular (RBC) Volume	MCH – Mean Corpuscular (RBC) Hemoglobin
8	MCH – Mean Corpuscular (RBC) Hemoglobin	MCHC – Mean Corpuscular (RBC) Hemoglobin Concentration
9	MCHC – Mean Corpuscular (RBC) Hemoglobin Concentration	RDW – RBC distribution width spread
10	PLT – Platelet or thrombocyte count	RDW-SD – RBC distribution width – standard deviation
11	Na	PLT – Platelet or thrombocyte count
12	Na	MPV – Mean platelet volume
13	Na	NE% – Neutrophil percent
14	Na	LY% – Lymphocyte percent
15	Na	MO% – Monocyte percent
16	Na	EO% – Eosinophil percent
17	Na	BA% – Basophil percent
18	Na	NE# – Neutrophil absolute count
19	Na	LY# – Lymphocyte absolute count
20	Na	MO# – Monocyte absolute count
21	Na	EO# - Eosinophil absolute count
22	Na	BA# – Basophil absolute count
23	Na	NRBC – Number of nucleated RBCs per 100 WBCs
24	Na	NRBC# - Nucleated RBC absolute count
25	Na	RET% - Reticulocyte percent
26	Na	RET# - Reticulocyte absolute count
27	Na	MRV – Mean reticulocyte volume
28	Na	IRF – Immature reticulocyte fraction

B. Assay Results Performed on Blood Samples

Many clinical chemistry, environmental biomarkers and toxicants, and nutritional analytes were measured on whole blood, blood spots, and blood components (plasma, serum, plasma, buffy coats) from NCS participants. NCEH measured many of the same toxicological and nutritional analytes on NCS pregnant women, newborns, and children as they periodically measure on NHANES participants. Quest Diagnostics measured many routine (and some non-routine) clinical chemistry analytes under a contract with the NCS Program office to assess the quality of NCS protocols and procedures. Table 5 lists the numbers of blood/blood component results by study phase and participant type.

Table 5: NCS Vanguard Blood Assays by Study Phase and Participant

Study Phase	Participant Type	Number of Results
IVS	Child	45
IVS	Adult	34,512
ARS	Child	5,452
ARS	Adult	2,216
PBS	Child	0
PBS	Adult	194
PRF	Child	648
PRF	Adult	1,880
TOTAL	…	44,947

C. Assay Results Performed on Urine Samples

Many clinical chemistry and toxicological analytes were measured on random spot urine samples from NCS participants. Similar to the blood analyses described above, NCEH measured many of the same toxicological analytes on urine from NCS pregnant women, newborns, and children as they periodically measure on urine from NHANES participants. Quest Diagnostics measured many routine (and some non-routine) clinical chemistry analytes under a contract with the NCS Program office to assess the quality of NCS protocols and procedures. Table 6 lists the numbers of urine results by study phase and participant type.

Urine results are reported as mass per volume analyzed but also a creatinine adjusted value is provided for those samples with a measured creatinine.

Table 6: NCS Vanguard Urine Assays by Study Phase and Participant

Study Phase	Participant Type	Number of Results
IVS	Child	5,493
IVS	Adult	49,822
ARS	Child	0
ARS	Adult	0
PBS	Child	N/A
PBS	Adult	62
PRF	Child	0
PRF	Adult	0
TOTAL	…	55,377

D. Assay Results Performed on Other Sample Types

Analytes were measured on saliva, breast milk and vaginal swab samples. Examples include measurement of bisphenol A, glucose, interleukin-6, triglycerides, and vitamin C in breast milk, measurement of cortisol in saliva, and interleukin-6 in vaginal swabs. Table 7 lists the numbers of results from other sample types, by study phase and participant type.

Table 7: NCS Vanguard Assays on Other Sample Types by Study Phase and Participant

Study Phase	Participant Type	Number of Results
IVS	Child	N/A
IVS	Adult	4,981
ARS	Child	0
ARS	Adult	0
PBS	Child	N/A
PBS	Adult	N/A
PRF	Child	256
PRF	Adult	319
TOTAL		5,556

III. Appendix

A. Variable Descriptions

This composite list reflects all variables in all four biological Analyte Data Files.

Variable Name	Variable Label	Variable Description
ANALYTE	Biomarker of interest	Substance or chemical constituent undergoing laboratory testing
CHILD_AGE_MONTHS	Child's age in months at the time of the sample collection	The calculated child's age in months using the child's date of birth and the date of the sample collection.
EVENTTYPE	Event type	NCS defined Data Collection activity by pregnancy status or age of child
GEST_AGEWEEKS_SAMPLE	Gestational age at the time of the sample collection	Gestational age in number of weeks calculated using the due date and the sample collection date.
INSTRUMENT_NAME	Instrument Name	Specifies test instrument used in hematology as Coulter AcT Analyzer or as DxH 800.
LAB	Analysis Laboratory	Facility where laboratory testing took place
LOD	Limit of Detection	Lowest measured level at which the laboratory can reproducibly report a result.
MATERIALTYPE	Material Type	Matrix or substance being analyzed i.e. Plasma or Tissue-Fixed
MONTHS_PRIOR_PREGNANCY	Approximate months prior to becoming pregnant at the time of the sample collection	For women who become pregnant, this is the number of months between the sample collection date and the month of becoming pregnant.
ORIGINATINGMATERIALTYPE	Parent Material Type	Source material for fractionated or processed item i.e. Whole Blood – EDTA (Blood collection tube contained EDTA anticoagulant)
PARTICIPANTTYPE	Participant Type	Mother, Child, Father, Other Enrolled Woman
PID	Universal PID	Universal **P**articipant **ID** Unique identifier for each individual enrolled in the NCS
RESULT_RAW	Text result as received from the analysis laboratory	Text result value as received from the instrument or analysis data file from the laboratory
REFERENCE_RANGE	Reference Range	Range of values from instrumentation, laboratory reporting or clinical reference resources

Variable Name	Variable Label	Variable Description
REFERENCE_RANGE_COMMENT	Reference Range Comment	Additional information to define Reference Range and/or source of the range
RESULT	Numeric result from the QC review processing done by Westat	Numeric result from the QC review processing done by Westat
RESULT_CR	Creatinine result for the aliquot	Result for urine creatinine if measured
RESULT_CR_ADJ	Creatinine adjusted result	Often used to adjust analyte concentrations for the effects of fluid balance, units are mass/mass as appropriate for the analyte
RESULT_FLAG	Result Flag	Indicator from hematology instrument that a clinical condition may exist with a specimen based on an abnormal cell distribution or population. Beckman Coulter recommends the review of results displaying a suspect message appropriate to the patient population.
ROOTID	Root ID	Two alpha characters followed by seven numbers BA1234567 EA9876543
SAMPLEID	Sample ID	Unique identification number for each item RootID + SeqID =SampleID i.e. BA1234567-LP10 or BA1234567-0101 Alternate value is labeled Specimen ID in some datasets.
SEQID	Sequence ID	Four alpha numeric characters -LV10, -CB17
TEST_LONG_NAME	Laboratory designation (long)	Full name of laboratory assay, e.g. Arachidonic acid, Estradiol, LC/MS/MS
TEST_NAME	Laboratory designation	Name or number found in raw dataset from laboratory e.g. 806000327, CBC; FOL2
TEST_PANEL	Test Panel	Specifies hematology test panel as CBC or Diff.
UNITS	Units of results	Standard units of measure for analyte

B. Biologic Sample Assay Methodologies

Adrenocorticotropic Hormone (ACTH) (Plasma)

Laboratory - Quest Diagnostics

Method - Concentrations of adrenocorticotropic hormone (ACTH) were measured in EDTA anticoagulated plasma from NCS participants - first and third trimester pregnant women, mothers at their 3-year visit, and 3-year old children. Immunoassay was the test method.

Allergens (Serum)

Laboratory - Quest Diagnostics

Method – Levels of IgE antibodies (ABs) to 8 allergens were measured in serum collected into red top tubes from first and third trimester pregnant women using the ImmunoCAP immunoassay (Thermo Fisher Scientific, Waltham, MA). The ABs tested were for cat dander (E1), Dermatophagoides pteronyssinus (dust mite D1), Dermatophagoides farinae (dust mite D2), Alternaria (mold), dog dander (E5); mouse urine proteins (E72), cockroach (I6), and rat urine proteins (E74).

The ImmunoCAP assay is a sandwich assay. Patient's serum is incubated with the allergen (AG) coupled to cellulose solid-phase polymer. The patient's specific IgE AB binds to the AG forming an AG-AB-solid phase complex. The complex is washed to remove nonspecific IgE ABs. Next an enzyme-labelled nonspecific AB antibody to IgE is added and binds with the AG-AB-solid phase complex to form a sandwich (AB-AG-AB-solid phase) complex. Unbound enzyme-labelled IgE AB is washed way and the sandwich complex is incubated with a fluorescent developing agent. After stopping the reaction, fluorescence of eluate is directly proportional to the level of specific IgE AB in the patient's serum.

Antioxidants (Plasma)

Laboratory - National Center for Environmental Health

Method - Concentrations of various antioxidants were measured in EDTA plasma from third trimester pregnant women and heparinized cord blood plasma from newborns using high performance liquid chromatography with photodiode array detection (Sowell et al., 1994). The oxidants were α-carotene, β-carotene, cis-β-carotene, β-cryptoxanthin, γ-tocopherol, lutein/zeaxanthin, trans-lycopene, retinyl palmitate, retinyl stearate, total lycopene, vitamin A, and vitamin E.

A small volume (100 mL) of plasma was mixed with an ethanol solution containing two internal standards, namely, retinyl butyrate and nonapreno-β-carotene. The micronutrients were extracted from the aqueous phase into hexane and dried under vacuum. The extract was re-dissolved in ethanol and acetonitrile and filtered to remove any insoluble material. An aliquot of the filtrate was injected onto a C18 reversed phase column and isocratically eluted with a mobile phase consisting of equal parts of ethanol and acetonitrile. Absorbance of these substances in solution is linearly proportional to concentration (within limits), thus spectrophotometric methods were used for quantitative analysis. Three wavelengths, approximately corresponding to vitamin E, vitamin A and carotenoid absorption maxima, namely, 300, 325, and 450 nm, were simultaneously monitored and chromatograms recorded. Quantitation was accomplished by comparing the peak height or peak area of the analyte in serum with the peak height or area of a known amount of the same analyte in a calibrator solution. Calculations were corrected for recovery based on the peak height or area of the internal standard in the serum compared with the peak height or area of the internal standard in the calibrator solution. Retinol and

the retinyl esters were compared with retinyl butyrate at 325 nm, α- and γ-tocopherol were compared with retinyl butyrate at 300 nm, and the carotenoids were compared with C45 at 450 nm. Calibration is single-point, forced through zero.

References

Sowell AL, Huff DL, Yeager PR, Caudill SP, and Gunter EW. Retinol, α-tocopherol, β-cryptoxanthin, lycopene, α-carotene, trans-β-carotene, and four retinyl esters in serum determined simultaneously by reversed-phase HPLC with multi-wavelength detection. *Clin Chem.* 1994; 40(3): 411-416.

Arsenic Speciation (Urine)

Laboratory - National Center for Environmental Health

Method - Concentrations of arsenic species were measured in random spot urines collected from third trimester pregnant women and 6-month old children using high performance liquid chromatography coupled to inductively-coupled-plasma dynamic-reaction-cell mass spectrometry (ICP-DRC-MS). The arsenic species were arsenic (V) acid, arsenobetaine, arsenocholine, arsenous (III) acid, dimethylarsinic acid, monomethylarsonic acid, and trimethylarsine oxide. This analytical technique is based on separation by anion-exchange chromatography (IC), followed by detection using quadrupole ICP-MS technology, and includes DRC™ technology (Baranov et al., 1999). This method minimizes or eliminates interference from argon-based polyatomic compounds (Tanner et al., 2000) and requires only 0.5 mL of urine. Arsenic species column separation is achieved due to differences in charge-charge interactions of each negatively-charged arsenic component in the mobile phase with the positively-charged quaternary ammonium groups bound at the column's solid-liquid interface. Upon exit from the column, the chromatographic eluant goes through a nebulizer, where it is converted into an aerosol upon entering the spray chamber.

Carried by a stream of argon gas, a portion of the aerosol is transported through the spray chamber and then through the central channel of the plasma, where it is heated to temperatures of 6000-8000°K. This thermal energy atomizes and ionizes the sample. The ions and the argon enter the mass spectrometer through an interface that separates the ICP, which is operating at atmospheric pressure (approximately 760 torr), from the mass spectrometer, which is operating at approximately 10^{-5} torr.

The mass spectrometer permits detection of ions at each mass-to-charge ratio in rapid sequence, which allows the determination of individual isotopes of an element. Once inside the mass spectrometer, the ions pass through the ion optics, then through the DRC™, and finally through the mass-analyzing quadrupole before being detected as they strike the surface of the detector. The ion optics uses an electrical field to focus the ion beam into the DRC™. The DRC™ component is pressurized with an appropriate reaction gas and contains a quadrupole. In the DRC™, elimination or reduction of argon-based polyatomic interferences takes place through the interaction of the reaction gas with the interfering polyatomic species in the incoming ion beam. The quadrupole in the DRC™ allows elimination of unwanted reaction by-products that would otherwise react to form new interferences.

References

Baranov VI and Tanner SD. A dynamic reaction cell for inductively coupled plasma mass spectrometry (ICP-DRC-MS). Part 1. The rf-field energy contribution in thermodynamics of ion-molecule reactions. *J Anal Atom Spectrom.* 1999; 14: 1133-1142.

Tanner S, Baranov VI, and Vollkopf U. A dynamic reaction cell for inductively coupled plasma mass spectroscopy (ICP-DRC-MS). Part III. Optimization and analytical performance. *J Anal Atom Spectrom* 2000; 15: 1261-1269.

Center for Disease Control. National Health and Nutrition Examination Survey. 2009-2010 Data Documentation, Codebook, and Frequencies. Arsenics – Total & Speciated – Urine. First Published: September 2011. Found at http://wwwn.cdc.gov/nchs/nhanes/2009-2010/UAS_F.htm. Last accessed September 3, 2015.

Blood Metals (Whole Blood)

Laboratory - National Center for Environmental Health

Method - Concentrations of cadmium, lead, manganese, selenium, and mercury were measured in EDTA-anticoagulated whole blood from first trimester pregnant women using high performance liquid chromatography coupled to inductively-coupled-plasma dynamic-reaction-cell mass spectrometry (ICP-DRC-MS). The lavender top EDTA blood collection tubes were prescreened for trace amounts of these metals prior to blood collection. This multi-element analytical technique is based on quadrupole ICP-MS technology. Coupling radio frequency power into a flowing argon stream seeded with electrons creates the plasma. Predominate species in the plasma are positive argon ions and electrons.

Diluted whole blood samples are converted into an aerosol using a nebulizer inserted within a spray chamber. A portion of the aerosol is transported through the spray chamber and then through the central channel of the plasma, where it experiences temperatures of 6000–8000^{o}K. This thermal energy atomizes and ionizes the sample. The ions, along with the argon, enter the mass spectrometer through an interface that separates the ICP, operating at atmospheric pressure (approximately 760 torr), from the mass spectrometer, operating at approximately 10-5 torr. The mass spectrometer permits detection of ions at each mass-to-charge ratio in rapid sequence, allowing individual isotopes of an element to be determined. Once inside of the mass spectrometer, the ions pass through the ion optics, then the mass analyzing quadrupole before being detected as they strike the surface of the detector. The ion optics focuses the ion beam using an electrical field. Electrical signals resulting from the detection of the ions are processed into digital information that is used to indicate the intensity of the ions and subsequently the concentration of the element. In this method blood samples are diluted with 18 M-ohm water and with diluent, containing 1% v/v tetramethylammonium hydroxide (TMAH), 0.05% disodium ethylenediamine tetraacetate (EDTA), 5% ethyl alcohol, and 0.05% Triton X-100. Au is added to reduce intrinsic Hg memory effects, Rh for internal standardization of Cd, and Bi for internal standardization of Hg and Pb.

Reference

Center for Disease Control. National Health and Nutrition Examination Survey. 2011-2012 Data Documentation, Codebook, and Frequencies. Blood Lead, Cadmium, Total Mercury, Selenium, and Manganese (PbCd_G). First Published: September 2013. Found at http://www.cdc.gov/nchs/nhanes/nhanes2011-2012/PbCd_G.htm. Last accessed September 3, 2015.

Cortisol (Saliva)

Laboratory – Quest Diagnostics

Method – Concentrations of cortisol were measured in saliva samples collected from first and third trimester pregnant women, parents/caregivers during the 3-year visit, and 3-year old children using liquid chromatography coupled with tandem mass spectrometry (LC/MSMS).

Cortisol Binding Globulin (CBG) (Plasma)

Laboratory – Quest Diagnostics

Method – Concentrations of cortisol binding globulin (CBG, transcortin) were measured in heparinized cord blood plasma from NCS newborn participants using radioimmunoassay.

Cotinine (Adult Serum and Child Urine)

Laboratory - National Center for Environmental Health

Method - Concentrations of cotinine were measured in serum samples collected into red top tubes from third trimester pregnant women. Since the assay is used for smokers and non-smokers, a large range of serum cotinine values was expected, from <0.1 ng/mL to >1000 ng/mL. Therefore, the assay used both an EIA pre-screening analysis and LC/MS analysis for samples with "low" cotinine concentrations in the passive exposure range and samples with “high” cotinine concentrations, generally those from active smokers. The pre-screening EIA assay relies on the competition between free cotinine in the sample and cotinine bound to enzyme, for antibody fixed on a polystyrene plate. After excess enzyme is washed away and substrate added, the amount of free cotinine present is inversely proportional to the amount of free cotinine in the biological sample.

The LC/MS assay uses isotope dilution - high performance liquid chromatography / atmospheric pressure chemical ionization tandem mass spectrometry (ID HPLC-APCI MS/MS). The serum sample is spiked with methyl-D3 cotinine as an internal standard, and following an equilibration period, the sample is applied to a basified solid phase extraction column. Cotinine is extracted off the column with methylene chloride, the organic extract is concentrated, and the residue is injected onto a short, C18 HPLC column. The eluant from these injections is monitored by APCI-MS/MS, and the m/z 80 daughter ion from the m/z 177 quasi-molecular ion is quantitated, along with additional ions for the internal standard, external standard, and for confirmation. Cotinine concentrations are derived from the ratio of native to labeled cotinine in the sample by comparisons to a standard curve.

Concentrations of cotinine were also measured in spot urine samples collected from six-month-old children. We could find no description for a urine assay for cotinine. However, in a memo accompanying the child cotinine results, NCEH states that the child urine samples were analyzed by LC/MS/MS using Method 2012, Cotinine and Hydroxycotinine in Urine. The samples were analyzed after hydrolysis with β-glucuronidase, thus these results represent total urinary cotinine levels.

Reference

Center for Disease Control. Laboratory Procedure Manual for Cotinine, Serum, ID HPLA-APCI MS/MS Method. Found at http://www.cdc.gov/nchs/data/nhanes/nhanes_01_02/l06_b_met_cotinine.pdf. Last accessed September 8, 2015.

Center for Disease Control. National Health and Nutrition Examination Survey. 2011-2012 Data Documentation, Codebook, and Frequencies. Cotinine – Serum & Total NNAL – Urine (COTNAL_G). First

Published: September 2013. Found at http://wwwn.cdc.gov/nchs/nhanes/2011-2012/COTNAL_G.htm. Last accessed September 8, 2015.

C-Reactive Protein (CRP) (Serum, Cord Blood Plasma)

Laboratory – Quest Diagnostics

Method - Concentrations of c-reactive protein (CRP) were measured in serum collected in red top tubes from first and third trimester pregnant women, women during the 3-year visit, and 3-year-old children, and in heparinized cord blood plasma from newborns. The method was nephelometry.

Creatinine (Urine)

Laboratory - National Center for Environmental Health

Method - Concentrations of creatinine were measured in random spot urine samples from pregnant women and 6-month old children using the Roche Hitachi Modular P Chemistry Analyzer and Creatinine Plus Assay reagents. In this enzymatic method creatinine is converted to creatine by creatininase. Creatine is then acted upon by creatinase to form sarcosine and urea. Sarcosine oxidase converts sarcosine to glycine and hydrogen peroxide, and the hydrogen peroxide reacts with a chromophore in the presence of peroxidase to produce a colored product that is measured at 546 nm (secondary wavelength = 700 nm). This is an endpoint reaction that agrees well with recognized HPLC methods, and it has the advantage over Jaffe picric acid-based methods that are susceptible to interferences from non-creatinine chromogens.

Creatinine (Urine)

Laboratory – Quest Diagnostics

Method – Concentrations of creatinine were measured in random spot urine samples from first and third trimester pregnant women, and birth mothers using a kinetic colorimetric assay.

Copper and Selenium (Serum)

Laboratory - National Center for Environmental Health

Method – Concentrations of copper and selenium were measured in serum collected into red top tubes from third trimester pregnant women using inductively coupled dynamic reaction cell plasma mass spectrophotometry (ICP-DRC-MS). This is a multi-element analytical technique capable of trace level elemental analysis. Liquid samples are introduced into the ICP through a nebulizer and spray chamber carried by a flowing argon stream. By coupling radio-frequency power into flowing argon, plasma is created in which the predominant species are positive argon ions and electrons and has a temperature of 6,000-8,000 K. The sample passes through a region of the plasma and the thermal energy atomizes the sample and then ionizes the atoms. The ions, along with the argon, enter the mass spectrometer through an interface that separates the ICP (at atmospheric pressure, ~760 torr) from the mass spectrometer (operating at a pressure of 10^{-5} torr). The ions pass through a focusing region, the dynamic reaction cell (DRC), the quadrupole mass filter, and finally are counted in rapid sequence at the detector allowing individual isotopes of an element to be determined. In this method, the instrument is operated in 'DRC' mode where the cell is pressurized with 99.99% ammonia gas which collides or reacts with the incoming ions to eliminate interfering ions and leave the ion of interest to be detected. After

leaving the DRC cell, the ions are focused with ion optics into a quadrupole mass analyzer with a nominal mass resolution of 0.7amu. The quadrupole is sequentially scanned to specific mass to charge ratio of each analyte and intensity is detected with a pulse detector. Electrical signals resulting from the detection of ions are processed into digital information that is used to indicate first the intensity of the ions and then the concentration of the element. The isotopes measured by this method include copper (m/z 65) and selenium (m/z 78) and the internal standard gallium (m/z 71). Serum samples are diluted 1+1+28 with water and diluent containing gallium (Ga) for multi-internal standardization.

Reference

Center for Disease Control. Laboratory Procedure Manual: Serum Zinc, Copper, and Selenium: Serum Multi-Element ICP-DRC-MS (Method No: ICPCRCMS-3006.7). Found at http://www.cdc.gov/nchs/data/nhanes/nhanes_11_12/CUSEZN_G_met_serum_elements.pdf. Last accessed September 8, 2015.

Dioxins, Furans, and Coplanar-PCBs (Breast Milk)

Laboratory - National Center for Environmental Health

Method - Concentrations of 7 PCDDs (dioxins), 10 PCDFs (furans), and 4 cPCBs were measured in breast milk samples collected by NCS mothers 1 month post-birth. The breast milk samples were spiked with 13C12-labeled internal standards and the analytes of interest were isolated by a liquid-liquid extraction (LLE) procedure (potassium oxalate, ethanol and diethyl ether/hexane). The solvent was evaporated and the lipid content determined gravimetrically. The residues were reconstituted in hexane followed by an automated (Fluid Management Systems Power-Prep/6) cleanup and enrichment procedure using multi-layered silica gel (acidic, basic, and neutral silica) and alumina columns coupled to AX-21 carbon columns. PCDDs/PCDFs/cPCBs were collected in the reverse direction with toluene from the AX-21 columns.

Following sample cleanup, excess solvent for each eluant was evaporated to 350 µL using a Caliper Life Science TurvoVap II and the remaining solvent was transferred to silanized auto sampler vials containing 1µL of dodecane "keeper" and allowed to evaporate to "dryness." Before quantification, the vials were reconstituted with 13C-labeled external standard.

PCDD/PCDD/cPCB congeners were analyzed by gas chromatography/isotope-dilution high-resolution mass spectrometry (GC/ID-HRMS). Samples were injected into a Hewlett-Packard 7890 gas chromatograph equipped with a DB-5ms capillary column coupled to a Thermo Electron DFS mass spectrometer operated in EI mode using selected ion monitoring (SIM) at 10,000 resolving power. The concentration of each analyte was calculated from its linear calibration curve. Analytical results were reported on a lipid-adjusted basis. International toxicity equivalents (I-TEQs) were also reported for PCDDs, PCDFs, cPCBs and other "dioxin-like" PCBs, based on the WHO-TEQ system. Lipid-adjusted detection limits were reported for each congener in each sample, corrected for sample weight and analyte recovery.

References

Patterson DG Jr, Alexander LR, Turner WE, Isaacs SG, and Needham LL. (1990). The Development and Application of a High Resolution Mass Spectrometry Method for Measuring Polychlorinated Dibenzo p Dioxins and Dibenzofurans in Serum. Chapter 9 in Instrumentation for Trace Organic Monitoring. Clement R.E., Sui K.M., and Hill H.H. Jr., eds, Lewis Publishers.

Van den Berg M, Birnbaum L, Denison M, et al. The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors (TEFs) for dioxins

Estradiol (Serum and Plasma)

Laboratory – Quest Diagnostics

Method - Concentrations of estradiol were measured in serum collected into red top tubes from first and third trimester pregnant women using liquid chromatography coupled to tandem mass spectrometry (LC/MSMS).

Fatty Acids (Serum and Plasma)

Laboratory - National Center for Environmental Health

Method - Concentrations of 30 fatty acids were measured in serum samples collected in serum separator tubes from third trimester pregnant women and heparinized cord blood plasma from newborns using capillary gas chromatography separation followed by electron capture negative-ion mass spectrometry. . Esterified fatty acids were hydrolyzed primarily from triglycerides, phospholipids and cholesteryl esters using sequential treatment with mineral acid and base in the presence of heat. Using a modification of Lagerstedt *et al.* method, total fatty acids were hexane-extracted from the matrix (100uL serum or plasma) along with an internal standard solution containing eighteen fatty acids labeled with stable isotopes to account for recovery. The extract was derivatized with pentafluorobenzyl bromide (PFBBr) in the presence of triethylamine to form pentafluorobenzyl esters. The reaction mixture was injected onto a capillary gas chromatograph column to resolve individual fatty acids of interest from other matrix constituents. Fatty acids were detected using electron capture negative-ion mass spectrometry within 34 minutes. Eleven saturated, six monounsaturated, and thirteen polyunsaturated fatty acids (30 fatty acids in total) were measured using selected ion monitoring. Quantitation was accomplished by comparing the peak area of the analyte in the unknown with the peak area of a known amount in a calibrator solution. Calculations were corrected based on the peak area of the internal standard in the unknown compared with the peak area of the internal standard in the calibrator solution. The assay was calibrated using a 5-point calibration curve.

Reference

Lagerstedt S, Hinrichs D, Batt S, Magera M, Rinaldo P, and McConnell J. Quantitative determination of plasma C8-C26 total fatty acids for the biochemical diagnosis of nutritional and metabolic disorders. *Mol Genet & Metab*. 2001; 73: 38-45.

Folate (Plasma)

Laboratory - National Center for Environmental Health

Method - Concentrations of 6 folate metabolites were measured in heparinized plasma cord blood samples from newborns using an isotope-dilution tandem mass spectrometry method coupled to liquid chromatography (LC-MS/MS). The method performance has been described in detail (method 2; scaled down specimen volume and 8-probe SPE) in a recent paper by Fazili *et al.* The method quantifies five folate forms and one oxidation product of 5-methyltetrahydrofolate known as MeFox. Total folate is calculated as the sum of the 6 folate metabolites and reported as well.

For sample preparation, plasma (150 µL) was mixed with ammonium formate buffer and amended with internal standard mixture that contained $^{13}C5$-labeled folate forms. Sample clean-up was performed using a 50-mg phenyl solid phase extraction (SPE) 96-well plate (Bond Elut 96; Agilent Technologies) and an automated 8-probe SPE system (Gilson 215; Gilson Inc.). Samples were eluted from the SPE plate with an organic elution buffer containing ascorbic acid and analyzed overnight by LC-MS/MS in positive ion mode using electrospray ionization on a Sciex API 5500 triple-quadrupole MS system (Applied Biosystems) coupled to a HP1200C LC system (Agilent Technologies). Chromatographic separation was achieved using a Luna C-8 analytical column (Phenomenex) with an isocratic mobile phase and a total run time of 7 min. Quantitation was performed by peak area ratio (analyte to internal standard) and based on a 6-point aqueous calibration curve that was carried through all sample preparation steps.

References

Pfeiffer CM, Fazili Z, McCoy L, Zhang M, and Gunter EW. Determination of folate vitamers in human serum by stable-isotope-dilution tandem mass spectrometry and comparison with radioassay and microbiologic assay. *Clin Chem.* 2004; 50: 423–32.

Fazili Z and Pfeiffer CM. Measurement of folates in serum and conventionally prepared whole blood lysates: application of an automated 96-well plate isotope-dilution tandem mass spectrometry method. *Clin Chem.* 2004; 50: 2378–81.

Fazili Z and Pfeiffer CM. Accounting for an isobaric interference allows correct determination of folate vitamers in serum by isotope dilution-liquid chromatography-tandem mass spectrophotometry. *J Nutr* 2012; 143: 108-113.

Free Thyroxine (free T4) (Serum)

Laboratory – Quest Diagnostics

Method - Concentrations of free thyroxine (free T4) were measured in serum collected in red top or serum separator tubes from third trimester pregnant women, birth mothers, mothers at the 3-year visit, and 3-year old children using a direct equilibrium dialysis immunoassay.

Glucose (Serum and Cord Blood Plasma and Serum)

Laboratory – Quest Diagnostics

Method - Concentrations of glucose were measured in serum collected in serum separator tubes from third trimester pregnant women, birth mothers, and mothers during their 3-year visit, and in cord blood serum or cord blood citrate-phosphate-dextrose anticoagulated plasma cord blood from NCS newborns. These samples were analyzed on the Beckman Coulter/Olympus Diagnostics (Melville, NY) AU-Series

Platform Analyzer using the hexokinase method. Briefly, glucose is phosphorylated by hexokinase in the presence of adenosine triphosphate (ATP) and magnesium ions to produce glucose-6-phosphate and adenosine diphosphate (ADP). Glucose-6-phosphate dehydrogenase (G6P-DH) specifically oxidizes glucose-6-phosphate to gluconate-6-phosphate with the concurrent reduction of NAD+ to NADH. The increase in absorbance at 340nm is proportional to the glucose concentration in the sample.

Lipids - Triglycerides and Total Cholesterol (Serum)

Laboratory - National Center for Environmental Health

Method - Concentrations of triglycerides and total cholesterol were measured in serum collected from third trimester pregnant women in red top tubes using the Roche Modular P Chemistry Analyzer. The triglycerides assay uses an enzymatic method with glycerol blanking. For glycerol blanking, reagent 1 is added to the sample. Then free glycerol in the sample is converted to glycerol-3-phosphate (G3P) using glycerol kinase. G3P is acted upon by glycerol phosphate oxidase to produce dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide combines with 4-chlorophenol under the action of peroxidase to produce an oxidation product that that does not react with the colorimetric component of reagent 2. After this initial reaction sequence is completed, the Mod P Analyzer records a blank absorbance reading and relates this back to free glycerol in the sample. Next reagent 2 is added which contains lipase to convert triglycerides in the sample to glycerol, and 4-aminophenzone which reacts with the hydrogen peroxide produced in the first reaction. The reaction is measured at 505 nm (secondary wavelength = 700 nm). This method is a two-reagent, endpoint reaction that is specific for triglycerides.

The total cholesterol assay is also enzymatic. Esterified cholesterol is converted to cholesterol by cholesterol esterase. The resulting cholesterol is then acted upon by cholesterol oxidase to produce cholest-4-en-3-one and hydrogen peroxide. The hydrogen peroxide then reacts with 4-aminophenazone in the presence of peroxidase to produce a colored product that is measured at 505 nm (secondary wavelength = 700 nm). The final step is known as the Trinder reaction. This method is a single reagent, endpoint reaction that is specific for cholesterol.

References

Center for Disease Control. National Health and Nutrition Examination Survey. 2009-2010 Data Documentation, Codebook, and Frequencies: Triglycerides and LDL-Cholesterol (TRIGL_F). First Published: December 2011. Found at http://www.cdc.gov/nchs/nhanes/nhanes2009-2010/TRIGLY_F.htm. Last accessed September 8, 2015.

Center for Disease Control. National Health and Nutrition Examination Survey. 2009-2010 Data Documentation, Codebook, and Frequencies: Cholesterol – Total (TCHOL_F). First Published: September 2011. Found at http://wwwn.cdc.gov/nchs/nhanes/2009-2010/TCHOL_F.htm. Last accessed September 8, 2015.

Hemoglobin A1c (Hb A1c) (Whole Blood)

Laboratory – Quest Diagnostics

Method - Concentrations of hemoglobin A1c (relative to total hemoglobin) were measured in EDTA anti-coagulated blood collected into lavender top tubes from first and third trimester pregnant women, birth mothers, mothers during their 3-year visit, and 3-year old children. These samples were analyzed using

the Roche Diagnostics Tina-quant® Hemoglobin A1cDx Gen-2 kit (Indianapolis, IN) and the Roche Cobas Integra 800 Clinical Chemistry Analyzer. This assay is a quantitative turbidometric inhibition immunoassay used for determination of mmol/mol hemoglobin A1c and % hemoglobin A1c. Briefly, anticoagulated whole blood is hemolyzed using a hemolyzing agent (active ingredient is Hb1qc antibody (bovine serum, pH 6.2). A second reagent is added to the liberated hemoglobin (active ingredient is HbA1c polyhapten, pH 6.2) to convert glycated hemoglobin to a derivative having a characteristic absorption spectrum. The sample is measured biochromatically and the Roche Cobas Analyzer calculates the %HbA1c from the HbA1c/total hemoglobin ratio.

References

Roche Diagnostics. Evaluation of Automatic Class III Designation for Cobas Integra 800 Tina-Quant HbA1c Dx. Gen.2 Assay – Decision Summary. Found at http://www.accessdata.fda.gov/cdrh_docs/reviews/K121291.pdf. Last accessed September 14, 2015.

Insulin (Serum)

Laboratory – Quest Diagnostics

Method - Concentrations of insulin were measured in serum collected into serum separator tubes from first and third trimester pregnant women, and mothers at their 3-year visit, and in heparinized or EDTA anti-coagulated cord blood plasma from newborns. The samples were analyzed using the Siemens IMMULITE® 2000 Immunoassay System which is a solid-phase, two-site sequential chemiluminescent immunometric assay.

Interleukin-6 (IL-6) (Breast Milk and Vaginal Swabs)

Laboratory – Quest Diagnostics

Method – Interleukin-6 concentrations were measured in breast milk collected by mothers 1 month post birth and vaginal swabs collected by first trimester pregnant women. The method used was an enzyme-linked immunosorbent immunoassay (ELISA).

Metals – Arsenic (As), Mercury (Hg), and Lead (Pb) (Whole Blood)

Laboratory – Quest Diagnostics

Method – Concentrations of arsenic, mercury, and lead were measured in EDTA-anticoagulated whole blood collected into royal blue tubes from third trimester pregnant women using inductively coupled plasma/mass spectrometry (ICP/MS).

Mercury (Hg) and Iodine (I) (Urine)

Laboratory - National Center for Environmental Health

Method - Concentrations of mercury and iodide were measured on random spot urine samples collected from third trimester pregnant women and 6-month old children using Inductively Coupled Plasma Dynamic Reaction Cell Mass Spectroscopy (ICP-DRC-MS). This multi-element analytical technique is based on quadrupole ICP-MS technology and includes DRC™ technology. Coupling radio frequency power into a flowing argon stream seeded with electrons creates the plasma, the heat source, which is ionized gas suspended in a magnetic field. Predominant species in the plasma are positive argon ions and electrons.

Diluted urine samples are converted into an aerosol using a nebulizer inserted within the spray chamber. A portion of the aerosol is transported through the spray chamber and then through the central channel of the plasma, where it is exposed to temperatures of 6000-8000 K. This thermal energy atomizes and ionizes the sample. The ions and the argon enter the mass spectrometer through an interface that separates the ICP, which is operating at atmospheric pressure (approximately 760 torr), from the mass spectrometer, which is operating at approximately 10^{-5} torr. The mass spectrometer permits detection of ions at each mass-to-charge ratio in rapid sequence, which allows the determination of individual isotopes of an element. Once inside the mass spectrometer, the ions pass through the ion optics, then through DRC™, and finally through the mass-analyzing quadrupole before being detected as they strike the surface of the detector. The ion optics uses an electrical field to focus the ion beam into the DRC™. The DRC™ component is pressurized with an appropriate reaction gas and contains a quadrupole. Electrical signals resulting from the detection of the ions are processed into digital information that is used to indicate the intensity of the ions and subsequently the concentration of the element.

Traditionally ICP-MS has been a trace analysis technique and the typical measurement ranges from < 1 µg/L to around 100 µg/L. DRC technology provides additional control of ICP-MS sensitivity; therefore, appropriate adjustments of the reaction cell parameters can significantly extend the useful concentration measurement range. In this method, iodine (isotope mass 127) and mercury (isotope mass 202) are measured in urine by ICP-DRC-MS using 100% argon as the Dynamic Reaction Cell™ (DRC) gas utilizing collisional focusing. Urine samples are diluted 1+1+ 8 (sample+ water + diluent) with water and diluent containing tellurium and bismuth for internal standardization.

Reference

Center for Disease Control. Laboratory Procedure Manual: Iodine & Mercury: Urine: Inductively Coupled Plasma Dynamic Reaction Cell Mass Spectrophotometry (ICP-DRC-MS) (Method ITU007B). Found at http://www.cdc.gov/nchs/data/nhanes/nhanes_05_06/urinary_iodine_mercury_met_d.pdf.

Last accessed September 8, 2015.

Mercury Speciation (Whole Blood)

Laboratory - National Center for Environmental Health

Method - Concentration of three mercury species (ethyl, inorganic, and methyl mercury) were measured in EDTA-anticoagulated whole blood samples collected from first trimester pregnant women using Species-Specific Isotope Dilution Gas Chromatography Inductively Coupled Plasma Dynamic Reaction Cell Mass Spectrometry (SSID-GC-ICP-DRC-MS) (DLS 3020.1). The method described below is from CDC SOP DLS 3020.5 but the principle is the same. Quantification of Inorganic Hg, Methyl Hg, and Ethyl Hg species was done using a triple spike isotope dilution (TSID) method employing gas chromatography (GC) to separate the species followed by introduction into an ICP-DRC-MS for detection. TSID is a specialized extension of the Isotope Dilution (ID) technique. TSID measures individual chemical species (inorganic, methyl and ethyl mercury species) in samples using ID principles. The blood sample is spiked with known amounts of each Hg species that have been enriched with isotopic variants of the target element of interest.

The first step of this method involves adding ("spiking") enriched isotopes ($^{199}Hg^{2+}$, $CH_3{}^{200}Hg^{+}$, and $C_2H_5{}^{201}Hg^{+}$) to the blood sample. Each Hg species spike is labeled with an enriched Hg isotope such that its isotopic pattern is unique to the species' chemical identity, i.e., the manner of isotope spiking is "species specific". Next, the spiked sample is digested in tetramethylammonium hydroxide (TMAH)

which disassociates bound mercury species from proteins, polypeptides, and other biomolecules. The digested blood sample with freed mercury species is chemically reacted ("derivatized") with a reagent that adds 3-carbon chains (n-propyl groups) to the mercury atom of each species molecule without compromising species identity. This type of chemical derivatization results in loss of ionic charge and reduced polarity; the net effect is to make each mercury species molecule volatile so it can escape the liquid phase and accumulate in the gas phase ("headspace") directly above the sample. Derivatization is performed inside a partially filled vial sealed with a rubber septa cap that can be penetrated by a needle. Solid Phase Microextraction (SPME) is a sampling technique that uses a thin polymer fiber with a hydrophobic coating; the method described here uses a SPME fiber with a 100 µm coating of polydimethylsiloxane (PDMS). The SPME assembly consists of the fiber inserted through the inside a stainless steel needle. A key design feature is the fiber can be mechanically withdrawn into the needle during vial septum penetration and then pushed out to expose the fiber to the headspace. During headspace exposure (the "extraction" step), the gaseous derivatized Hg species adsorb onto the PDMS coating of the SPME fiber. When other factors held are constant, the adsorbed mass increases as a function of sample concentration. After a predetermined time, the SPME fiber is retracted into the injection needle, and the needle is withdrawn from the sample vial. Subsequently, the needle moves to the injector port of the programmable temperature gradient gas chromatograph (GC) and, on programmatic command, performs a programmed temperature ramp injection sequence. This action transfers the propylated inorganic, methyl and ethyl Hg species to the head of a 30 m capillary GC column which, using He as the carrier gas, ramps the column temperature to 280°C. The order of chromatographic separation of the Hg species is based on increasing molecular weight: methylpropylmercury (derivatized methyl Hg), ethylpropylmercury (derivatized ethyl Hg), and dipropylmercury (derivatized inorganic Hg) is last. Hg species exiting the GC column are seen as chromatographic peaks detected using inductively-couple argon plasma (ICP) as the ion source and a quadrupole mass spectrometer (Q-MS) for mass specific quantification. Species identification is based on chromatographic retention time; species specific isotope ratios are calculated from integrated peak areas derived from m/z signals corresponding to ^{199}Hg, ^{200}Hg, ^{201}Hg and ^{202}Hg isotopes. The ICP-MS is equipped with a Dynamic Reaction Cell (DRC™) for minimizing polyatomic interferences.

Reference

Center for Disease Control. Laboratory Procedure Manual: Inorganic Mercury, Methyl Mercury, Ethyl Mercury: Blood: Blood mercury Speciation ISID-GC-ICP-DRC-MS (Triple Spike Isotope Dilution Gas Chromatography-Inductively Coupled Plasma Dynamic Reaction Cell Mass Spectrometry (DLS-3020.5). Found at http://www.cdc.gov/nchs/data/nhanes/nhanes_11_12/IHgEM_met_G_mercuryspecies.pdf. Last accessed September 8, 2015.

Metals (Urine)

Laboratory - National Center for Environmental Health

Method - Concentrations of 12 metals were measured on random spot urines collected from third trimester pregnant women and six-month-old children using inductively coupled plasma-mass spectrometry (ICP-MS). The twelve metals were antimony (Sb), barium (Ba), beryllium (Be), cadmium (Cd), cesium (Cs), cobalt (Co), manganese (Mn), molybdenum (Mo), lead (Pb), platinum (Pt), tin (Sn), thallium (TI), tungsten (W), and uranium (U). This method is based on the method by (Mulligan KJ, et al. 1990). This ICP-MS assay is a multi-element method where urine is introduced into the ICP through a nebulizer and spray chamber carried by a flowing argon stream. By coupling radio-frequency power into flowing argon, plasma is created in which the predominant species are positive argon ions and electrons.

The sample passes through a region of the plasma that has a temperature of 6000–8000°K. The thermal energy atomizes the sample and then ionizes the atoms. The ions, along with the argon, enter the mass spectrometer through an interface that separates the ICP from the mass spectrometer, which is operating at an atmospheric pressure of 10^{-5} torr. The mass spectrometer permits ions at each mass to be detected in rapid sequence, allowing individual isotopes of an element to be determined. Electrical signals resulting from the detection of the ions are processed into digital information that is used to indicate first the intensity of the ions and then the concentration of the element. Urine samples are diluted 1+9 with 2% (v/v), double-distilled, concentrated nitric acid containing both iridium (Ir) and rhodium (Rh) for multi-internal standardization. This procedure can be used for all 12 elements or for subsets of the 12 elements.

References

Center for Disease Control. National Health and Nutrition Examination Survey. 2009-2010 Data Documentation, Codebook, and Frequencies: Urine Heavy Metals (UHM_F). First Published: September 2011. Found at http://www.cdc.gov/nchs/nhanes/nhanes2009-2010/UHM_F.htm. Last accessed September 9, 2015.

Mulligan KJ, Davidson TM, and Caruso JA. Feasibility of the direct analysis of urine by inductively coupled argon plasma mass-spectrometry for biological monitoring of exposure to metals. *J Anal Atom Spectrom.* 1990; 5(4): 301-306.

4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) (Urine)

Laboratory - National Center for Environmental Health

Method - Concentrations of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) were measured on random spot urines collected from third trimester pregnant women and six-month-old children using liquid chromatography linked to tandem mass spectrometry (LC/MS-MS). For "total" NNAL assays, the urine sample is fortified with an NNAL-13C6 internal standard, and then hydrolyzed using β-glucuronidase in incubations for at least 24 hours. The samples are then extracted and cleaned up on a specially-designed solid-phase molecularly-imprinted polymer (MIP) column, after which the analyte is eluted and analyzed by LC/MS/MS, monitoring the m/z 210->180 native, and m/z 216->186 internal standard transition ions. NNAL concentrations are derived from the ratio of the integrated peaks of native to labeled ions by comparison to a standard calibration curve.

References

Center for Disease Control. Laboratory Procedure Manual: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL): Urine: MIP Column and LC API MS/MS: Method 2002.01. Found at http://www.cdc.gov/nchs/data/nhanes/nhanes_11_12/COTNAL_G_met_NAL.pdf. Last accessed September 9, 2015.

Xia Y, McGuffey JE, Bhattacharyya S, Sellergren B, Yilmaz E, Wang L, and Bernert JT. Analysis of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol in urine by extraction on a molecularly-imprinted polymer column and liquid chromatography/atmospheric pressure ionization tandem mass spectrometry. *Anal Chem. 2005;* 77: 7639-7645.

Organic Phosphorus Insecticides (Urine)

Laboratory - National Center for Environmental Health

Method - Concentrations of 6 organophosphorus insecticides were measured on random spot urines collected from third trimester pregnant women and 6-month old children using a solid phase extraction using weak anion exchange sorbent on a 96-well plate followed by a highly selective and sensitive isotope dilution and high-performance liquid chromatography with electrospray ionization-tandem mass spectrometry (HPLC/ESI-MS/MS). This was based on the method described in Odetokum et al. (2010). The organophosphorus insecticides were diethyldithiophosphate, diethylphosphate, dimethyldithiophosphate, dimethylphosphate, dimethlythiophosphate, and diethylthiophosphate).

References

Odetokum MS, Montesano MA, Weerasekera G, Whitehead RD Jr, Needham LL, and Barr DB. Quantification of dialkylphosphate metabolites of organophosphorus insecticides in human urine using 96-well plate sample preparation and high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry. *J Chromatrogr B Analyt Technol Biomed Life Sci*. 2010; 878(27): 2567-2574.

Perchlorate, Thiocyanate, Nitrate, and Iodide (Urine)

Laboratory - National Center for Environmental Health

Method - Concentrations of perchlorate, thiocyanate, nitrate, and iodine were measured in random spot urines collected from third trimester pregnant women and 6-month old children using atmospheric pressure ionization tandem mass spectrophotometry. Separation of the 3 metabolites was achieved using an Ion Pac AS16 column with sodium hydroxide as the eluent. The eluent from the column is ionized using an electrospray interface to generate and transmit negative ions into the mass spectrometer. Comparison of relative response factors (ratio of native analyte to stable isotope-labeled internal standard) of unknowns with known standard concentrations yields individual analyte concentrations.

Child urines were further analyzed for iodine concentrations using Inductively Coupled Plasma Dynamic Reaction Cell Mass Spectroscopy (ICP-DRC-MS). This analytical technique is based on quadrupole ICP-MS technology and includes DRC™ technology. Coupling radio frequency power into a flowing argon stream seeded with electrons creates the plasma, the heat source, which is ionized gas suspended in a magnetic field. Predominant species in the plasma are positive argon ions and electrons. Diluted urine samples are converted into an aerosol using a nebulizer inserted within the spray chamber. A portion of the aerosol is transported through the spray chamber and then through the central channel of the plasma, where it is exposed to temperatures of 6000-8000°K. This thermal energy atomizes and ionizes the sample. The ions and the argon enter the mass spectrometer through an interface that separates the ICP, which is operating at atmospheric pressure (approximately 760 torr), from the mass spectrometer, which is operating at approximately 10^{-5} torr. The mass spectrometer permits detection of ions at each mass-to-charge ratio in rapid sequence, which allows the determination of individual isotopes of an element. Once inside the mass spectrometer, the ions pass through the ion optics, then through DRC™, and finally through the mass-analyzing quadrupole, before being detected as they strike the surface of the detector. The ion optics uses an electrical field to focus the ion beam into the DRC™. The DRC™ component is pressurized with an appropriate reaction gas and contains a quadrupole. Electrical signals resulting from the detection of the ions are processed into digital information that is used to indicate the intensity of the ions and subsequently the concentration of the element. Traditionally ICP-MS has been a trace analysis technique and the typical measurement ranges from < 0.1 µg/L to around 100 µg/L. In this method, iodine (isotope mass 127)is measured in urine by ICP-DRC-MS using 100% argon as the Dynamic Reaction Cell™ (DRC) gas utilizing collisional focusing. Urine samples are diluted 1+1+ 8

(sample+ water + diluent) with water and diluent containing tellurium and bismuth for internal standardization.

References

Center for Disease Control. Laboratory Procedure Manual: Perchlorate, Nitrate, and Thiocyanate: Urine: Ion Chromatography with Tandem Mass Spectrometry (IC-MS/MS): Method 2150.03bg (revised 1/31/2011). Found at http://www.cdc.gov/nchs/data/nhanes/nhanes_11_12/PERNT_G_met.pdf. Last accessed September 9, 2015.

Center for Disease Control. National Health and Nutrition Examination Survey. 2007-2008 Data Documentation, Codebook, and Frequencies: Urinary Iodine (UIO_E). First Published: December 2009. Found at http://www.cdc.gov/nchs/nhanes/nhanes2007-2008/UIO_E.htm. Last accessed September 9, 2015.

Phenols (Breast Milk)

Laboratory - National Center for Environmental Health

Method - Concentrations of bisphenol A were measured on breast milk samples from women collected 1 month after birth using solid phase extraction (SPE) coupled on-line to high performance liquid chromatography (HPLC) and tandem mass spectrometry (MS/MS). The method used is a modification of that reported in Ye et al (2005) for urines.

References

Ye X, Kuklenyik Z, Needham LL, and Calafat AM. Automated on-line column-switching HPLC-MS/MS method with peak focusing for the determination of nine environmental phenols in urine. Anal Chem. 2005; 77: 5407-54134.

Center for Disease Control. National Health and Nutrition Examination Survey. 2011-2012 Data Documentation, Codebook, and Frequencies: Environmental Phenols and Parabens (EPH_G). First Published: November 2013. Found at http://www.cdc.gov/nchs/nhanes/nhanes2011-2012/EPH_G.htm. Last accessed September 9, 2015.

Phenols (Urine)

Laboratory - National Center for Environmental Health

Method - Concentrations of 8 phenols were measured on random spot urines collected from third trimester pregnant women and six-month-old children using solid phase extraction (SPE) coupled on-line to high performance liquid chromatography (HPLC) and tandem mass spectrometry (MS/MS). The phenols are benzophenone-3, bisphenol A, triclosan, butyl paraben, methyl paraben, n-propyl paraben, 2,4-dichlorophenol, and 2.5-dichlorophenol. With the use of isotopically labeled internal standards, the detection limits in 100 µL of urine are 0.1-4 nanograms per milliliter (ng/mL), sufficient for measuring urinary levels of phenols in non-occupationally exposed participants. The method used is a modification of that reported in Ye et al (2005).

References

Ye X, Kuklenyik Z, Needham LL, and Calafat AM. Automated on-line column-switching HPLC-MS/MS method with peak focusing for the determination of nine environmental phenols in urine. Anal Chem. 2005; 77: 5407-54134.

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Phthalates (Urine)

Laboratory - National Center for Environmental Health

Method - Concentrations of 11 phthalates were measured on random spot urines collected from third trimester pregnant women and 6-month old children using high performance liquid chromatography-electrospray ionization-tandem mass spectrometry (HPLC-ESI-MS/MS). The phthalates were mono-n-butyl phthalate, mono benzyl phthalate, mono carboxynonyl phthalates, mono carboxyisooctyl phthalates, mono-3-carboxypropyl phthalate, mono-2-ethyl-5-carboxypentyl phthalate, mono-2-ethyl-5-hydroxyhexyl phthalate, mono-2-ethylhexyl phthalate, mono-2-ethyl-5-oxohexyl phthalate, mono ethyl phthalate, and mono isobutyl phthalate. Urine samples were processed using enzymatic deconjugation of the glucuronidated analytes followed by on-line solid phase extraction (SPE) coupled with reversed phase HPLC-ESI-MS/MS. Assay precision was improved by incorporating isotopically-labeled internal standards of the target analytes. In addition, 4-methyl umbelliferyl glucuronide was used to monitor deconjugation efficiency. This selective method allowed for rapid detection of metabolites of phthalate diesters or other alternative plasticizers in human urine with limits of detection in the low ng/mL range. This method is a modification of that reported in Silva et al (2007).

References

Silva MJ, Samandar E, Preau JL, Reidy JA, Needham LL, and Calafat AM. Quantification of 22 phthalate metabolites in human urine. *J Chromatogr B*. 2007; 860: 106-112.

Center for Disease Control. Laboratory Procedure Manual: Metabolites of Phthalates and Phthalate Alternatives: Urine: HPLC/ESI-MS/MS (Method No. 6306.04). Found at http://www.cdc.gov/nchs/data/nhanes/nhanes_11_12/PHTHTE_G_met.pdf. Last accessed September 9, 2015.

Phytoestrogens (Urine)

Laboratory - National Center for Environmental Health

Method - Concentrations of 6 phytoestrogens were measured on random spot urines collected from third trimester pregnant women and 6-month old children using high performance liquid chromatography-atmospheric pressure photoionization-tandem mass spectrometry (LC-APPI-MS/MS). The phytoestrogens were daidzein, o-desmethylangolensin, equol, enterodiol, enterolactone, and genistein. Urine specimens were first spiked with $^{13}C_3$-labeled internal standards of each phytoestrogen analyte, a mixture of glucuronidated and sulfated methyumbelliferone, and a concentrated aliquot of ammonium acetate buffer (pH 5.0). Samples were then treated with a β-glucuronidase/sulfatase enzyme to hydrolyze all glucuronide- and sulfate-conjugated analytes to their aglycone forms, using the methylumbelliferone as an indicator of the deconjugation process. Finally, samples were filtered using a

size-exclusion molecular weight cutoff filter to remove particulates and proteinaceous matter prior to LC-APPI-MS/MS analysis. Analytes were chromatographically using a solid-core C_{18} column and a water/methanol gradient. Analytes were quantified by interpolation of peak area (analyte/internal standard) ratios against a nine-point calibration curve obtained from synthetic urine matrix-based standards.

References

Parker DL, Rybak ME, and Pfeiffer CM. Phytoestrogen biomonitoring: an extractionless LC-MS/MS method for measuring urinary isoflavones and lignans by use of atmospheric pressure photoionization (APPI). *Anal Bioanal Chem*. 2012; 402: 1123-1136.

Polycyclic Aromatic Hydrocarbons (PAH) (Urine)

Laboratory - National Center for Environmental Health

Method - Concentrations of 9 polycyclic aromatic hydrocarbons (PAHs) were measured in random spot urines from third trimester mothers and infants using gas chromatography isotope dilution high resolution mass spectrometry (GC-IDHRMS) based on the method by Li *et al* (2006). The PAHs were 1-naphthol, 2-naphthol, 3-hydroxyfluorene, 9-hydroxyfluorene, 1-hydroxyphenanthrene, 2-hydroxyphenanthrene, 3-hydroxyphenanthrene, 1-hydroxypyrene, and 2-hydroxypyrene. Urine samples were spiked with a mixture of ^{13}C-labeled internal standards, and then subjected to overnight enzymatic deconjugation to yield free OH-PAHs. This was followed by semi-automated liquid-liquid extraction using a Gilson 215 Liquid Handler (Gilson Inc., Middleton, WI). The sample extracts were next evaporated to remove the solvent and re-constituted in toluene. Finally, the samples were derivatized to yield the trimethylsilyl derivatives of the OH-PAHs. Analytical determination of the target analytes was performed by gas chromatography isotope dilution tandem mass spectrometry employing a Thermo TSQ Quantum instrument.

References

Li Z, Romanoff LC, Trinidad DA, Hussain N, Jones RS, Porter EN, Patterson DG Jr, and Sjödin A. Measurement of urinary mono-hydroxy polycyclic aromatic hydrocarbons using automated liquid-liquid extraction and gas chromatography/isotope dilution high resolution mass spectrometry. *Anal Chem*.2006; 78(16): 5744-5751.

Polyfluoroalkyl Compounds (Serum)

Laboratory - National Center for Environmental Health

Method - Concentrations of 8 polyfluoroalkyl compounds (PFCs) were measured in serum collected in red top tubes from third trimester mothers using solid phase extraction high performance liquid chromatography isotope dilution tandem mass spectrophotometry. The PFCs were 2-(N-ethyl-perfluorooctane sulfonamido) acetic acid, 2-(N-methyl-perfluorooctane sulfonamido) acetic acid, perfluorodecanoic acid, perfluorohexane sulfonic acid, perfluorononanoic acid, perfluorooctanoic acid, perfluorooctane sulfonic acid, and perfluorooctane sulfonamide.

References

Kato K, Basden BJ, Needham LL, and Calafat AM. Improved selectivity for the analysis of maternal serum and cord serum for polyfluoroalkyl chemicals. *J Chromatogr A*. 2011; 1218(15): 2133-2137.

Pro B-Type Natriuretic Peptide (ProBNP) (Plasma)

Laboratory – Quest Diagnostics

Method – Concentrations of Pro-B-type natriuretic peptide were measured in EDTA anticoagulated plasma from first and third trimester pregnant women and heparinized cord blood plasma from newborns using an Electrochemiluminescent immunoassay (ECLIA).

Progesterone (Plasma)

Laboratory – Quest Diagnostics

Method – Concentrations of progesterone were measured in heparinized cord blood plasma from newborns using liquid chromatography tandem mass spectrometry (LC/MSMS).

Strontium (Urine)

Laboratory - National Center for Environmental Health

Method - Concentrations of strontium were measured on random spot urines from third trimester pregnant women and six-month-old children using inductively-coupled-plasma dynamic-reaction-cell mass spectrometry (ICP-DRC-MS). Liquid samples are introduced into the ICP through a nebulizer and spray chamber carried by a flowing argon stream. By coupling radio-frequency power into flowing argon, plasma is created in which the predominant species are positive argon ions and electrons and has a temperature of 6000-8000°K. The sample passes through a region of the plasma and the thermal energy atomizes the sample and then ionizes the atoms. The ions, along with the argon, enter the mass spectrometer through an interface that separates the ICP (at atmospheric pressure, ~760 torr) from the mass spectrometer (operating at a pressure of 10^{-5} torr). The ions pass through a focusing region, the dynamic reaction cell, the quadrupole mass filter, and finally are counted in rapid sequence at the detector allowing individual isotopes of an element to be determined. For strontium, the dynamic reaction cell is operated in the 'standard' mode where the cell is not pressurized and ions pass through the cell to the quadrupole mass filter unaffected.

Electrical signals resulting from the detection of ions are processed into digital information that is used to indicate first the intensity of the ions and then the concentration of the element. Urine samples are diluted 1+ 9 with 2% (v/v) concentrated nitric acid (and 1.5% ethanol in the case of arsenic). The diluent contains iridium (Ir), rhodium (Rh) for multi°-internal standardization. Internal standards are a constant concentration in all blanks, calibrators and samples. Monitoring the instrument signal ratio of a metal to its internal standard allows correction for instrument noise and drift, and sample-to-sample matrix differences.

References

Center for Disease Control. Laboratory Procedure Manual: Antimony, Arsenic, Barium, Beryllium, Cadmium, Cesium, Cobalt, Lead, Manganese, Molybdenum, Platinum, Strontium, Thallium, Tin, Tungsten, and Uranium: Urine: Urine Multi-Element ICP-DRC-MS (renamed from "Inductively Coupled Plasma-Mass Spectrometry (ICP-DRC-MS): Method No. 30183 (15 element panel). Revised March 18.2012. Found at http://www.cdc.gov/nchs/data/nhanes/nhanes_11_12/UHM_G_met_heavy_metals.pdf. Last accessed September 9, 2015.

Thyroid Stimulating Hormone (TSH) (Serum)

Laboratory – Quest Diagnostics

Method – Concentrations of thyroid stimulating hormone (TSH) were measured in serum collected into serum separator tubes from third trimester pregnant women, mothers during their 3-year visit, and 3-year old children using a chemiluminescence immunoassay.

Total Arsenic (As) (Urine)

Laboratory - National Center for Environmental Health

Method - Concentrations of total arsenic were measured on random spot urines from third trimester pregnant women and six-month-old children using inductively-coupled-plasma dynamic-reaction-cell mass spectrometry (ICP-DRC-MS). This multi-element analytical technique is based on quadrupole ICP-MS technology and includes DRC™ technology, which minimizes or eliminates much argon-based polyatomic interference. Coupling radio frequency power into a flowing argon stream seeded with electrons creates the plasma, the heat source, which is ionized gas suspended in a magnetic field. Predominant species in the plasma are positive argon ions and electrons. Diluted urine samples are converted into an aerosol by using a nebulizer inserted within a spray chamber. A portion of the aerosol is transported through the spray chamber and then through the central channel of the plasma, where it is exposed to temperatures of 6000-8000°K. This thermal energy atomizes and ionizes the sample. The ions and the argon enter the mass spectrometer through an interface that separates the ICP, which is operating at atmospheric pressure (approximately 760 torr), from the mass spectrometer, which is operating at approximately 10^{-5} torr. The mass spectrometer permits detection of ions at each mass-to-charge ratio in rapid sequence, which allows the determination of individual isotopes of an element. Once inside the mass spectrometer, the ions pass through the ion optics, then through DRC™, and finally through the mass-analyzing quadrupole before being detected as they strike the surface of the detector. The ion optics uses an electrical field to focus the ion beam into the DRC™. The DRC™ component is pressurized with an appropriate reaction gas and contains a quadrupole. In the DRC™, elimination or reduction of argon-based polyatomic interferences takes place through the interaction of the reaction gas with the interfering polyatomic species in the incoming ion beam. The quadrupole in the DRC™ allows elimination of unwanted reaction by-products that would otherwise react to form new interferences. Electrical signals, resulting from the detection of the ions, are processed into digital information that is used to indicate the intensity of the ions, and subsequently the concentration of the element. In this method, arsenic (isotope mass 75) and gallium (isotope mass 71) or tellurium (isotope mass 126) is measured in urine by ICP-DRC-MS, using argon/hydrogen (90%/10%, respectively) as a reaction gas. Urine samples are diluted 1:9 with 2% (v/v) double-distilled nitric acid containing gallium or tellurium for internal standardization.

References

Center for Disease Control. National Health and Nutrition Examination Survey. 2009-2010 Data Documentation, Codebook, and Frequencies: Urinary Total Arsenic and Speciated Arsenics (UAS_F). First Published: September 2011. Found at http://www.cdc.gov/nchs/nhanes/nhanes2009-2010/UAS_F.htm. Last accessed September 9, 2015.

Universal Pesticides (Urine)

Laboratory - National Center for Environmental Health

Method - Concentrations of 8 pesticides and 2 herbicides were measured on random spot urines from third trimester pregnant women and 6-month old children using semi-automated solid phase extraction and mass spectrometric quantification based on the methods reported in Davis *et al* (2013). The insecticides were 2,4-dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, 4-fluoro-3-phenoxybenzoic acid, cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylic acid, 3,5,6-trichloro-2-pyridinol, malathion dicarboxylic acid, 3-phenoxybenzoic acid, 2-isopropyl-4-methyl-6-hydroxypyrimidine, para-nitrophenol, and trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid. Organophosphate and pyrethroid insecticides and phenoxyacetic acid herbicides represent important classes of pesticides applied in commercial and residential settings. These 10 target analytes were extracted from one milliliter of urine by a semi-automated solid phase extraction technique, separated from each other and from other urinary biomolecules by reversed-phase high performance liquid chromatography (HPLC), and detected using tandem mass spectrometry with isotope dilution quantitation. This method can be used to measure all the target analytes in one injection with similar repeatability and detection limits of previous methods which required more than one injection. Each step of the procedure was optimized to produce a robust, reproducible, accurate, precise and efficient method. The required selectivity and sensitivity for trace-level analysis (e.g., limits of detection <0.5 ng/mL) were achieved using a narrow diameter analytical column, higher than unit mass resolution for certain analytes, and stable isotope labeled internal standards.

References

Davis MD, Wade EL, Restrepo PR, Roman-Esteva W, Bravo R, Kuklenyik P, and Calafat AM. Semi-automated solid phase extraction method for the mass spectrometric quantification of 12 specific metabolites of organophosphorus pesticides, synthetic pyrethroids, and select herbicides in human urine. *J Chromatogr B*. 2013; 929: 18-26.

Varicella Antibodies IgG and IgM (Serum)

Laboratory – Quest Diagnostics

Method – Levels of Varicella antibodies (IgG and IgM) were measured in serum collected into serum separator tubes from first and third trimester pregnant women, and mothers at their 3-year visit using immunoassays.

Vitamin C (Serum)

Laboratory - National Center for Environmental Health

Method - Concentrations of vitamin C were measured in serum collected into red top tubes from third trimester pregnant women using isocratic high-performance liquid chromatography (HPLC) with electrochemical detection at 650 mV. One part serum was mixed with four parts 6% MPA to acidify the

serum and stabilize ascorbate. The samples were frozen at -70°C until analysis. Prior to analysis, the samples were thawed at room temperature and centrifuged at 2,500 rpm, and the supernatants were decanted. The supernatants were mixed with a solution containing trisodium phosphate and dithiothreitol (to reduce dehydroascorbate to ascorbate) and an internal standard (1-methyl uric acid). The supernatants were re-acidified with 40% MPA to stabilize the ascorbate and filtered to remove insoluble material. A 10 µL aliquot was injected onto a C-18 reversed-phase column and eluted with a mobile phase containing 14.1 g/L monochloroacetic acid, 0.76 g/L disodium ethylenediamine tetraacetate, 1% (by volume) 10 N sodium hydroxide, and 1.5% (by volume) methanol. Quantitation was based on analyte peak area ratios interpolated from a three-point calibration curve (0.025, 0.150, and 0.500 mg/dL) obtained from matrix-based standards that had been extracted in the same manner as the specimens.

References

McCoy LF, Bowen MB, Xu M, Chen H, and Schleicher RL. Improved HPLC assay for measuring serum vitamin C with 1-methyluric acid used as an electrochemically active internal standard. *Clin Chem.* 2005; 51: 1062-1064.

Vitamin C (Breast Milk and Cord Blood Plasma)

Laboratory – Quest Diagnostics

Method – Concentrations of Vitamin C in breast milk collected by mothers 1 month post birth and heparinized cord blood plasma from newborns were measured using liquid chromatography coupled to tandem mass spectrometry (LC/MSMS).

Vitamin D (Serum)

Laboratory - National Center for Environmental Health

Method - Concentrations of 3 vitamers (vitamin D) were measured in serum collected into red top tubes from third trimester pregnant women and heparinized plasma from cord blood of newborns using ultra-high-performance liquid chromatographic-tandem mass spectrometry method (UHPLC-MS/MS). The 3 vitamers were 25-hydroxyvitamin D2 (VID2), 25-hydroxyvitamin D3 (VID3), and the C3-epimer, epi-25-hydroxyvitamin D3 (VID3E). The serum and plasma samples were subjected to liquid-liquid extraction using a robotic liquid handler. Stable isotopically labeled internal standards were mixed with serum prior to the addition of hexane to extract the fat-soluble compounds. The hexane/serum mixture was removed from the liquid handler, shaken vigorously, centrifuged to break up any emulsions and returned to the liquid handler. The hexane fraction was transferred to a 96-well plate which was then dried completely under a nitrogen stream. The final step was the reconstitution of each specimen using a solution of methanol and water. The three hydroxylated vitamin D forms listed above were quantitated using reversed-phase UHPLC and atmospheric pressure chemical ionization tandem mass spectrometry. Quantitation was based on analyte peak area ratios interpolated from a six-point calibration curve obtained from matrix-based standards that had been extracted in the same manner as the specimens.

Total vitamin D levels were calculated from the sum of 2 of the vitamers (25-hydroxyvitamin D2 and 25-hydroxyvitamin D3).

References

Schleicher RL, Encisco SE, Chaudhary-Webb M, Paliakov E, McCoy LF, and Pfeiffer CM. Isotope dilution ultra performance liquid chromatography-tandem mass spectrometry method for simultaneous measurement of 25-hydroxyvitamin D2, 25-hydroxyvitamin D3 and 3-epi-25-hydroxyvitamin D3 in human serum. *Clinica Chimica Acta*. 2011; 412: 1594–1599.

Vitamin E (Breast Milk)

Laboratory – Quest Diagnostics

Method – Concentrations of Vitamin E (alpha-tocopherol and beta-gamma-tocopherol) in breast milk collected by mothers 1 month post birth were measured using high performance liquid chromatography (HPLC).

Volatile Organic Compounds (Urine)

Laboratory - National Center for Environmental Health

Method - Concentrations of 28 volatile organic compounds (VOCs) were measured in random spot urines from third trimester pregnant women and six-month-old children using a modified version of NCEH's established UPLC-electrospray tandem mass spectrometry (UPLC/ESI-MSMS) method. Details about this method can be found in Alwis et al (2012). The VOCs were N-acetyl-S-(2-carbamoylethyl)-L-cysteine, N-acetyl-S-(N-methylcarbamoyl)-L-cysteine, 2-aminothiazoline-4-carboxylic acid, N-acetyl-D-(benzyl)-L-cysteine, N-acetyl-D-(N-propyl)-L-cysteine, N-acetyl-D-(2-carboxyethyl)-L-cysteine, N-acetyl-S-(2-cyanoethyl)-L-cysteine, N-acetyl-S-(1,2-dichlorovinyl)-L-cysteine, N-acetyl-D-(2,2-dichlorovinyl)-L-cysteine, N-acetyl-S-(3,4-dihydroxybutyl)-L-cysteine, N-acetyl-S-(dimethylphenyl)-L-cysteine, N-acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine, N-acetyl-S-(2-hydroxyethyl)-L-cysteine, N-acetyl-S-(3-hydroxypropyl)-L-cysteine, N-acetyl-S-(2-hydroxypropyl)-L-cysteine, N-acetyl-S-(3-hydroxypropyl-1-methyl)-L-cysteine, mandelic acid, 2-methylhippuric acid, 3-methylhippuric acid, 4-methylhippuric acid, N-acetyl-S-(1-hydroxymethyl-2-propenyl)-L-cysteine, N-acetyl-S-(2-hydroxy-3-butenyl)-L-cysteine, N-acetyl-S-(4-hydroxy-2-butenyl)-L-cysteine, t,t-muconic acid, N-acetyl-S-(phenyl-2-hydroxyethyl)-L-cysteine, phenylglyoxylic acid, N-acetyl-S-(phenyl)-L-cysteine, N-acetyl-S-(trichlorovinyl)-L-cysteine, and 2-thioxothiazolidine-4-carboxylic acid.

References

Alwis KU, Blount BC, Britt AS, Patel D, and Ashley DL. Simultaneous analysis of 28 urinary VOC metabolites using ultra high-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (UPLC-ESI/MSMS). *Anal Chim Acta*. 2012; 750: 152-160.