NHANES 2001-2002 Data Release
May 2004
Documentation for Laboratory Results

Laboratory 6 – Blood Lead and Cadmium, Blood Total Mercury, Blood Inorganic Mercury, RBC folate, Serum folate, Methylmalonic acid, Vitamin B_{12}, Ferritin, Homocysteine, and Urinary Mercury

(1) Documentation File Date- September 19, 2003

(2) Documentation File Name- Laboratory 6 – Blood Lead and Cadmium, Blood Total Mercury, Blood Inorganic Mercury, RBC folate, Serum folate, Methylmalonic acid, Vitamin B_{12}, Ferritin, and Homocysteine

(3) Survey Years Included in this File Release-2001-2002

(4) Component Description

4.1 Lead

Lead is a known environmental toxin that has been shown to deleteriously affect the nervous, hematopoietic, endocrine, renal and reproductive systems. In young children, lead exposure is a particular hazard because children more readily absorb lead than do adults, and children's developing nervous systems also make them more susceptible to the effects of lead. The primary sources of exposure for children are lead-laden paint chips and dust as a result of deteriorating lead-based paint. The risk for lead exposure is disproportionately higher for children who are poor, non-Hispanic black, living in large metropolitan areas, or living in older housing. Among adults, the most common high exposure sources are occupational.

Blood lead levels measured in previous NHANES programs have been the cornerstone of lead exposure surveillance in the U.S. The data have been used to document the burden of and dramatic decline of elevated blood lead levels; to promote the reduction of lead use; and to help to redefine national lead poisoning prevention guidelines, standards, and abatement activities.

4.2 Cadmium

Cadmium is performed to identify cases of cadmium toxicity. Occupational exposure is the most common cause of elevated cadmium levels.

4.3 Total Blood Mercury, Inorganic Mercury, and Urinary Mercury

Uncertainties exist regarding levels of exposure to methyl mercury from fish consumption and potential health effects resulting from this exposure. Past estimates of exposure to methyl mercury has been obtained from results of food

consumption surveys and measures of methyl mercury in fish. Measures of a biomarker of exposure are needed for improved exposure assessments. Blood mercury levels will be assessed in two subpopulations particularly vulnerable to the health effects from mercury exposure: children 1-5 years old and women of childbearing age. Women of childbearing age will also have a urine mercury test. Blood measures of total and inorganic mercury will be important for evaluation of exposure from exposure to mercury in interior latex paints.

4.4 **RBC folate, Serum folate, Vitamin B12, Ferritin, Homocysteine and Methylmalonic Acid**

The objectives of this component are: 1) to provide data for monitoring secular trends in measures of nutritional status in the U.S. population; 2) to evaluate the effect of people's habits and behaviors such as physical activity and the use of alcohol, tobacco, and dietary supplements on people's nutritional status; and 3) to evaluate the effect of changes in nutrition and public health policies including welfare reform legislation, food fortification policy, and child nutrition programs on the nutritional status of the U.S. population.

These data will be used to estimate deficiencies and toxicities of specific nutrients in the population and subgroups, to provide population reference data, and to estimate the contribution of diet, supplements, and other factors to serum levels of nutrients. Data will be used for research to further define nutrient requirements as well as optimal levels for disease prevention and health promotion.

(5) Sample Description:

5.1 Blood lead, cadmium, and ferritin

Participants aged 1 year and older were tested.

5.2 RBC folate, serum folate, vitamin B_{12}, homocysteine, and methylmalonic acid

Participants aged 3 year and older were tested.

5.3 Total blood mercury and inorganic mercury

Participants aged 1–5 years and females aged 16-49 years were tested.

5.4 Urinary mercury

Female participants aged 16-49 years were tested.

(6) Description of the Laboratory Methodology

6.1 Lead and cadmium

Cadmium and lead were simultaneously measured in whole blood using adaptations of the methods of Miller, et al[11a]; Parsons, et al[21b] and Stoeppler et al[11c]. Cadmium and lead quantification was based on the measurement of light absorbed at 228.8 nm and 283.3 nm, respectively, by ground state atoms of cadmium and lead from either an electrodeless discharge lamp (EDL) or hollow cathode lamp (HCL) source. Human blood (patient or study) samples, bovine blood quality control pools, and aqueous standards were diluted with a matrix modifier (nitric acid, Triton X-100, and ammonium phosphate). The cadmium and lead contents were determined on a Perkin-Elmer Model SIMAA 6000 simultaneous multi-element atomic absorption spectrometer with Zeeman background correction.

6.2 Total blood mercury and inorganic mercury

Total mercury in whole blood was measured by flow injection cold vapor atomic absorption analysis with on-line microwave digestion, based on the method by T. Guo and J. Bassner[11d]. Decomposition of organic mercury compounds in blood occurred mainly while the sample (mixed with bromate-bromide reagent and hydrochloric acid) flowed through the digestion coil in the microwave. Further decomposition of organic mercury was achieved by on-line addition of potassium permanganate. The total (organic + inorganic) mercuric mercury released was reduced to mercury vapor by sodium tetrahydroborate. The mercury vapor was measured by the spectrometer at 253.7 nm. Inorganic mercury in whole blood was measured by using stannous chloride as reductant without employing microwave digestion system. Mercury vapor (reduced from inorganic mercury compounds) was measured via the same quartz cell at 253.7 nm. The difference in the total reduced mercury (by sodium tetrahydroborate) and inorganic reduced mercury (by stannous chloride) was taken to represent organic mercury in whole blood[11e].

Mercury analysis was performed to identify cases of mercury toxicity. Urine mercury (total), can also be analyzed on the same subjects for NHANES, on the Perkin-Elmer FIMS.

6.3 RBC folate, serum folate, and vitamin B_{12}

Both vitamins were measured by using the Bio-Rad Laboratories "Quantaphase II Folate/vitamin B12" radioassay kit[11f]. The assay was performed by combining serum or a whole blood hemolysate sample with ^{125}I-folate and ^{57}Co-vitamin B12 in a solution containing dithiothreitol (DTT) and cyanide. The mixture was boiled to inactivate endogenous folate-binding proteins and to convert the various forms of vitamin B12 to cyanocobalamin. The reduced folate and its analogs were stabilized by DTT during the heating. The mixture was cooled and then combined with immobilized affinity-purified porcine intrinsic factor and folate-binding proteins. The addition of these substances adjusts and buffers the pH of the reaction mixture to 9.2. The reaction mixture was then incubated for 1 hour at room temperature.

During incubation, the endogenous and labeled folate and B_{12} competed for the limited number of binding sites on the basis of their relative concentrations. The reaction mixtures were then centrifuged and decanted. Labeled and unlabeled folate and vitamin B_{12}, binding to immobilized binding proteins, were concentrated in the bottom of the tube in the form of a pellet. The unbound folate and B_{12} in the supernatant were discarded, and the radioactivity associated with the pellet was counted. Standard curves were prepared by using the pre-calibrated folate/ B_{12} standards in a human serum albumin base. The concentration of the folate and vitamin B_{12} in the participant's serum or folate in a participant's whole blood was calculated from the standard curve.

In the erythrocyte folate procedure, the sample was first diluted 1:11 with a solution of 1 g/dL ascorbic acid in water and either incubated for 90 min prior to assay or frozen immediately for later assay. The 90-minute incubation or the freeze-thaw was necessary for hemolysis of the red blood cells; either allowed the endogenous folate conjugates to hydrolyze the conjugated pterylpolyglutamates prior to assay. The sample was further diluted 1:2 with a protein diluent (human serum albumin), resulting in a matrix similar to that of the standards and serum samples.

6.4 Ferritin

Ferritin was measured by using the Bio-Rad Laboratories' "QuantImune Ferritin IRMA" kit[11g], which was a single-incubation two-site immunoradiometric assay (IRMA) based on the general principles of assays as described by Addison et al.[11h] and Miles[11i] and modified by Jeong et al.[11j]. In this IRMA, which measured the most basic isoferritin, the highly purified ^{125}I-labeled antibody to ferritin was the tracer, and the ferritin antibodies were immobilized on polyacrylamide beads as the solid phase. Serum or ferritin standards (made from human liver) were mixed with the combined tracer/solid-phase antibody reagent, and the mixture was incubated. During incubation, both the immobilized and the ^{125}I-labeled antibodies bound to the ferritin antigen in the serum or standards, thus creating a "sandwich."

After incubation, the beads were diluted with saline, centrifuged, and decanted. The level of ^{125}I-labeled ferritin found in the pellets was measured by using a gamma counter. There was a direct relationship between the radioactive levels of the pellets and the amount of endogenous ferritin in the serum or standards, rather than the inverse relationship measured by most radioimmunoassay (RIAs).

6.5 Homocysteine

Total homocysteine (tHcy) in plasma was measured by the "Abbott Homocysteine (HCY) assay", a fully automated fluorescence polarization immunoassay (FPIA) from Abbott Diagnostics[11k]. In brief, dithiothreitol (DTT) reduced homocysteine bound to albumin and to other small molecules, homocysteine, and mixed disulfides, to free thiol. S-adenosylhomocysteine (SAH) hydrolase catalyzed conversion of

homocysteine to SAH in the presence of added adenosine. In the subsequent steps, the specific monoclonal antibody and the fluoresceinated SAH analog tracer constituted the FPIA detection system[11l]. Plasma total homocysteine concentrations were calculated by the Abbott IMx® using a machine-stored calibration curve.

An international round robin performed in 1998[11m] demonstrated that this method was fully equivalent to other most frequently used methods in this field (i.e., HPLC-FD, HPLC-ED, GC/MS). Thus, the Abbott Homocysteine (HCY) assay can be used as primary method for the determination of plasma total homocysteine in NHANES 1999-2000. The HPLC assay can be used as a reference method and can be performed on a subset of NHANES 1999 –2000 for continuing method comparison and on smaller studies.

6.6 Methylmalonic acid

Methylmalonic acid (MMA) is extracted from plasma or serum along with an added internal standard using a commercially available strong anion exchange resin. The extracted acid is then derivatized with cyclohexanol to form a dicyclohexyl ester. The derivatized samples are injected onto a gas chromatograph for separation from other constituents. The effluent from the gas chromatograph is monitored with a mass selective detector using selected ion monitoring. Results are quantitated by internal calibration using peak area ratios of MMA and the internal standard (d3MMA).

Increased concentrations of methylmalonic acid in plasma or serum and excessive urinary excretion of MMA are believed to be direct measures of tissue stores of cobalamin (vitamin B12) and to be the first indication of cobalamin deficiency. The concentration of MMA in plasma or serum was found to be a useful indicator of cobalamin deficiency, especially in patients with few or no hematological abnormalities, normal results for the Schilling test, or normal or only slightly depressed serum cobalamin concentrations. In folate deficiency, methylmalonic acid is normal. Methylmalonic acid may be elevated due to inborn errors of metabolism. The range of methylmalonic acid in plasma or serum from "healthy adults" is 0.05 to 0.26 mol/L .

6.7 Urinary mercury

Mercury in urine was measured by flow injection cold vapor atomic absorption analysis, which was based on the method that Guo and Bassner developed [11n]. Since mercury in urine was found almost entirely in the inorganic form, Guo and Bassner's method did use microwave digestion, and decomposition of mercury compounds was achieved by manually adding mixed bromate-bromide reagent and concentrated hydrochloric acid (HCl). Further decomposition of mercury compounds was achieved by adding potassium permanganate online. The mercury

vapor (reduced from inorganic mercury compounds by sodium tetrahydroborate) was measured by the spectrophotometer at 253.7 nm.

(7) Laboratory Quality Control and Monitoring

The NHANES quality control and quality assurance protocols (QA/QC) meet the 1988 Clinical Laboratory Improvement Act mandates. A detailed quality control and quality assurance instruction was discussed in the NHANES Laboratory/Medical Technologists Procedures Manual (LPM). Read the LABDOC file for detailed QA/QC protocols.

(8) Data Processing and Editing

Specimens were processed, stored and shipped to Division of Laboratory Sciences, National Center for Environmental Health, National Centers for Disease Control and Prevention, Atlanta, Georgia. Detailed specimen collection and processing instructions was discussed in the NHANES Laboratory/Medical Technologists Procedures Manual (LPM). Read the LABDOC file for detailed data processing and editing protocols. The analytical methods were described in the Description of the Laboratory Methodology section.

(9) Data Access:

All data are publicly available.

(10) Analytic Notes for Data Users:

The analysis of NHANES 2001-2002 laboratory data must be conducted with the key survey design and basic demographic variables. The NHANES 2001-2002 Household Questionnaire Data Files contain demographic data, health indicators, and other related information collected during household interviews. They also contain all survey design variables and sample weights for these age groups. The phlebotomy file includes auxiliary information such as the conditions precluding venipuncture. The household questionnaire and phlebotomy files may be linked to the laboratory data file using the unique survey participant identifier SEQN.

(11) References

11a. Miller DT, Paschal DC, Gunter EW, Stroud PE, D'Angelo J. Determination of blood lead with electrothermal atomic absorption using a L'vov platform and matrix modifier. Analyst 1987;112:1701-4.

11b. Parsons PJ, Slavin W. A rapid Zeeman graphite furnace atomic absorption spectrometric method for the determination of lead in blood. Spectrochimica Acta 1993;48B (No. 6/7):925-39.

11c. Stoeppler, M. and Brandt, K., Determination of cadmium in whole blood and urine by electrothermal atomic-absorption spectrophotometry. Fresenius Z. Anal. Chem. 1980;300:372-80.

11d. Guo,Tiezheng and Baasner, Jjorn. On-Line Microwave Sample Pretreatment for the Determination of Mercury in Blood by Flow Injection Cold Vapor Atomic Absorption Spectrometry. Talanta 1993. 40(12):1927-1936.

11e. Chen HP, Paschal DC, Miller DT, Morrow JC. Determination of total and inorganic mercury in whole blood by on-line digestion with flow-injection. Atom Spectrosc 19/5; 1998, 176-9.

11f. Instruction Manual, Bio-Rad Quantaphase II Folate Radioassay Kit. Bio-Rad Laboratories, Hercules, CA: Bio-Rad Laboratories, 1993.

11g. Instruction Manual, Bio-Rad QuantImune Ferritin IRMA. Hercules (CA): Bio-Rad Laboratories, 1986.

11h. Addison G, Beamish M, Hales C, et al. An immunoradiometric assay for ferritin in the serum of normal patients and patients with iron deficiency and iron overload. J Clin Path 1972; 25:326.

11i. Miles L. Measurement of serum ferritin by a 2-site immunoradiometric assay. In: Handbook of Radioimmunoassay (Abraham, G ed.). New York: Marcel Dekker, Inc., 1977: Chapter 4.

11j. Jeong H, Blackmore J, Lewin N. Ferritin immunoradiometric assay, U.S. Patent No. 4,244,940

11k. Abbott Homocysteine (HCY) assay package insert.

11l. Shipchandler, MT, Moore EG. Rapid, fully automated measurement of plasma homocysteine with the Abbott IMx® analyzer. Clin Chem 1995;41(7):991-994.

11m. Pfeiffer CM, Huff DL, Smith SJ, Miller DT, Gunter EW. An international comparison of plasma total homocysteine laboratory measurements. Clin Chem 1999;45: in press.

11n. Guo T, Baasner J. Determination of mercury by flow-injection cold vapor atomic absorption spectrometry. Analytica Chimica Acta 1993; 278: 189-96.