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

Laboratory 39 - Erythrocyte Protoporphyrin

(1) Documentation File Date- September 22, 2004

(2) Documentation File Name – Laboratory 39 - Erythrocyte Protoporphyrin

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

(4) Component Description

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 Eligible Sample

Participants aged 1 year and older were tested.

(6) Description of the Laboratory Methodology

Division of Laboratory Sciences, National Centers for Disease Control

Free erythrocyte protoporphyrin (FEP) is measured by a modification of the method of Sassa et al. Protoporphyrin is extracted from EDTA whole blood into a 2:1 (v/v) mixture of ethyl acetate acetic acid, then back extracted into diluted hydrochloric acid. The protoporphyrin in the aqueous phase is measured fluorometrically at excitation and emission wavelengths of 404 and 658 nm, respectively. Calculations are based on a processed protoporphyrin IX (free acid) standard curve. After a correction for the individual hematocrit is made, the final concentration of protoporphyrin in a specimen is expressed as micrograms per deciliter (g/dL) of packed red blood cells (RBC).

State of New York Department of Health, Wadsworth Center, Trace Elements Laboratory

Porphyrins and heme components are extracted from whole blood into a 4:1 mixture of ethyl acetate-acetic acid. Porphyrins are then separated from heme by back-extraction into a 1.5 M hydrochloric acid solution, and quantitatively determined by molecular fluorometry using a spectrofluorometer calibrated with protoporphyrin IX (PPIX) standard solutions; however, the exact concentration of the standards must first be established using molecular absorbance, Beer's Law, and the millimolar absorptivity of PPIX.

The analytical method for EP routinely employed by the EP Lab is based largely on those originally described by Sassa et al. (1973) and Chisolm and Brown (1975). New York State's extraction method owes much to contributions from other public health labs, including the CDC, and closely follows the key elements of the consensus method for EP as published by the National Committee for Clinical Laboratory Standards (NCCLS C42-A*, 2001). At the invitation of Dr. Sassa, the EP Laboratory's routine method for EP was published as Unit 8.8 in Current Protocols in Toxicology, 1999 by J. Wiley & Sons, Inc. Elements of this protocol are reproduced below, but a reprint of the original publication is available from the EP lab director.

(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 in 2001 and to the State of New York Department of Health, Wadsworth Center, Trace Metals Laboratory, Albany, New York in 2002. Detailed specimen collection and processing instructions was discussed in the NHANES 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.

The Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention performed testing from 1999-2001. The State of New York Department of Health, Wadsworth Center, Trace Elements Laboratory began testing in 2002.

(11) References

Sassa S, Granick JL, Granick S, Kappas A, Levere RD. Microanalyses of erythrocyte protoporphyrin levels by spectrophotometry in the detection of chronic lead intoxication in the subclinical range. Biochem Med 1973;8: 135-48.

Committee on Specifications and Criteria for Biochemical Compounds, National Research Council. Specifications and criteria for biochemical compounds, 3rd ed. Washington, D.C.: National Academy of Sciences, 1972.

Culbreth P, Walter G, Carter R, Burtis C. Separation of protoporphyrin and related compounds by reversed-phase liquid chromatography. Clin Chem 1979; 25(6):605-10.

Gunter EW, Turner WE, Neese JW, Bayse DD. Laboratory procedures used by the Clinical Chemistry Division, Centers for Disease Control, for the Second Health and Nutrition Examination Survey (NHANES 1999+ II) 1976-80. Atlanta: Centers for Disease Control, 1981: 8-12.

Gunter EW, Miller DT. Laboratory procedures used by the Division of Environmental Health Laboratory Sciences, Center for Environmental Health, Centers for Disease Control, for the Hispanic Health and Nutrition Examination Survey (HHANES) 1982-84. Atlanta: Centers for Disease Control, 1986: 9-13.

Gunter EW, Turner WE, Huff DL. Investigation of protoporphyrin IX standard materials used in acid-extraction methods, and a proposed correction for the millimolar absorptivity of protoporphyrin IX. Clin Chem 1989;35(8):1601-9.

Fulwood R, Johnson CL, Bryner JD, Gunter EW, McGrath CR. Hematological and Nutritional Biochemistry Reference Data for Persons 6 Months-74 Years of Age: United States, 1976-80. National Center for Health Statistics. Vital and Health Statistics. Series 11-No. 232. DHHS Pub. No. (PHS) 83-162. Public Health Service.

Washington, D.C.: U.S. Government Printing Office, 1982.

Gunter EW, Lewis BL, Koncikowski SM. Laboratory methods used for the Third National Health and Nutrition Examination Survey (NHANES III), 1988-1994. Centers for Disease Control and Prevention, Hyattsville, MD., 1996. VII-A-1 to VII-A-XII.

State of New York Department of Health, Wadsworth Center, Trace Metals Laboratory:

Blumberg, W.E., Eisinger, J., Lamola, A.A., and Zuckerman, D.M. 1977a. The hematofluorometer. Clinical Chemistry 23:270-274.

Blumberg, W.E., Eisinger, J., Lamola, A.A., and Zuckerman, D.M. 1977b. Zinc protoporphyrin level in blood determined by a portable hematofluorometer: a screening device for lead poisoning. Journal of Laboratory & Clinical Medicine 89:712-723.

Bowers, M.A., Aicher, L.D., Davis, H.A., and Woods, J.S. 1992. Quantitative determination of porphyrins in rat and human urine and evaluation of urinary porphyrin profiles during mercury and lead exposures. J Lab Clin Med 120:272-281.

Centers for Disease Control, 1975. Increased lead absorption and lead poisoning in young children [Report]. U.S. Department of Health, Education, and Welfare, Atlanta, GA.

Centers for Disease Control, 1978. Preventing lead poisoning in young children [Report]. U.S. Department of Health, Education, and Welfare, Atlanta, GA.

Centers for Disease Control, 1985. Preventing lead poisoning in young children [Report]. U.S. Department of Health and Human Services, Atlanta, GA.

Centers for Disease Control, 1991. Preventing lead poisoning in young children [Report]. U.S. Department of Health and Human Services, Atlanta, GA.

Chiba, M. and Sassa, S. 1982. Analysis of porphyrin carboxylic acids in biological fluids by high- performance liquid chromatography. Anal Biochem 124:279-285.

Chisolm, J.,Jr. and Brown, D.H. 1975. Micro-scale photofluorometric determination of "free erythrocyte pophyrin" (protoporphyrin IX). Clin Chem 21:1669-1682.

Doran, D. and Mitchell, D.G. 1984. Problems in the determination of erythrocyte protoporphyrin by ethyl acetate-acetic acid extraction. Ann Clin Biochem 21:141-145.

Gunter, E.W., Turner, W.E., and Huff, D.L. 1989. Investigation of protoporphyrin IX standard materials used in acid- extraction methods, and a proposed correction for the millimolar absorptivity of protoporphyrin IX. Clin Chem 35:1601-1608.

Ho, J., Guthrie, R., and Tieckelmann, H. 1987. Quantitative determination of

porphyrins, their precursors and zinc protoporphyrin in whole blood and dried blood by high-performance liquid chromatography with fluorimetric detection. J Chromatogr. 417:269-276.

Kammholz, L.P., Thatcher, L.G., Blodgett, F.M., and Good, T.A. 1972. Rapid protoporphyrin quantitation for detection of lead poisoning. Pediatrics. 50:625-631.

National Committee for Clinical Laboratory Standards., 1996. Erythrocyte protoporphyrin testing; Approved guideline. 1 pp. NCCLS Document C42-A, Wayne, PA.

Parsons, P.J., Reilly, A.A., and Hussain, A 1991. Observational Study of Erythrocyte Protoporphyrin as a Screening Test for Detecting Lead Exposure in Children: Impact of Lowering the Blood Lead Action Threshold. Clin. Chem. 37:216-225.

Piomelli, S. 1973. A micromethod for free erythrocyte porphyrins: the FEP test. J Lab Clin Med 81:932-940.

Sagen, E. and Romslo, I. 1985. Determination of porphyrins by high performance liquid chromatography: fluorescence detection compared to absorbance detection. Scand. J Clin Lab Invest. 45:309-314.

Sassa, S., Granick, J.L., Granick, S., Kappas, A., and Levere, R.D. 1973. Studies in lead poisoning. I. Microanalysis of erythrocyte protoporphyrin levels by spectrophotometry in the detection of chronic lead intoxication in the subclinical range. Biochem Med 8:135-148.

Sato, H., Ido, K., and Kimura, K. 1994. Simultaneous separation and quantification of free and metal-chelated protoporphyrins in blood by three-dimensional HPLC. Clin Chem 40:1239-1244.

Schwartz, S., Berg, M.H., Bossenmaier, I., and Dinsmore, H. 1960. Determination of porphyrins in biological materials. In Methods of biochemical analysis, Vol VIII, D. Glick ed.) pp. 221-294. Interscience Publishers, Inc., New York.

Scoble, H.A., McKeag, M., Brown, P.R., and Kavarnos, G.J. 1981. The rapid determination of erythrocyte porphyrins using reversed-phase high performance liquid chromatography. Clin Chim Acta 113:253-265.
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