National Health and Nutrition Examination Survey

2015-2016 Data Documentation, Codebook, and Frequencies

Folate - RBC (FOLATE_I)

Data File: FOLATE_I.xpt

First Published: February 2019

Last Revised: NA

Component Description

The objectives of this component are to: 1) provide data for monitoring secular trends in measures of nutritional status in the U.S. population; 2) evaluate the effect of people's habits and behaviors, such as physical activity and the use of alcohol, tobacco, and dietary supplements on nutritional status; and 3) 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 subgroup, 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 in research to further define nutrient requirements as well as optimal levels for disease prevention and health promotion.

Eligible Sample

Examined participants aged 1 year and older were eligible.

Description of Laboratory Methodology

Same as in NHANES 2011-2014, population folate status in the 2015-2016 survey cycle was assessed by a combination of two analytical methods: whole-blood folate was measured by microbiologic assay, while serum folate forms were measured by isotope-dilution high performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). RBC folate was then calculated using the data from both assays.

There were no changes to the lab method, lab equipment, or laboratory performing the analyses for this component in the NHANES 2015-2016 cycle.

Whole Blood Folate

Microbiological assays have been used for many years to estimate the concentration of folate in blood and other tissues. In the 1990s, more robust and reliable procedures were developed which use microtiter plates for higher throughput and a cryopreserved antibiotic resistant microorganism to avoid having to work under aseptic conditions. The described procedure is an adaptation of such a method, and was introduced by the National Center for Environmental Health (NCEH) to NHANES in 2007. Diluted whole blood is added to an assay medium containing Lactobacillus rhamnosus (formerly known as Lactobacillus casei) (NCIB 10463) and all of the nutrients necessary for the growth of L. rhamnosus except folate. The inoculated medium is incubated for 45 hours at 37^{o}C. Since the growth of L. rhamnosus is proportional to the amount of total folate present in whole blood sample; the folate level can be assessed by measuring the turbidity of the inoculated medium at 590 nm in a PowerWave X340

Microplate reader (Bio-Tek Instrument). The assay was calibrated with 5-methyl-tetrahydrofolate from Merck Cie (Eprova).

Serum Total Folate

Serum total folate was calculated as the sum of individual folate forms. Five folate forms, 5-methyl-tetrahydrofolate, folic acid, 5-formyl-tetrahydrofolate, tetrahydrofolate, and 5,10-methenyl-tetrahydrofolate are measured by isotope-dilution high performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) (Fazili, et al. 2013). The assay is performed by combining specimen (150 µL serum) with ammonium formate buffer and an internal standard mixture. Sample extraction and clean-up is performed by automated 96-probe solid phase extraction (SPE) using 96-well phenyl SPE plates and takes ~1 h for a 96-well plate. Folate forms are separated within 6 min using isocratic mobile phase conditions and measured by LC-MS/MS. Quantitation is based on peak area ratios interpolated against a five-point aqueous linear calibration curve using 1/x2 weighting.

Refer to the Laboratory Method Files section for detailed description on the laboratory methods used.

Laboratory Method Files

Whole Blood Folate (December 2018)

Folate Vitamers (December 2018)

Laboratory Quality Assurance and Monitoring

Whole blood and blood serum are processed, stored, and shipped to the Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA for analysis.

Detailed instructions on specimen collection and processing are discussed in the NHANES Laboratory Procedures Manual (LPM). Vials are stored under appropriate frozen (–20°C) conditions until they are shipped to National Center for Environmental Health for testing. The NHANES quality assurance and quality control (QA/QC) protocols meet the 1988 Clinical Laboratory Improvement Act mandates. Detailed QA/QC instructions are discussed in the NHANES LPM.

Mobile Examination Centers (MECs)

Laboratory team performance is monitored using several techniques. NCHS and contract consultants use a structured quality assurance evaluation during unscheduled visits to evaluate both the quality of the laboratory work and the quality-control procedures. Each laboratory staff member is observed for equipment operation, specimen collection and preparation; testing procedures and constructive feedback are given to each staff member. Formal retraining sessions are conducted annually to ensure that required skill levels were maintained.

Analytical Laboratories

NHANES uses several methods to monitor the quality of the analyses performed by the contract laboratories. In the MEC, these methods include performing blind split samples collected during “dry run” sessions. In addition, contract laboratories randomly perform repeat testing on 2% of all specimens.

NCHS developed and distributed a quality control protocol for all the contract laboratories, which outlined the use of Westgard rules (Westgard, et al. 1981) when running NHANES specimens. Progress reports containing any problems encountered during shipping or receipt of specimens, summary statistics for each control pool, QC graphs, instrument calibration, reagents, and any special considerations are submitted to NCHS quarterly. The reports are reviewed for trends or shifts in the data. The laboratories are required to explain any identified areas of concern.

All QC procedures recommended by the manufacturers were followed. Reported results for all assays meet the Division of Laboratory Sciences' quality control and quality assurance performance criteria for accuracy and precision, similar to the Westgard rules (Caudill, et al. 2008).

Data Processing and Editing

The data were reviewed. Incomplete data or improbable values were sent to the performing laboratory for confirmation.

One derived variable was created in this data file. The variable was created using the following formula:

LBDRFO: The RBC folate value in nmol/L RBC (LBDRFOSI) was converted to ng/mL RBC (LBDRFO) by dividing LBDRFOSI by 2.265 (rounded to 3 significant figures).

Analytic Notes

Same as in NHANES 2011-2012 and 2013-2014, red blood cell (RBC) folate in NHANES 2015-2016 was calculated from the whole blood folate concentration as measured by microbiologic assay by adjusting for RBC volume and correcting for serum total folate concentration which was calculated as the sum of individual folate forms. The amounts of individual serum folate forms were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS). For folate forms with results lower than limit of detection (LOD), an imputed value of LOD divided by the square root of 2 was used. Serum folate forms used to calculate serum total folate concentration were: 5-methyl-tetrahydrofolate, folic acid, 5-formyl-tetrahydrofolate, tetrahydrofolate, and 5,10-methenyl-tetrahydrofolate. For more detailed information regarding folate forms data in NHANES 2015-2016, please refer to the documentation accompanying the Folate Forms – Serum (FOLFMS_I) file.

Refer to the 2015-2016 Laboratory Data Overview for general information on NHANES laboratory data.

Examined sample weights should be used for analyses. Please refer to the NHANES Analytic Guidelines and the on-line NHANES Tutorial for details on the use of sample weights and other analytic issues.

Demographic and Other Related Variables

The analysis of NHANES laboratory data must be conducted using the appropriate survey design and demographic variables. The 2015-2016 Demographics File contains demographic data, health indicators, and other related information collected during household interviews as well as the sample design variables. The recommended procedure for variance estimation requires use of stratum and PSU variables (SDMVSTRA and SDMVPSU, respectively) in the demographic data file.

The Fasting Questionnaire File includes auxiliary information such as fasting status, the time of venipuncture, and the conditions precluding venipuncture.

This laboratory data file can be linked to the other NHANES data files using the unique survey participant identifier (i.e., SEQN).

Detection Limits

An exact lower limit of detection (LLOD) for RBC folate cannot be calculated because the value is a composite of whole blood folate, serum folate, and hematocrit. Therefore, there is no LLOD for the calculated value of RBC folate. Furthermore, the LOD of this method for whole blood folate depends on the dilution factor (i.e., LOD = 44 nmol/L whole blood if whole blood hemolysate is only diluted 1/40; assuming a hematocrit of 40%, this would correspond to a RBC folate concentration of 110 nmol/L RBC).

References

- Caudill, S.P., Schleicher, R.L., Pirkle, J.L. Multi-rule quality control for the age-related eye disease study. Statist. Med. (2008) 27(20):4094-40106.
- Fazili Z, Whitehead RD Jr, Paladugula N, Pfeiffer CM. A high-throughput LC-MS/MS method suitable for population biomonitoring measures five serum folate vitamers and one oxidation product. Anal Bioanal Chem. 2013;405:4549–60.
- Westgard J.O., Barry P.L., Hunt M.R., Groth T. A multi-rule Shewhart chart for quality control in clinical chemistry. Clin Chem (1981) 27:493-501.

Codebook and Frequencies

SEQN - Respondent sequence number

Variable Name:	SEQN
SAS Label:	Respondent sequence number
English Text:	Respondent sequence number
Target:	Both males and females 1 YEARS - 150 YEARS

LBDRFO - RBC folate (ng/mL)

Variable Name: LBDRFO

SAS Label: RBC folate (ng/mL)

English Text: RBC folate (ng/mL)

Target: Both males and females 1 YEARS - 150 YEARS

Code or Value	Value Description	Count	Cumulative	Skip to Item
83.4 to 2970	Range of Values	8099	8099	
.	Missing	1066	9165	

LBDRFOSI - RBC folate (nmol/L)

Variable Name: LBDRFOSI

SAS Label: RBC folate (nmol/L)

English Text: RBC folate (nmol/L)

Target: Both males and females 1 YEARS - 150 YEARS

Code or Value	Value Description	Count	Cumulative	Skip to Item
189 to 6730	Range of Values	8099	8099	
.	Missing	1066	9165