National Health and Nutrition Examination Survey

August 2021-August 2023 Data Documentation, Codebook, and Frequencies

Folate - RBC (FOLATE_L)

Data File: FOLATE_L.xpt

First Published: September 2024

Last Revised: NA

Component Description

Folate belongs to the group of water-soluble B vitamins that occur naturally in food. It is required in cellular one carbon metabolism and hematopoiesis. Prolonged folate deficiency leads to megaloblastic anemia (Bailey, 2015). Low folate status has been shown to increase the risk of women of childbearing age to have an offspring with neural tube defects. Low folate status also increases plasma homocysteine levels, a potential risk factor for cardiovascular disease, in the general population. Potential roles of folate and other B vitamins in modulating the risk for diseases (e.g., heart disease, cancer, and cognitive impairment) are currently being studied.

The measurement of total folate (TFOL) provides information on the folate status of the individual. Serum folate is an indicator of short-term status, while red blood cell (RBC) folate is an indicator of long-term status. 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

All examined participants aged 1 year and older were eligible.

Description of Laboratory Methodology

Population folate status in the NHANES August 2021–August 2023 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), this is the same method used in NHANES 2011-March 2020. RBC folate was then calculated using the data from both assays.

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 that 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 this 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 42 hours at 37°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 an 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 a detailed description on the laboratory methods used.

There were no changes to the lab method, lab equipment, or lab for this component during the NHANES August 2021-August 2023 cycle.

Laboratory Method Files

Folate - RBC (September 2024)

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 (–30°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 Amendments 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 QA evaluation during unscheduled visits to evaluate both the quality of the laboratory work and the QC 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 QC protocol for all the contract laboratories, which outlined the use of Westgard rules (Westgard, et. al., 1981) when testing 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' QA/QC 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 variable was created in this data file. The variable (LBDRFO) 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

Red blood cell (RBC) folate in NHANES August 2021 – August 2023 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 August 2021—August 2023, please refer to the documentation accompanying the Folate Forms – Serum (FOLFMS_L) file.

There are over 800 laboratory tests performed on NHANES participants. However, not all participants provided biospecimens or enough volume for all the tests to be performed. The specimen availability can also vary by age or other population characteristics. Analysts should evaluate the extent of missing data in the dataset related to the outcome of interest as well as any predictor variables used in the analyses to determine whether additional re-weighting for item non-response is necessary.

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.

Phlebotomy Weights

For the August 2021-August 2023 cycle, analysis of nonresponse patterns for the phlebotomy component in the MEC examination revealed differences by age group and race/ethnicity, among other characteristics. For example, approximately 67% of children aged 1-17 years who were examined in the MEC provided a blood specimen through phlebotomy, while 95% of examined adults aged 18 and older provided a blood specimen. Therefore, an additional phlebotomy weight, WTPH2YR, has been included in this data release to address possible nonresponse bias. Participants who are eligible but did not provide a blood specimen have their phlebotomy weight assigned a value of "0" in their records. The phlebotomy weight should be used for analyses that use variables derived from blood analytes, and is included in all relevant data files.

Demographic and Other Related Variables

The analysis of NHANES laboratory data must be conducted using the appropriate survey design and demographic variables. The August 2021—August 2023 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

- Bailey LB, Stover PJ, McNulty H, Fenech MF, Gregory III JF, Mills JL, Pfeiffer CM, Fazili Z, Zhang M, et al. Biomarkers of nutrition for development – folate review. J Nutr. 2015;145:1636S–80S.
- 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

WTPH2YR - Phlebotomy 2 Year Weight

Variable Name: WTPH2YR

SAS Label: Phlebotomy 2 Year Weight

English Text: Phlebotomy 2 Year Weight

Target: Both males and females 1 YEARS - 150 YEARS

Code or Value	Value Description	Count	Cumulative	Skip to Item
4391.8220579 to 253478.77765	Range of Values	7626	7626	
0	No blood sample provided	1101	8727	
.	Missing	0	8727	

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
63.6 to 3240	Range of Values	7506	7506	
.	Missing	1221	8727	

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
144 to 7330	Range of Values	7506	7506	
.	Missing	1221	8727	