National Health and Nutrition Examination Survey

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

Serum Folate Forms - Total & Individual - Serum (FOLFMS_L)

Data File: FOLFMS_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 (Bailey, 2015). Prolonged folate deficiency leads to megaloblastic anemia. 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

Examined participants aged 1 year and older were eligible.

Description of Laboratory Methodology

Six folate forms, 5-methyltetrahydrofolate, folic acid, tetrahydrofolate, 5-formyltetrahydrofolate, 5,10-methenyltetrahydrofolate, and an oxidation product of 5-methyltetrahydrofolate called MeFox (pyrazino-s-triazine derivative of 4-α-hydroxy-5-methyltetrahydrofolate) 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 $1/x^2$ weighting.

This method distinguishes 5-formylTHF from 10-formylTHF on the basis of mass transitions, however, during LC-MS/MS in the acidic mobile phase, 10-formylTHF converts within minutes to 5,10-methenylTHF and trace amounts of 5-formylTHF, THF, and 10-formyl-folic acid. Thus, this method is not capable of quantifying 10-formylTHF; formylated folates are quantified as 5-

formylTHF and 5,10-methenylTHF, which are stable under these conditions (Pfeiffer, et. al., 2004). Because concentrations of the three minor folate forms THF, 5-formylTHF, and 5,10-methenylTHF are typically below the limit of detection (LOD) and can be a result of folate interconversions at slightly acidic pH during sample preparation, it is recommended to express the sum of these three forms as non-methyl folate (Pfeiffer, et. al., 2015).

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 site for this component during the NHANES August 2021-August 2023 cycle.

Laboratory Method Files

Folate Forms (September 2024)

Laboratory Quality Assurance and Monitoring

Serum specimens 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 the 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 (LBDFOT) was created using the following formula:

LBDFOT: The serum folate value in nmol/L (LBDFOTSI) was converted to ng/mL (LBDFOT) by dividing LBDFOTSI by 2.265 (rounded to 3 significant figures).

Analytic Notes

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.

Serum Folate Forms for NHANES August 2021-August 2023

In NHANES August 2021-August 2023, a comprehensive list of serum folate forms were measured by isotope-dilution high performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) (Table 1). Serum total folate (LBDFOTSI) was calculated by adding LBXSF1SI-LBXSF5SI. LBXSF6SI was not included in the total folate calculation, due to evidence that it may already be present in vivo (Pfeiffer, et. al., 2015). An imputed value of LOD divided by the square root of 2 was used for individual folate forms with results that were less than the limit of detection (LOD).

Please refer to the Analytic Notes for the 2011-2012 Folate Forms – Serum (FOLFMS_G) file for additional details on the comparability in serum total folate and folate forms measured between NHANES 2011–2020 and the previous survey cycles.

Table 1. Folate forms measured by LC-MS/MS

Analyte	Abbreviation	Variable Name
5-Methyltetrahydrofolate	5-methylTHF	LBXSF1SI
Pteroylglutamic acid (Folic Acid)	Folic acid	LBXSF2SI
5-Formyltetrahydrofolate	5-formylTHF	LBXSF3SI
Tetrahydrofolate	THF	LBXSF4SI
5,10-Methenyltetrahydrofolate	5,10-methenylTHF	LBXSF5SI
Pyrazino-s-triazine derivative of 4-a-hydroxy-5-methyltetrahydrofolate	MeFox	LBXSF6SI
Serum total folate (sum of folate forms)	tFOL	LBDFOTSI

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

The detection limits were constant for all of the analytes in the data set. Two variables are provided for each of these analytes. The variable name ending in "LC" (ex., LBDSF1LC) indicates whether the result was below the limit of detection: the value "0" means that the result was at or above the limit of detection, "1" indicates that the result was below the limit of detection. The other variable prefixed LBX (ex., LBXSF1SI) provides the analytic result for that analyte. For analytes with analytic results below the lower limit of detection (ex., LBDSF1LC=1), an imputed fill value was placed in the analyte results field. This value is the lower limit of detection divided by the square root of 2 (LLOD/sqrt[2]).

The lower limit of detection (LLOD, in nmol/L) for the 6 folate forms are shown below. Because total folate is calculated from the sum of folate forms SF1-SF5, a lower limit of detection does not apply.

Variable Name	Analyte Description	LLOD
LBXSF1SI	5-Methyltetrahydrofolate	0.13
LBXSF2SI	Folic acid	0.14
LBXSF3SI	5-Formyltetrahydrofolate	0.20
LBXSF4SI	Tetrahydrofolate	0.25
LBXSF5SI	5,10-Methenyltetrahydrofolate	0.20
LBXSF6SI	MeFox	0.10
LBDFOTSI	Total folate, serum	n/a

References

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- 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.
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- Pfeiffer CM, Fazili Z, McCoy LF, Gunter EW. Determination of folate vitamers in human serum by stable-isotope dilution tandem mass spectrometry and comparison to radioassay and microbiologic assay. Clin Chem. 2004;50(2):423-432.
- 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

LBDFOTSI - Serum total folate (nmol/L)

Variable Name: LBDFOTSI

SAS Label: Serum total folate (nmol/L)

English Text: Serum total folate (nmol/L)

Target: Both males and females 1 YEARS - 150 YEARS

Code or Value	Value Description	Count	Cumulative	Skip to Item
3.89 to 445	Range of Values	7348	7348	
.	Missing	1379	8727	

LBDFOT - Serum total folate (ng/mL)

Variable Name: LBDFOT

SAS Label: Serum total folate (ng/mL)

English Text: Serum total folate (ng/mL)

Target: Both males and females 1 YEARS - 150 YEARS

Code or Value	Value Description	Count	Cumulative	Skip to Item
1.72 to 196	Range of Values	7348	7348	
.	Missing	1379	8727	

LBXSF1SI - 5-Methyl-tetrahydrofolate (nmol/L)

Variable Name: LBXSF1SI

SAS Label: 5-Methyl-tetrahydrofolate (nmol/L)

English Text: 5-Methyl-tetrahydrofolate (nmol/L)

Target: Both males and females 1 YEARS - 150 YEARS

Code or Value	Value Description	Count	Cumulative	Skip to Item
2.7 to 338	Range of Values	7348	7348	
.	Missing	1379	8727	

LBDSF1LC - 5-Methyl-tetrahydrofolate cmt

Variable Name: LBDSF1LC

SAS Label: 5-Methyl-tetrahydrofolate cmt

English Text: 5-Methyl-tetrahydrofolic comment code

Target: Both males and females 1 YEARS - 150 YEARS

Code or Value	Value Description	Count	Cumulative	Skip to Item
0	At or above the detection limit	7348	7348	
1	Below lower detection limit	0	7348	
.	Missing	1379	8727	

LBXSF2SI - Folic acid (nmol/L)

Variable Name: LBXSF2SI

SAS Label: Folic acid (nmol/L)

English Text: Folic acid (nmol/L)

Target: Both males and females 1 YEARS - 150 YEARS

Code or Value	Value Description	Count	Cumulative	Skip to Item
0.056 to 317	Range of Values	7348	7348	
.	Missing	1379	8727	

LBDSF2LC - Folic acid cmt

Variable Name: LBDSF2LC

SAS Label: Folic acid cmt

English Text: Folic acid comment code

Target: Both males and females 1 YEARS - 150 YEARS

Code or Value	Value Description	Count	Cumulative	Skip to Item
0	At or above the detection limit	7272	7272	
1	Below lower detection limit	76	7348	
.	Missing	1379	8727	

LBXSF3SI - 5-Formyl-tetrahydrofolate (nmol/L)

Variable Name: LBXSF3SI

SAS Label: 5-Formyl-tetrahydrofolate (nmol/L)

English Text: 5-Formyl-tetrahydrofolate (nmol/L)

Target: Both males and females 1 YEARS - 150 YEARS

Code or Value	Value Description	Count	Cumulative	Skip to Item
0.141 to 1.48	Range of Values	7348	7348	
.	Missing	1379	8727	

LBDSF3LC - 5-Formyl-tetrahydrofolate cmt

Variable Name: LBDSF3LC

SAS Label: 5-Formyl-tetrahydrofolate cmt

English Text: 5-Formyl-tetrahydrofolate comment code

Target: Both males and females 1 YEARS - 150 YEARS

Code or Value	Value Description	Count	Cumulative	Skip to Item
0	At or above the detection limit	17	17	
1	Below lower detection limit	7331	7348	
.	Missing	1379	8727	

LBXSF4SI - Tetrahydrofolate (nmol/L)

Variable Name: LBXSF4SI

SAS Label: Tetrahydrofolate (nmol/L)

English Text: Tetrahydrofolate (nmol/L)

Target: Both males and females 1 YEARS - 150 YEARS

Code or Value	Value Description	Count	Cumulative	Skip to Item
0.177 to 31	Range of Values	7348	7348	
.	Missing	1379	8727	

LBDSF4LC - Tetrahydrofolate cmt

Variable Name: LBDSF4LC

SAS Label: Tetrahydrofolate cmt

English Text: Tetrahydrofolate comment code

Target: Both males and females 1 YEARS - 150 YEARS

Code or Value	Value Description	Count	Cumulative	Skip to Item
0	At or above the detection limit	7033	7033	
1	Below lower detection limit	315	7348	
.	Missing	1379	8727	

LBXSF5SI - 5,10-Methenyl-tetrahydrofolate (nmol/L)

Variable Name: LBXSF5SI

SAS Label: 5,10-Methenyl-tetrahydrofolate (nmol/L)

English Text: 5,10-Methenyl-tetrahydrofolate (nmol/L)

Target: Both males and females 1 YEARS - 150 YEARS

Code or Value	Value Description	Count	Cumulative	Skip to Item
0.141 to 7.14	Range of Values	7348	7348	
.	Missing	1379	8727	

LBDSF5LC - 5,10-Methenyl-tetrahydrofolate cmt

Variable Name: LBDSF5LC

SAS Label: 5,10-Methenyl-tetrahydrofolate cmt

English Text: 5,10-Methenyl-tetrahydrofolate comment code

Target: Both males and females 1 YEARS - 150 YEARS

Code or Value	Value Description	Count	Cumulative	Skip to Item
0	At or above the detection limit	270	270	
1	Below lower detection limit	7078	7348	
.	Missing	1379	8727	

LBXSF6SI - Mefox oxidation product (nmol/L)

Variable Name: LBXSF6SI

SAS Label: Mefox oxidation product (nmol/L)

English Text: Mefox oxidation product (nmol/L)

Target: Both males and females 1 YEARS - 150 YEARS

Code or Value	Value Description	Count	Cumulative	Skip to Item
0.062 to 38.9	Range of Values	7348	7348	
.	Missing	1379	8727	

LBDSF6LC - Mefox oxidation product cmt

Variable Name: LBDSF6LC

SAS Label: Mefox oxidation product cmt

English Text: Mefox oxidation product comment code

Target: Both males and females 1 YEARS - 150 YEARS

Code or Value	Value Description	Count	Cumulative	Skip to Item
0	At or above the detection limit	7348	7348	
1	Below lower detection limit	0	7348	
.	Missing	1379	8727	