# National Health and Nutrition Examination Survey

## 2015-2016 Data Documentation, Codebook, and Frequencies

## Folate Forms - Total & Individual - Serum (FOLFMS_I)

**Data File: FOLFMS_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

Five folate forms, 5-methyl-tetrahydrofolate, folic acid, 5-formyl-tetrahydrofolate, tetrahydrofolate, 5,10-methenyl-tetrahydrofolate, and an oxidation product of 5-methyl-tetrahydrofolate called MeFox (pyrazino-s-triazine derivative of 4-α-hydroxy-5-methyl-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 $1/x^2$ weighting.

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

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

## Laboratory Method Files

Folate Vitamers (December 2018)

## 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 (–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 variable was created in this data file. The variable (LBDFOT) was created using the following formula:

**LBDFOT:** The serum total 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

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

Exam 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.

**Serum Folate Forms for NHANES 2015–2016**

In NHANES 2015–2016, a comprehensive list of serum folate forms was 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 < LOD. No LBDFOTSI was calculated if the result for one or more of the folate forms was missing.

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 2011–2016 and the previous survey cycles.

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

| Analyte | Abbreviation | Variable Name |
|---|---|---|
| 5-Methyl-tetrahydrofolate | 5-methylTHF | LBXSF1SI |
| Pteroylglutamic acid | Folic acid | LBXSF2SI |
| 5-Formyl-tetrahydrofolate | 5-formylTHF | LBXSF3SI |
| Tetrahydrofolate | THF | LBXSF4SI |
| 5,10-Methenyl-tetrahydrofolate | 5,10-methenylTHF | LBXSF5SI |
| Pyrazino-s-triazine derivative of 4-α-hydroxy-5-methyl-tetrahydrofolate | 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 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**

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, a lower limit of detection does not apply.

| Variable Name | SAS Label | LLOD |
|---|---|---|
| LBXSF1SI | 5-Methyl-tetrahydrofolate | 0.13 |
| LBXSF2SI | Folic acid | 0.14 |
| LBXSF3SI | 5-Formyl-tetrahydrofolate | 0.20 |
| LBXSF4SI | Tetrahydrofolate | 0.25 |
| LBXSF5SI | 5,10-Methenyl-tetrahydrofolate | 0.20 |
| LBXSF6SI | Mefox oxidation product | 0.10 |
| LBDFOTSI | Serum total folate | n/a |

## 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.
- Pfeiffer C, Sternberg M, Fazili M, Lacher D, Zhang M, Johnson C, Hammer H, Baily R, Rader J, Yamini S, Berry RJ, Yetley E. British Journal of Nutrition (2015) 113:1965:1977.
- 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 |
|---|---|---|---|---|
| 4.07 to 1390 | Range of Values | 8047 | 8047 | |
| . | Missing | 1118 | 9165 | |

# 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 |
|---|---|---|---|---|
| 1.8 to 614 | Range of Values | 8047 | 8047 | |
| . | Missing | 1118 | 9165 | |

# 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 |
|---|---|---|---|---|
| 3.16 to 1370 | Range of Values | 8057 | 8057 | |
| . | Missing | 1108 | 9165 | |

# 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 | 8057 | 8057 | |
| 1 | Below lower detection limit | 0 | 8057 | |
| . | Missing | 1108 | 9165 | |

# 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 |
|---|---|---|---|---|
| 0.099 to 418 | Range of Values | 8047 | 8047 | |
| . | Missing | 1118 | 9165 | |

# 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 | 8006 | 8006 | |
| 1 | Below lower detection limit | 41 | 8047 | |
| . | Missing | 1118 | 9165 | |

# 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 38.9 | Range of Values | 8057 | 8057 | |
| . | Missing | 1108 | 9165 | |

# 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 | 185 | 185 | |
| 1 | Below lower detection limit | 7872 | 8057 | |
| . | Missing | 1108 | 9165 | |

# 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 23.1 | Range of Values | 8057 | 8057 | |
| . | Missing | 1108 | 9165 | |

# 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 | 7642 | 7642 | |
| 1 | Below lower detection limit | 415 | 8057 | |
| . | Missing | 1108 | 9165 | |

# 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 7.13 | Range of Values | 8057 | 8057 | |
| . | Missing | 1108 | 9165 | |

# 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 | 875 | 875 | |
| 1 | Below lower detection limit | 7182 | 8057 | |
| . | Missing | 1108 | 9165 | |

# 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 |
|---|---|---|---|---|
| 0.0707 to 59.7 | Range of Values | 8055 | 8055 | |
| . | Missing | 1110 | 9165 | |

# 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 | 8053 | 8053 | |
| 1 | Below lower detection limit | 2 | 8055 | |
| . | Missing | 1110 | 9165 | |