Blood levels of folate over time, current US levels, and differences between assessment methods

Christine M Pfeiffer, PhD
Chief, Nutritional Biomarkers Branch

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May 11-12, 2015 in Bethesda, MD
Disclosure

Nothing to disclose
Presentation outline

- Assays for serum and red blood cell (RBC) folate
  - Brief overview of available methods
  - Issues with (lack of) comparability of data
  - Issues with cutoff values

- Serum and RBC folate status pre- vs. post-fortification

- Current US blood folate levels
  - Post-fortification concentrations of serum and RBC folate
  - Post-fortification prevalence estimates of low blood concentrations
  - Post-fortification concentrations of serum folate forms, including unmetabolized folic acid (UMFA)
  - Factors associated with higher UMFA concentrations
ASSAYS FOR SERUM AND RBC FOLATE
Folate structure allows variations in three areas

Reduction to 7,8 dihydro- (DHF) and 5,6,7,8 tetrahydrofolate (THF)

Polyglutamation

para-Aminobenzoic acid (pABA)

Glutamic acid

para-Aminobenzoylglutamic acid (pABG)

Pteridine

<table>
<thead>
<tr>
<th>Coenzyme</th>
<th>Substituent at</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-MethylTHF</td>
<td>N-5 CH₃</td>
</tr>
<tr>
<td>5,10-MethenylTHF</td>
<td>=CH</td>
</tr>
<tr>
<td>5,10-MethyleneTHF</td>
<td>CH₂</td>
</tr>
<tr>
<td>5-FormylTHF</td>
<td>CHO</td>
</tr>
<tr>
<td>10-FormylTHF</td>
<td>CHO</td>
</tr>
<tr>
<td>THF</td>
<td>H H</td>
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</table>
Serum and RBC folate – 2012 UK NEQAS PT program

**Serum folate (nmol/L):**
- Range: 5.4-10.2
- Range: 13.7-20.8
- Range: 22.1-30.8

**Whole blood samples:**
- Range: 236-1660
- Range: 368-1409
- Range: 322-1192
# Main laboratory methods for serum and RBC folate

<table>
<thead>
<tr>
<th>Method type</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
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</table>
| Microbiologic assay (MBA) for total folate | • Small sample volume  
• Inexpensive  
• Suited for low resource setting  
• Measures all biologically active forms approximately equally (however, calibration with 5-methylTHF generates lower results than calibration with folic acid) | • Relatively laborious and takes 2 days to result  
• Replicates needed due to higher imprecision  
• Multiple dilutions needed due to limited linear range  
• Inhibited by presence of antibiotics or antifolates |
| Competitive protein binding assay (CPBA) for total folate | • User friendly and minimum operator involvement  
• High sample throughput  
• Suited for clinical setting  
• Generally good precision (~5%) | • Questionable accuracy due to different affinities of folate forms to FBP  
• Less suited for long-term studies due to potential lot-to-lot variability  
• Matrix effects likely with sample dilution |
| Chromatography-based assay for folate (various detectors; recently MS/MS) | • Information on folate vitamers  
• Highly selective and specific  
• Good analytical sensitivity and precision  
• Suited for research setting | • Expensive instrumentation and technical service, experienced operator  
• Complex sample extraction/clean-up  
• Conversion of polyglutamates to monoglutamates needed for whole blood  
• Summation of folate forms to total folate |

*Pfeiffer et al. – Folate methods chapter in Bailey’s Folate in Health and Disease, 2nd ed.*
# Folate methods and data in NHANES

<table>
<thead>
<tr>
<th>NHANES</th>
<th>Serum folate</th>
<th>RBC folate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre-fortification</strong></td>
<td></td>
<td></td>
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<tr>
<td><strong>Post-fortification</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1999-2000</td>
<td>BioRad RIA</td>
<td>BioRad RIA</td>
</tr>
<tr>
<td>2001-2002</td>
<td>BioRad RIA</td>
<td>BioRad RIA</td>
</tr>
<tr>
<td>2003-2004</td>
<td>BioRad RIA</td>
<td>BioRad RIA</td>
</tr>
<tr>
<td>2005-2006</td>
<td>BioRad RIA</td>
<td>BioRad RIA</td>
</tr>
<tr>
<td>2007-2008</td>
<td>MBA (LC-MS/MS)</td>
<td>MBA</td>
</tr>
<tr>
<td>2009-2010</td>
<td>MBA</td>
<td>MBA</td>
</tr>
<tr>
<td>2011-2012</td>
<td>LC-MS/MS</td>
<td>MBA</td>
</tr>
<tr>
<td>2013-2014</td>
<td>LC-MS/MS</td>
<td>MBA</td>
</tr>
<tr>
<td>2015-2016</td>
<td>LC-MS/MS</td>
<td>MBA</td>
</tr>
</tbody>
</table>

Converted to MBA-equivalent data
*Pfeiffer et al. J Nutr 2012*

MBA approx equivalent to LC-MS/MS for serum folate
*Fazili et al. Clin Chem 2007*
*Yetley et al. AJCN 2011*
Folate methods and data in the literature

- MBA, CPBA, chromatography-based—little information on how the various methods compare at any given point in time
- MBA:
  - Limited number of labs using this assay
  - Assay results may vary depending on microorganism (antibiotic-resistant vs. wild type) and calibrator (folic acid vs. 5-methylTHF vs. 5-formylTHF)
  - Most comprehensive comparison data: Pfeiffer et al. J Nutr 2011
  - 2015 CDC Folate Round Robin for microbiologic assay labs
- CPBA:
- LC-MS/MS:
  - No comparison data available
  - 2015 CDC Serum Folate Round Robin for LC-MS/MS labs
Cutoff values for “abnormal” folate levels

**“Low” folate**
- Clinical deficiency: Megaloblastic anemia
- Negative balance: Serum folate < 3 ng/mL
- Onset of depletion: RBC folate < 160 ng/mL
- Deficient erythropoiesis: RBC folate < 120 ng/mL
- Deficient anemia: RBC folate < 100 ng/mL
- Lack of megaloblastic changes: RBC folate > 140 ng/mL

**“High” folate**
- Tissue deficiency: Increased tHcy
- Insufficiency: Neural-tube defects
- Low risk: Serum folate < 4 ng/mL, RBC folate < 151 ng/mL
- Lowest risk: RBC folate > 400 ng/mL
- No specific health outcome
- Serum folate > 20 ng/mL

MBA: chloramphenicol-resistant microorganism calibrated with 5-methylTHF

MBA: wild-type microorganism

BioRad radio protein-binding assay

MBA: chloramphenicol-resistant microorganism calibrated with folic acid
SERUM AND RBC FOLATE STATUS
PRE- VS. POST-FORTIFICATION

Fortification begins in 1998

Red blood cell folate*, ng/mL


Mexican American
Non-Hispanic black
Non-Hispanic white

*BioRad data

CDC’s 2012 Second National Nutrition Report at www.cdc.gov/nutritionreport
Post-fortification serum and RBC folate concentrations were ~2.5x and 1.5x pre-fortification concentrations, respectively.

* MBA-equivalent data

Pfeiffer et al. J Nutr 2012
Higher serum and RBC folate 95th percentile concentrations for supplement users vs. non-users

* MBA-equivalent data

Pfeiffer et al. J Nutr 2012
Relationship between blood folate concentrations and total vitamin intake quartiles in adult supplement users and non-users

NHANES 2003-2006

*BioRad data
CURRENT BLOOD FOLATE LEVELS

Folate status and concentrations of serum folate forms in the US population: National Health and Nutrition Examination Survey 2011–2

Christine M. Pfeiffer, Maya R. Sternberg, Zia Fazal, David A. Lacher, Minly Zhang, Clifford L. Johnson, Heather C. Hamner, Megan L. Bailey, Jeannette L. Ruder, Sedigheh Yamin, R. J. Berry and Elizabeth A. Yecley

1National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA, USA
2National Center for Health Statistics, Centers for Disease Control and Prevention, Hyattsville, MD, USA
3National Center for Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, Atlanta, GA, USA
4Office of Dietary Supplements, National Institutes of Health, Bethesda, MD, USA
5Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, USA

U.S. Women of Childbearing Age Who Are at Possible Increased Risk of a Neural Tube Defect-Affected Pregnancy Due to Suboptimal Red Blood Cell Folate Concentrations, National Health and Nutrition Examination Survey 2007 to 2012

Sarah C. Tinker, Heather C. Hamner, Yan Ping Qi, and Krista S. Crider
## Current folate status in the US population

**NHANES 2007 – 2012**

<table>
<thead>
<tr>
<th>Survey period</th>
<th>Serum folate (nmol/L)</th>
<th>RBC folate (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007 – 2008</td>
<td>39.5 (37.7 – 41.3)</td>
<td>1120 (1070 – 1160)</td>
</tr>
<tr>
<td>2009 – 2010</td>
<td>38.2 (37.2 – 39.3)</td>
<td>1040 (1010 – 1070)</td>
</tr>
<tr>
<td>2011 – 2012</td>
<td>41.4 (40.1 – 42.9)</td>
<td>1050 (1010 – 1090)</td>
</tr>
</tbody>
</table>

**WHO cutoff:**

<table>
<thead>
<tr>
<th>10 nmol/L</th>
<th>340 nmol/L</th>
<th>906 nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survey period</td>
<td>Serum folate &lt;13.7 nmol/L</td>
<td>RBC folate &lt;624 nmol/L</td>
</tr>
<tr>
<td>2007 – 2008</td>
<td>3.5%</td>
<td>7.6%</td>
</tr>
<tr>
<td>2009 – 2010</td>
<td>3.9%</td>
<td>9.4%</td>
</tr>
<tr>
<td>2011 – 2012</td>
<td>1.1%</td>
<td>9.0%</td>
</tr>
</tbody>
</table>

22.8% (women 12-49 y)

*Pfeiffer et al. Br J Nutr 2015*  
*Tinker et al. 2015*
Current blood folate concentrations
NHANES 2011-2012

- NHANES 2011-2012 provides the first data on serum folate forms for persons 1 y and older by demographic and selected physiologic and lifestyle variables
- Concentrations of 5-methylTHF (100%), UMFA (99.9%), MeFox (98.8%), and THF (85.2%) mostly detectable
- 5-FormylTHF (3.6%) and 5,10-methenylTHF (4.4%) rarely detected
- Contribution to total folate: 5-methylTHF (86.7%), UMFA (4%), non-methylfolate (4.7%), MeFox (4.5%)
- All biomarkers showed higher concentrations with recent folic acid-containing supplement use

Pfeiffer et al. Br J Nutr 2015
SERUM UNMETABOLIZED FOLIC ACID CONCENTRATIONS

UMFA
Serum UMFA contributed 4% to total folate, NHANES 2011-2012

Concentrations varied with age, sex, race-ethnicity, fasting status, eGFR, BMI, BSA, serum cotinine, alcohol intake, and folic acid supplement use

Pfeiffer et al. Br J Nutr 2015
Unmetabolized Folic Acid Is Detected in Nearly All Serum Samples from US Children, Adolescents, and Adults\textsuperscript{1-4}

Christine M. Pfeiffer,\textsuperscript{5} Maya R. Sternberg,\textsuperscript{5} Zia Fazili,\textsuperscript{5} Elizabeth A. Yetley,\textsuperscript{5} David A. Lacher,\textsuperscript{7} Regan L. Bailey,\textsuperscript{8} and Clifford L. Johnson\textsuperscript{9}

\textsuperscript{5}National Center for Environmental Health, CDC, Atlanta, GA; \textsuperscript{6}Office of Dietary Supplements, NIH, Bethesda, MD; and \textsuperscript{7}National Center for Health Statistics, CDC, Hyattsville, MD

- Prevalence of UMFA $>1$ nmol/L was 33% overall and 21% in fasting ($\geq 8$ h) adults in NHANES 2007-2008
- UMFA $>1$ nmol/L was largely but not entirely explained by fasting status and by total folic acid intake from diet and supplements

\textit{Pfeiffer et al. J Nutr 2015}
Summary

- Folate assays have not yet been standardized and results are not comparable across assays or laboratories
- Fortification has significantly increased blood folate levels in the US population
- Post-fortification prevalence of deficient blood folate levels is <10%
- Post-fortification blood folate levels have been fairly constant over a period of ~15 years
- Post-fortification detection of serum UMFA is nearly ubiquitous and concentrations >1 nmol/L are largely but not entirely explained by fasting status and by total folic acid intake from diet and supplements
Acknowledgments
Staff of the Nutritional Biomarkers Branch
Colleagues at NCHS/NHANES, NIH/ODS, and FDA
Questions?

For more information please contact Centers for Disease Control and Prevention

1600 Clifton Road NE, Atlanta, GA 30333
Telephone: 1-800-CDC-INFO (232-4636)/TTY: 1-888-232-6348
Visit: www.cdc.gov | Contact CDC at: 1-800-CDC-INFO or www.cdc.gov/info

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.