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

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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)

**Coenzyme**
- 5-MethylTHF
- 5,10-MethenylTHF
- 5,10-MethyleneTHF
- 5-FormylTHF
- 10-FormylTHF
- THF

**Substituents**
- N-5
- N-10
- CH₃
- =CH
- CH₂
- CHO
- CHO
- H
- H
Serum and RBC folate – 2012 UK NEQAS PT program

**Serum folate (nmol/L)**

- Range: 13.7-20.8
- Range: 5.4-10.2
- Range: 22.1-30.8

**RBC folate (nmol/L)**

- 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>
</thead>
</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 vitamins  
• 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>
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</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><strong>MBA</strong> (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
- Tissue deficiency: Increased tHcy
- Insufficiency: Neural-tube defects
- Lowest risk:
  - Serum folate <4 ng/mL
  - RBC folate <151 ng/mL

**“High” folate**
- No specific health outcome
- Serum folate >20 ng/mL

**CDC**
- 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

**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
SERUM AND RBC FOLATE STATUS 
PRE- VS. POST-FORTIFICATION

Fortification begins in 1998

*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. Pfeiffer1, Maya R. Sternberg2, Zia Fazil3, David A. Lacher2, Minly Zhang1, Clifford L. Johnson1, Heather C. Hamner4, Began L. Bailey3, Jeannine L. Radtke4, Sedigheh Yamin2, R. J. Berry2 and Elizabeth A. Yeeley5

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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. Tinker1, Heather C. Hamner2, Yan Ping Qi1, and Krista S. Crider1
# 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: 10 nmol/L, 340 nmol/L, 906 nmol/L

<table>
<thead>
<tr>
<th>Survey period</th>
<th>Serum folate &lt;13.7 nmol/L</th>
<th>RBC folate &lt;624 nmol/L</th>
<th>RBC folate &lt;748 nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007 – 2008</td>
<td>3.5%</td>
<td>7.6%</td>
<td></td>
</tr>
<tr>
<td>2009 – 2010</td>
<td>3.9%</td>
<td>9.4%</td>
<td>22.8% (women 12-49 y)</td>
</tr>
<tr>
<td>2011 – 2012</td>
<td>1.1%</td>
<td>9.0%</td>
<td></td>
</tr>
</tbody>
</table>

*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 year 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
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.
Unmetabolized Folic Acid Is Detected in Nearly All Serum Samples from US Children, Adolescents, and Adults

Christine M Pfeiffer, Maya R Sternberg, Zia Fazilk, Elizabeth A Yetley, David A Lacher, Regan L Bailey, and Clifford L Johnson

Prevalence of UMFA >1 nmol/L was 33% overall and 21% in fasting (≥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

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

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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.