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Applying inappropriate cutpoints leads to misinterpretation of folate status in the U.S. population[1–,5]

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Abstract

Background—Folate cutpoints for risk of deficiency vs. possible deficiency were originally derived differently (experimental vs. epidemiologic data) and their interpretation is different. Matching cutpoints derived from one assay with population-based data derived from another assay requires caution.

Objective—We assessed the extent of folate status misinterpretation using inappropriate cutpoints.

Methods—In the cross-sectional NHANES, serum and red blood cell (RBC) folate were first measured using a radioproteinbindingassay (RPBA, 1988–2006), then using a microbiologic assay (MBA, 2007–2010). We compared prevalence estimates for assay-matched (e.g., using RPBA cutpoint with RPBA data) and assay-mismatched (e.g., using MBA cutpoint with RPBA data) cutpoints for risk of deficiency based on megaloblastic anemia as a hematologic indicator in persons ≥4 y [e.g., <7 nmol/L serum folate, <305 nmol/L RBC folate, derived by MBA], possible deficiency based on rising homocysteine as a metabolic indicator in persons ≥4 y (e.g., <10 nmol/L serum folate, <340 nmol/L RBC folate, derived by RPBA), and insufficiency based on elevated risk of neural tube defects in women 12–49 y (e.g., <906 nmol/L RBC folate, derived by MBA).

Results—Pre-folic acid fortification (1988–1994), risk of deficiency for assay-matched vs. assay-mismatched cutpoints was 5.6% vs. 16% (serum folate) and 7.4% vs. 28% (RBC folate); it declined post-fortification (1999–2006) to <1% vs. <1% (serum folate) and <1% vs. 2.5% (RBC

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[5]Running head: Folate cutpoints

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[2]The findings and conclusions in this manuscript are those of the authors and do not necessarily represent the official views or positions of the Centers for Disease Control and Prevention/Agency for Toxic Substances and Disease Registry, the National Institutes of Health, or the Department of Health and Human Services. One author is a staff member of the World Health Organization; the author alone is responsible for the views expressed in this article and they do not necessarily represent the decisions, policy or views of the World Health Organization.

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folate). Pre-fortification (1988–1994), risk of possible deficiency for assay-matched vs. assay-mismatched cutpoints was 35% vs. 56% (serum folate) and 37% vs. 84% (RBC folate); it declined post-fortification (1999–2006) to 1.9% vs. 7.0% (serum folate) and 4.8% vs. 53% (RBC folate). Post-fortification (2007–2010), risk of insufficiency was 23% (assay-matched) vs. 39% (assay-mismatched).

Conclusions—Applying assay-mismatched cutpoints leads to misinterpretation of folate status. This likely applies to clinical assays as no comparability data are available.

Keywords

NHANES; deficiency; insufficiency; microbiologic assay; radioproteinbindingassay

Introduction

Low folate status has been associated with increased risk of adverse health outcomes from megaloblastic anemia and hyperhomocysteinemia to elevated risk for a neural tube birth defect affected pregnancy (1). Using appropriate cutpoints is essential to correctly assess folate status (2). The World Health Organization (WHO)[6] summarized historical information on serum and red blood cell (RBC) folate concentrations for assessing folate status in populations (3). A recent folate review stated that the inconsistent use of cutpoints over time has led to scientific confusion (4). Earlier reports concluded that because of large assay differences, method-specific reference ranges should be used (5,6). This is still true today as can ben seen from proficiency testing data for clinical assays. The 2015 College of American Pathologists Ligand Survey shows large within-platform (~7–30% and ~10–50% for serum and RBC folate, respectively) and even larger across-platform variability (~1.5- to 6-fold and ~8- to 40-fold differences for serum and RBC folate, respectively) (*unpublished results, 2016*). We know how folate assays used in the National Health and Nutrition Examination Survey (NHANES) compare (7) and can calculate assay-adjusted cutpoints; however, this type of information is lacking for commonly used clinical assays, resulting in an inability to appropriately compare study data produced with different clinical assays or interpret patient data.

The 1998 Institute of Medicine (IOM) Dietary Reference Intakes report (1) and WHO guidelines (3,8,9) are primary sources for folate cutpoints (Table 1). Folate depletion experiments using a microbiologic assay [wild-type microorganism and folic acid calibrator, MBA-1, (10)] defined stages of deficiency, with megaloblastic anemia being the final stage of deficiency based on a hematologic indicator. Serum folate <7 nmol/L indicated negative balance (10). RBC folate <363, <272, and <227 nmol/L marked the onset of depletion, the beginning of deficient erythropoiesis, and megaloblastic anemia, respectively (10). An RBC folate cutpoint of <305 nmol/L indicating the appearance of hypersegmented neutrophils was commonly used (1,5). A 2005 WHO Technical Consultation recommended cutpoints for possible deficiency based on rising plasma homocysteine (Hcy) as a metabolic indicator

[6]Abbreviations: 5-methylTHF, 5-methyltetrahydrofolate; Hcy, plasma homocysteine; IOM, Institute of Medicine; MBA-1, microbiologic assay with wild-type microorganism and folic acid calibrator; MBA-2, microbiologic assay with chloramphenicol-resistant strain and folic acid calibrator; MBA-3, microbiologic assay with chloramphenicol-resistant strain and 5-methyltetrahydrofolate calibrator; NTD, neural tube defects; RBC, red blood cell; RPBA, radioproteinbindingassay

(serum folate <10 nmol/L and RBC folate <340 nmol/L) (11,12). These cutpoints were derived from cross-sectional NHANES 1988–1994 data using the BioRad radioproteinbindingassay (RPBA). In 2015, the WHO recommended a population cutpoint for folate insufficiency in women of reproductive age based on elevated risk of neural tube defects (NTD) [RBC folate <906 nmol/L, (9)] derived from epidemiologic data produced by a microbiologic assay [chloramphenicol-resistant strain and folic acid calibrator, MBA-2 (13)].

This study employs commonly used folate cutpoints derived experimentally or from epidemiologic data and cutpoint adjustments to obtain assay matching. We applied assay-matched (e.g., RPBA cutpoint with RPBA data) and assay-mismatched (e.g., MBA-1 cutpoint with RPBA data) cutpoints for different levels of folate status (risk of deficiency based on megaloblastic anemia, possible deficiency based on rising Hcy, and insufficiency based on elevated NTD risk) to serum and RBC folate data from persons ≥4 y participating in the NHANES 1988–2010, and compared resulting prevalence estimates and the extent of misinterpretation of folate status.

Materials and Methods

Participants and study design

The NHANES, conducted by the National Center for Health Statistics at the Centers for Disease Control and Prevention (CDC), collects nationally representative cross-sectional data on the health and nutritional status of the civilian non-institutionalized U.S. population using a stratified, multistage, probability sample design. Survey participants are first interviewed in their homes to collect information on demographic characteristics, health-related issues, and dietary supplement use. Participants then undergo a physical examination and a blood draw in a Mobile Examination Center about 1–3 wk after the household interview. All NHANES participants provided written informed consent, and all procedures were approved by the National Center for Health Statistics Research Ethics Review Board.

Biomarker measurement

The BioRad Quantaphase I RIA was used during 1988–1991 and the Quantaphase II during 1991–2006 to measure folate in serum and whole blood hemolysate samples for RBC folate determination (Bio-Rad Laboratories, Hercules, CA) (Supplemental Table 1). The Quantaphase I data were assay-adjusted prior to their public release to account for assay differences between the Quantaphase I and II (14). The CDC microbiologic assay using a chloramphenicol-resistant strain of *L. rhamnosus* and 5-methyltetrahydrofolate (5-methylTHF) for calibration (MBA-3) was used during 2007–2010 to measure folate in serum and whole blood hemolysate samples (15).

Study variables

This study used data from persons ≥4 y participating in NHANES 1988–2010. For sample sizes, see Supplemental Table 2. We used the demographic variables age, sex, and race-ethnicity, and categorized them as follows: age (4–11, 12–19, 20–39, 40–59, and ≥60 years), sex (males and females), and race-ethnicity (Mexican American, non-Hispanic Black, and

non-Hispanic White; other race-ethnicity groups were included in overall estimates). Women of reproductive age (12–49 y) were considered separately to assess elevated NTD risk. Use of any dietary supplement was ascertained from self-reported use over the past 30 d (yes and no; using data from the Dietary Supplement Questionnaire).

Statistics

Statistical analyses were performed using SAS (version 9, SAS Institute Inc., Cary, NC) and SUDAAN (version 9.2, RTI, Research Triangle Park, NC) software. We applied no a priori exclusion criteria to our data analysis and used pairwise deletion for missing values in a particular bivariate analysis. Mobile Examination Center statistical weights were used to account for differential nonresponse or noncoverage, and to adjust for oversampling of some groups. We assessed the proportion of individuals at different levels of folate status using 3 approaches. First, we calculated the weighted prevalence of low serum and RBC folate concentrations at various assay-matched (e.g., BioRad RIA cutpoint with BioRad RIA data) cutpoints using the *original data as measured* by the laboratory. Second, we applied various assay-mismatched (e.g., MBA-1 cutpoint with BioRad RIA data) cutpoints to the *original data as measured* by the laboratory. Third, we *adjusted the original data* and applied assay-matched cutpoints to calculate the weighted prevalence. We did not assess the fourth possibility of applying assay-mismatched cutpoints to adjusted data. To facilitate data review, we compiled the cutpoints used for each of these 3 approaches (Table 2) and summarized information on the assays underlying the cutpoints and the NHANES data (Table 3) in the same format as we later present the data. We used the MBA-1 cutpoints for risk of deficiency based on megaloblastic anemia with MBA-3 data and considered it to be likely assay-matched. We know of no regression equation that links the MBA-1 and MBA-3 assays and we consider these 2 assays to produce similar concentrations (see discussion). We “forward” adjusted the BioRad RIA data from the NHANES 1988–1994 and 1999–2006 to MBA-3 units (Supplemental Table 1; fractional polynomial regression for serum folate and linear regression for whole blood folate) using previously published regression equations (7) to have data for the full 1988–2010 time period in the same MBA-3 units. We also used these regression equations to calculate assay-adjusted MBA-3 cutpoints for risk of possible deficiency based on rising Hcy. When we adjusted the BioRad RIA data, we excluded serum folate data from 2 participants in 1988–1994 because their unadjusted concentrations were <1 nmol/L and the adjustment formula requires logarithmic transformation (which produces a negative number) and then calculating the square root. We also excluded RBC folate data for a small fraction (1–2%) of participants in 1988–1994 (558 of 23404) and 1999–2006 (469 of 31278) because the RBC folate adjustment formula requires serum folate, RBC folate, and hematocrit, and for these participants 1 of these tests was missing. To provide a visual representation of where in the distribution the cutpoints lie, we generated cumulative frequency distribution curves of original and adjusted serum and RBC folate concentrations for the 3 time periods (1988–1994, 1999–2006, and 2007–2010) (Supplemental Figure 1). Regression equations showing the relationships between 3 microbiologic assays used presently [MBA-2 (13), MBA-3 (15), and a variation of MBA-1 calibrated with 5-formyl-tetrahydrofolate (19)], while beyond the scope of this paper, are often required by researchers to interpret published data (Supplemental Text 1).

Results

Cutpoint adjustments to achieve assay matching

The folate cutpoints published by the IOM and WHO have to be assay-matched when used with NHANES data to assess long-term temporal trends (Table 1). The cutpoints for risk of deficiency based on megaloblastic anemia as a hematologic indicator (<7 nmol/L serum folate and <305 nmol/L RBC folate) were mainly derived from MBA-1 data and cannot be used directly with BioRad RPBA data from the NHANES 1988–1994 and 1999–2006. An earlier report (14) published a regression equation to convert BioRad RPBA Quantaphase I to Quantaphase II data based on results from nearly 1800 serum samples and showed that the Quantaphase II assay measured ~35% lower. Because the Quantaphase I assay was originally calibrated to the MBA-1, the investigators used the same regression equation to calculate adjusted cutpoints for Quantaphase II data (<5 nmol/L serum folate and <215 nmol/L RBC folate) (14,16).

The WHO cutpoints for risk of possible deficiency based on rising Hcy as a metabolic indicator (<10 nmol/L serum folate and <340 nmol/L RBC folate) were derived from pre-fortification BioRad RPBA data (3,11,12). These cutpoints can be used directly with data from the NHANES 1988–1994 and 1999–2006, however adjusted cutpoints have to be used with MBA-3 data from the NHANES 2007–2010 (3). The MBA-3 measured ~40% (serum) and 80% (RBC) higher than the BioRad RPBA (7) and the adjusted MBA-3 cutpoints were <14 nmol/L serum folate and <624 nmol/L RBC folate.

Finally, the WHO cutpoint for insufficiency based on elevated NTD risk in women of reproductive age (<906 nmol/L RBC folate) was derived from MBA-2 data [assay calibrated with folic acid (13)] and cannot be used directly with NHANES data. The MBA-3 assay used in the NHANES 2007–2010 (15) was calibrated with 5-methylTHF, which –compared to calibration with folic acid– produced higher microorganism growth response (i.e., higher calibration curve) and ~25% lower folate concentrations in patient samples (17). The adjusted MBA-3 cutpoint was <748 nmol/L RBC folate (18).

Risk of folate deficiency based on hematologic indicator using original data

Risk of deficiency based on megaloblastic anemia for assay-matched cutpoints (e.g., BioRad RPBA cutpoint with BioRad RPBA data) was 5.6% (serum folate) and 7.4% (RBC folate) during pre-folic acid fortification (1988–1994); it declined post-fortification (1999–2006 and 2007–2010) to <1% (serum and RBC folate) (Table 4). The same pre- to post-fortification pattern, but slightly different magnitude, was observed for different age and race-ethnicity groups, males and females, and supplement users and nonusers (Table 5). When we used assay-mismatched cutpoints (e.g., MBA-1 cutpoint with BioRad RIA data), risk of folate deficiency based on megaloblastic anemia was overestimated during the pre-fortification period (16% for serum folate and 28% for RBC folate) and slightly overestimated for RBC folate during the 1999–2006 post-fortification period (2.5%) (Table 4). Estimates by population subgroup varied to the greatest extent by age group in the pre-fortification period: 1.3% for 4–11 y olds vs. 23% for 20–39 y olds for serum folate; 12% for 4–11 y olds vs. 39% for 12–19 y olds for RBC folate (Supplemental Table 3).

Risk of possible folate deficiency based on metabolic indicator using original data

When we used assay-matched cutpoints, risk of possible deficiency based on rising Hcy declined from 35% (serum folate) and 37% (RBC folate) pre-fortification to 1.9% (1999–2006) and 4.2% (2007–2010) for serum folate and 4.8% (1999–2006) and 8.6% (2007–2010) for RBC folate post-fortification (Table 4). As noted with risk of deficiency based on megaloblastic anemia, we observed the same pre- to post-fortification patterns for different demographic groups and supplement users and nonusers (Table 6). When we used assay-mismatched cutpoints, risk of possible folate deficiency based on rising Hcy was overestimated during 1988–1994 (56% for serum folate and 84% for RBC folate) and 1999–2006 (7.0% for serum folate and 53% for RBC folate) and underestimated during 2007–2010 (<1% for serum and RBC folate) (Table 4). Estimates by population subgroup varied to the greatest extent by age group in the early post-fortification period (1999–2006): 0.3% for 4–11 y olds vs. 10% for 20–39 y olds for serum folate; 32% for ≥60 y olds vs. 72% for 12–19 y olds for RBC folate (Supplemental Table 4).

Risk of folate insufficiency based on elevated NTD risk using original data

During 2007–2010, elevated NTD risk in women of reproductive age (12–49 y) was 23% vs. 39% when we used an assay-matched vs. assay-mismatched cutpoint, respectively (Table 4). In both cases, estimates varied by race-ethnicity and supplement use status, but the pre- to post-fortification patterns were consistent with our observation in the total population (Supplemental Table 5).

Risk of folate deficiency and possible deficiency using assay-adjusted data

When we used adjusted data (i.e., applied regression equations to BioRad RIA data to adjust it to MBA-3 units) and applied MBA-1 cutpoints (Table 4), we observed different estimates for risk of deficiency based on megaloblastic anemia during the pre-fortification period compared to using original BioRad RIA data and adjusting the MBA-1 cutpoint to BioRad units (16% vs. 5.6% for serum folate and 2.0% vs. 7.4% for RBC folate). During the post-fortification period (1999–2006), we observed no difference in prevalence between these 2 approaches (<1% for serum and RBC folate). Similarly, the estimates for risk of possible deficiency based on rising Hcy were comparable between the 2 approaches both for the pre-fortification (1988–1994; 37% vs. 35% for serum folate and 37% vs. 37% for RBC folate) and post-fortification period (1999–2006; 2.2% vs. 1.9% for serum folate and 4.7% vs. 4.8% for RBC folate). Estimates by population subgroup varied to the greatest extent by age group in the pre-fortification period: 1.1% for 4–11 y olds vs. 22% for 20–39 y olds for serum folate risk of deficiency based on megaloblastic anemia (Supplemental Table 6); 8.7% for 4–11 y olds vs. 48% for 20–39 y olds for serum folate risk of possible deficiency based on rising Hcy (Supplemental Table 7). Risk of folate insufficiency based on elevated NTD risk varied by race-ethnicity both pre- and post-fortification, but by supplement use only post-fortification (Supplemental Table 8).

Discussion

This paper provides information on commonly used folate cutpoints derived from experimental or epidemiologic data and on adjustments to achieve assay matching, making it

an important tool for researchers and public health program and policy officials to evaluate population folate status. It also provides “correct” prevalence estimates for 3 levels of folate status by population subgroup (Tables 5–7). Using assay-mismatched cutpoints led to misinterpretation of folate status. The extent of misinterpretation depended largely on the presence of low folate concentrations in the population and was therefore different in the pre- compared to post-fortification periods and in different population subgroups. It appeared that subgroups with low prevalence of low folate concentrations (e.g., children 4–11 y of age) were more sensitive to misinterpretation. While this paper does not address the extent of misinterpretation at the individual level in a clinical setting or with other laboratory-developed assay, clearly there is a need for more research in this area.

Some previous reports used assay-mismatched cutpoints (20–25), sometimes intentionally because using assay-matched cutpoints with post-fortification NHANES data produced very low prevalence estimates for risk of deficiency based on megaloblastic anemia (small cell size) (23). Nonetheless, the use of assay-mismatched cutpoints may have been why prevalence estimates for serum folate were different from those for RBC folate (24). In our report, risk of deficiency was similar for both biomarkers pre-fortification (serum folate: 5.6%; RBC folate: 7.4%) and post-fortification (serum and RBC folate: <1%) when we used assay-matched cutpoints. Risk of possible deficiency based on rising Hcy was also similar for both biomarkers pre-fortification (serum folate: 35%; RBC folate: 37%) and of similar magnitude post-fortification (serum folate: 4.2%; RBC folate: 8.6%). Risk of insufficiency based on elevated NTD risk in women of reproductive age can only be interpreted using RBC folate; it was 23% during the 2007–2010 post-fortification period.

Several expert panels previously discussed the appropriateness of commonly used cutpoints to estimate the prevalence of low folate status (1,5,8,9,12,14). Some of those issues have been summarized (2), and a recent review article discussed general issues related to cutpoints for nutritional biomarkers (26). Because a cutpoint of adequacy should discriminate accurately (with few misclassification errors) between healthy and at-risk groups, it is preferred that cutpoints are identified and/or validated through clinical trials using accurate assays (2,26). The folate cutpoints for risk of deficiency based on megaloblastic anemia were derived from experimental data (limited sample size) and therefore most closely align with this requirement. However, this is not the case for the cutpoints for risk of possible deficiency based on rising Hcy, which were derived from cross-sectional pre-fortification NHANES data. A validation of these cutpoints through experimental data is lacking, and thus any resulting prevalence estimates should be interpreted with caution.

Another reason that cutpoints for risk of possible deficiency based on rising Hcy should be used with caution is the underlying assay (BioRad RPBA). For whole blood samples, the relationship between the BioRad RPBA and the MBA-3 depends on *MTHFR C677T* genotype, overestimating RBC folate concentrations in persons with *T/T* genotype and underestimating concentrations in persons with *C/C* genotype (27). However, *MTHFR C677T* genotype information is often not available, necessitating the use of an “all-genotype” rather than “genotype-specific” regression equations to adjust the data (7). Issues of inaccurate RBC folate concentrations also appear to be present in other protein binding

assays (28). Lastly, the BioRad RPBA was discontinued ~10 y ago and, to our knowledge, has not been used in other national nutrition surveys. Information on how the BioRad RPBA compares to assays used in other nutrition surveys is missing, making it impossible to suggest appropriate adjustment factors. All of these issues limit the implementation of the WHO cutpoints for possible deficiency based on rising Hcy internationally and raise questions for the U.S. data (1988–2006), specifically for RBC folate. Given both the non-experimental study design limitation and the known biases of the biomarker assay used, it is important to raise these points, particularly because some investigators are solely relying on the WHO cutpoints to interpret population folate status.

The idea that adjusting the data as compared to adjusting the cutpoint should make no difference in prevalence was indeed what we observed for risk of possible deficiency based on rising Hcy both pre- and post-fortification, where we used the same regression equations to either adjust the data or the cutpoints. However, it was not the case for risk of deficiency based on megaloblastic anemia pre-fortification (~3-fold over- and underestimation for serum and RBC folate, respectively), where we adjusted the data to the MBA-3, but used MBA-1 cutpoints. The regression equations that related the BioRad RPBA to the MBA-3 were generated using serum and whole blood samples collected in the post-fortification period (27,29), when folate concentrations were much higher than during pre-fortification. It is unclear whether the regression equations appropriately characterized the relationship between these 2 assays at low folate concentrations, particularly for serum folate where we applied a non-linear (fractional polynomial) equation.

A likely explanation for the discrepancy observed with RBC folate could be that the adjusted RBC folate cutpoint for risk of deficiency based on megaloblastic anemia for the BioRad RPBA (<215 nmol/L) may not be perfect because the regression equation was generated using serum samples (14). Thus, our pre-fortification prevalence using an assay-matched cutpoint and original data (7.4%) may not be accurate, and the comparison to the prevalence using adjusted data (2.0%) may not be warranted.

A third explanation could be that using MBA-1 cutpoints with MBA-3 data is not truly “assay-matched”. It is not certain how the “traditional” MBA-1, used to generate the IOM cutpoints for risk of deficiency based on megaloblastic anemia, related to the current MBA-3. The BioRad RPBA Quantaphase II to I downward adjustment was ~35% and the Quantaphase I assay was supposedly producing comparable results to the MBA-1 (14). The Quantaphase II assay produced ~30% (serum folate) and ~45% (RBC folate) lower concentrations than the MBA-3 (7). In a circular way this suggests that the “traditional” MBA-1 performed similarly to the current MBA-3. Thus, using the IOM cutpoints for risk of deficiency with MBA-3 measured data should be acceptable.

A minor challenge related to folate cutpoints is the use of different units (i.e., ng/mL vs. nmol/L) (26). The literature is inconsistent about the use of conversion factors (e.g., 2.266 is typically used, based on molecular weight of folic acid; 2.178 is sometimes used, based on molecular weight of 5-methylTHF) and whether to round the cutpoint (e.g., 6.8 vs. 7 nmol/L serum folate; 226.5 vs. 227 nmol/L RBC folate). Cutpoints that fall on the tail of a distribution can produce very different prevalence estimates even with minor changes in the

cutpoint. To avoid giving inappropriate implied precision, we rounded adjusted cutpoints to integers (Table 1).

In summary, our work serves to re-evaluate the folate status of the U.S. population pre- and post-fortification by applying our best understanding about folate cutpoints and describing their limitations. The results showed a great potential for misclassification when using inappropriate cutpoints, particularly at lower folate concentrations present during the pre-fortification time period. Given the importance of folate status and discussions about folic acid fortification, these findings are relevant nationally and internationally. Even though some questions remain, we can provide answers regarding the use of appropriate cutpoints in NHANES. However, our findings cannot be generalized to data from clinical assays or from laboratory-developed tests because the relationship of those assays to assays underlying cutpoints is unknown. There is a need for standardization of folate assays (30). Additionally, the overreliance on dichotomous cutpoints has limitations (26) beyond the ones discussed here and exploring alternative approaches to categorize population folate status, such as the use of multiple risk categories (18), would be beneficial.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Commonly used cutpoints to assess folate status and their assay-adjusted equivalents[1]

Type of deficiency Matrix	Published cutpoint, nmol/L (*Reference*)[2]	Assay for cutpoint[3]	Type of data	Interpretation	Adjusted cutpoint, nmol/L (*Reference*)[4,5]	Target assay[6]
Deficiency based on hematologic indicator						
Serum	<7 (1,3)	MBA-1	Experimental	Negative folate balance	<5 (14)	BioRad RPBA
RBC	<305 (1)	MBA-1	Experimental	Appearance of hypersegmented neutrophils[7]	<215 (16)	BioRad RPBA
RBC	<227 (3)	MBA-1	Experimental	Megaloblastic anemia	<156 (14)	BioRad RPBA
Possible deficiency based on metabolic indicator						
Serum	<10 (3)	BioRad RPBA	Epidemiologic	Rising homocysteine	<14 (7)[8]	MBA-3
RBC	<340 (3)	BioRad RPBA	Epidemiologic	Rising homocysteine	<624 (7)[8]	MBA-3
Insufficiency based on elevated neural tube defects risk						
RBC	<906 (9)	MBA-2	Epidemiologic	Limited to WRA at the population level	<748 (17,18)	MBA-3

[1] BioRad RPBA, radioproteinbindingassay; MBA-1, microbiologic assay, wild-type microorganism and folic acid calibrator; MBA-2, microbiologic assay, chloramphenicol-resistant strain and folic acid calibrator; RBC, red blood cell; WRA, women of reproductive age

[2] Cutpoint values expressed in conventional units (ng/mL) are as follows: deficiency: 3 (serum), 140 (RBC), and 100 (RBC); possible deficiency: 4 (serum) and 151 (RBC); insufficiency: 400 (RBC)

[3] Assay used to generate folate results on which cutpoint was based

[4] Cutpoint values expressed in SI units (nmol/L) shown in table have been rounded to integers to avoid giving inappropriate implied precision: deficiency: 4.5 rounded to 5 (serum), 215.3 rounded to 215 (RBC), and 156.4 rounded to 156 (RBC); possible deficiency: 13.7 rounded to 14 (serum) and 623.6 rounded to 624 (RBC); insufficiency: 747.8 rounded to 748 (RBC)

[5] Cutpoint values expressed in conventional units (ng/mL) are as follows: deficiency: 2 (serum), 95 (RBC), and 69 (RBC); possible deficiency: 6 (serum) and 275 (RBC); insufficiency: 330 (RBC)

[6] Assay to which adjusted cutpoint is applied: MBA-3, microbiologic assay, chloramphenicol-resistant strain and 5-methyltetrahydrofolate calibrator

[7] Lack of megaloblastic changes in subjects with RBC folate >140 ng/mL

[8] Reference provides regression equations to calculate adjusted cutpoints

Table 2

Assay-matched and assay-mismatched cutpoints for risk of folate deficiency, possible deficiency, or insufficiency applied to original or adjusted data from participants aged ≥4 y in the pre-fortification (1988–1994) and post-fortification (1999–2010) NHANES[1]

Type of deficiency	Serum folate			RBC[2] folate		
Cutpoint; data	**1988–1994**	**1999–2006**	**2007–2010**	**1988–1994**	**1999–2006**	**2007–2010**
Deficiency based on hematologic indicator (megaloblastic anemia)						
Assay-matched; original	<5[3]	<5[3]	<7	<215[3]	<215[3]	<305
Assay-mismatched; original	<7	<7	<5[3]	<305	<305	<215[3]
Assay-matched; adjusted	<7	<7	n.d.	<305	<305	n.d.
Possible deficiency based on metabolic indicator (rising homocysteine)						
Assay-matched; original	<10	<10	<14[3]	<340	<340	<624[3]
Assay-mismatched; original	<14[3]	<14[3]	<10	<624[3]	<624[3]	<340
Assay-matched; adjusted	<14[3]	<14[3]	n.d.	<624[3]	<624[3]	n.d.
Insufficiency based on elevated neural tube defects risk[4]						
Assay-matched; original	n/a	n/a	n/a	n.d.	n.d.	<748[3]
Assay-mismatched; original	n/a	n/a	n/a	<906	<906	<906
Assay-matched; adjusted	n/a	n/a	n/a	<748[3]	<748[3]	n.d.

[1] Original data for 1988–2006 were generated with the BioRad radioproteinbindingassay; original data for 2007–2010 were generated with the CDC microbiologic assay (MBA-3; chloramphenicol-resistant strain and 5-methyltetrahydrofolate calibrator)

[2] RBC, red blood cell

[3] Cutpoint adjusted

[4] Limited to women 12–49 y of age; n/a, not applicable for serum folate

n.d., not determined for 1988–1994 and 1999–2006 because no BioRad cutpoint is available for folate insufficiency; not determined for 2007–2010 because MBA-3 data were not adjusted

Table 3

Assay underlying each cutpoint and each NHANES data set to determine risk of folate deficiency, possible deficiency, or insufficiency for participants aged ≥4 y in the pre-fortification (1988–1994) and post-fortification (1999–2010) NHANES[1]

Type of deficiency	Assay for cutpoint/assay for NHANES data		
Cutpoint; data	**1988–1994**	**1999–2006**	**2007–2010**
Deficiency based on hematologic indicator (megaloblastic anemia)			
Assay-matched; original	$RPBA_{Adj}$/RPBA	$RPBA_{Adj}$/RPBA	MBA-1/MBA-3
Assay-mismatched; original	MBA-1/RPBA	MBA-1/RPBA	$RPBA_{Adj}$/MBA-3
Assay-matched; adjusted	MBA-1/$MBA\text{-}3_{Adj}$	MBA-1/$MBA\text{-}3_{Adj}$	n.d.
Possible deficiency based on metabolic indicator (rising homocysteine)			
Assay-matched; original	RPBA/RPBA	RPBA/RPBA	$MBA\text{-}3_{Adj}$/MBA-3
Assay-mismatched; original	$MBA\text{-}3_{Adj}$/RPBA	$MBA\text{-}3_{Adj}$/RPBA	RPBA/MBA-3
Assay-matched; adjusted	$MBA\text{-}3_{Adj}$/$MBA\text{-}3_{Adj}$	$MBA\text{-}3_{Adj}$/$MBA\text{-}3_{Adj}$	n.d.
Insufficiency based on elevated neural tube defects risk[4]			
Assay-matched; original	n.d.	n.d.	$MBA\text{-}3_{Adj}$/MBA-3
Assay-mismatched; original	MBA-2/RPBA	MBA-2/RPBA	MBA-2/MBA-3
Assay-matched; adjusted	$MBA\text{-}3_{Adj}$/$MBA\text{-}3_{Adj}$	$MBA\text{-}3_{Adj}$/$MBA\text{-}3_{Adj}$	n.d.

[1]Subscript "Adj" indicates that the cutpoint or the NHANES data were adjusted, while no subscript indicates that the published cutpoint or original NHANES data were used; MBA-1, microbiologic assay, wild-type microorganism and folic acid calibrator; MBA-2, microbiologic assay, chloramphenicol-resistant strain and folic acid calibrator; MBA-3; chloramphenicol-resistant strain and 5-methyltetrahydrofolate calibrator; RPBA, radioproteinbindingassay

n.d., not determined for 1988–1994 and 1999–2006 because no BioRad cutpoint is available for folate insufficiency; not determined for 2007–2010 because MBA-3 data were not adjusted

Table 4

Prevalence for risk of folate deficiency, possible deficiency, or insufficiency for the total population using assay-matched or assay-mismatched cutpoints and original or assay-adjusted data from participants aged ≥4 y in the pre-fortification (1988–1994) and post-fortification (1999–2010) NHANES[1]

Type of deficiency	Serum folate			RBC[2] folate		
Cutpoint; data	**1988–1994**	**1999–2006**	**2007–2010**	**1988–1994**	**1999–2006**	**2007–2010**
Deficiency based on hematologic indicator (megaloblastic anemia)						
Assay-matched; original	5.6 (4.8, 6.5)[3]	0.1 (0.0, 0.1)*[3]	0.1 (0.1, 0.2)	7.4 (6.5, 8.4)[3]	0.2 (0.2, 0.3)[3]	0.1 (0.1, 0.3)*
Assay-mismatched; original	16 (15, 18)	0.4 (0.3, 0.5)	<1†[3]	28 (26, 30)	2.5 (2.2, 2.8)	<1†[3]
Assay-matched; adjusted	16 (14, 17)	0.3 (0.3, 0.5)	n.d.	2.0 (1.6, 2.4)	0.1 (0.0, 0.1)	n.d.
Possible deficiency based on metabolic indicator (rising homocysteine)						
Assay-matched; original	35 (33, 38)	1.9 (1.7, 2.1)	4.2 (3.7, 4.8)[3]	37 (35, 39)	4.8 (4.3, 5.3)	8.6 (7.7, 9.5)[3]
Assay-mismatched; original	56 (53, 58)[3]	7.0 (6.4, 7.5)[3]	0.7 (0.5, 1.0)	84 (82, 85)[3]	53 (51, 55)[3]	0.2 (0.1, 0.3)
Assay-matched; adjusted	37 (35, 40)[3]	2.2 (1.9, 2.4)[3]	n.d.	37 (35, 39)[3]	4.7 (4.2, 5.2)[3]	n.d.
Insufficiency based on elevated neural tube defects risk[3]						
Assay-matched; original	n/a	n/a	n/a	n.d.	n.d.	23 (21, 25)[3]
Assay-mismatched; original	n/a	n/a	n/a	97 (96, 98)	89 (87, 90)	39 (36, 41)
Assay-matched; adjusted	n/a	n/a	n/a	59 (56, 63)[3]	15 (14, 17)[3]	n.d.

[1] Estimates are percentages (95% CI); for cutpoints used, see Table 2; for sample sizes, see Supplemental Table 2; "other" race-ethnicity group (persons with multi-ethnic background) is included in these total estimates; original data for 1988–2006 were generated with the BioRad radioproteinbindingassay; original data for 2007–2010 were generated with the CDC microbiologic assay (MBA-3; chloramphenicol-resistant strain and 5-methyltetrahydrofolate calibrator)

[2] RBC, red blood cell

[3] Cutpoint adjusted

[4] Limited to women 12–49 y of age; n/a, not applicable for serum folate

n.d., not determined for 1988–1994 and 1999–2006 because no BioRad cutpoint is available for folate insufficiency; not determined for 2007–2010 because MBA-3 data were not adjusted

* 30% ≤ relative standard error <40%

† Estimate suppressed, relative standard error ≥40%

Table 5

Prevalence for risk of folate deficiency by population subgroup using assay-matched cutpoints and original data from participants aged ≥4 y in the pre-fortification (1988–1994) and post-fortification (1999–2010) NHANES[1]

Population group	Serum folate cutpoint and time period			RBC folate cutpoint and time period		
	<5 nmol/L[2] 1988–1994	<5 nmol/L[2] 1999–2006	<7 nmol/L[3] 2007–2010	<215 nmol/L[2] 1988–1994	<215 nmol/L[2] 1999–2006	<305 nmol/L[3] 2007–2010
Age group						
4–11 y	<1[†]	<1[†]	<1[†]	1.5 (1.1, 2.2)	<1[†]	<1[†]
12–19 y	4.9 (3.8, 6.5)	<1[†]	<1[†]	12 (9.5, 14)	0.2 (0.1, 0.3)	<1[†]
20–39 y	8.7 (7.2, 10)	<1[†]	<1[†]	8.9 (7.6, 10)	0.2 (0.1, 0.4)	<1[†]
40–59 y	5.8 (4.9, 6.9)	<1[†]	<1[†]	8.0 (6.7, 9.4)	0.4 (0.2, 0.7)	<1[†]
≥60 y	2.8 (2.2, 3.8)	<1[†]	<1[†]	4.5 (3.8, 5.4)	0.2 (0.1, 0.3)	<1[†]
Sex						
Male	5.8 (4.8, 7.1)	<1[†]	0.1 (0.0, 0.2)[*]	6.6 (5.5, 7.8)	0.2 (0.2, 0.4)	<1[†]
Female	5.3 (4.5, 6.3)	<1[†]	0.1 (0.0, 0.2)[*]	8.2 (7.2, 9.4)	0.2 (0.1, 0.4)	0.2 (0.1, 0.4)[*]
Race-ethnicity						
Mexican American	5.8 (4.9, 6.7)	<1[†]	<1[†]	7.0 (5.5, 8.7)	0.3 (0.1, 0.5)[*]	<1[†]
Non-Hispanic Black	7.7 (6.7, 8.9)	<1[†]	0.3 (0.1, 0.6)[*]	18 (16, 20)	0.9 (0.6, 1.2)	<1[†]
Non-Hispanic White	5.5 (4.5, 6.6)	<1[†]	0.1 (0.0, 0.2)[*]	6.0 (5.0, 7.2)	<1[†]	<1[†]
Supplement use						
Yes	7.0 (5.8, 8.3)	<1[†]	<1[†]	6.5 (5.1, 8.3)	0.2 (0.1, 0.3)	<1[†]
No	4.9 (4.0, 6.0)	0.1 (0.1, 0.2)[*]	0.2 (0.1, 0.3)[*]	7.8 (6.7, 9.1)	0.3 (0.2, 0.4)	0.2 (0.1, 0.4)[*]

[1] Based on megaloblastic anemia as hematologic indicator; estimates are percentages (95% CI); data for 1988–2006 generated with BioRad radioproteinbindingassay (RPBA); data for 2007–2010 generated with CDC microbiologic assay (MBA-3; chloramphenicol-resistant strain and 5-methyltetrahydrofolate calibrator); RBC, red blood cell

[2] Microbiologic assay (MBA-1; wild-type microorganism and folic acid calibrator) cutpoints of 7 nmol/L (serum folate) and 305 nmol/L (RBC folate) adjusted to BioRad RPBA units (14)

[3] MBA-1 cutpoint used as is

[*] 30% ≤ relative standard error <40%

[†] Estimate suppressed, relative standard error ≥40%

Table 6

Prevalence for risk of possible folate deficiency by population subgroup using assay-matched cutpoints and original data from participants aged ≥4 y in the pre-fortification (1988–1994) and post-fortification (1999–2010) NHANES[1]

	Serum folate cutpoint and time period			RBC folate cutpoint and time period		
	<10 nmol/L[2]	<10 nmol/L[2]	<14 nmol/L[3]	<340 nmol/L[2]	<340 nmol/L[2]	<624 nmol/L[3]
	1988–1994	1999–2006	2007–2010	1988–1994	1999–2006	2007–2010
Age group						
4–11 y	7.8 (6.3, 9.5)	<1[†]	<1[†]	20 (16, 23)	0.9 (0.7, 1.3)	2.1 (1.5, 3.0)
12–19 y	38 (35, 42)	1.2 (0.9, 1.7)	2.1 (1.4, 3.1)	49 (45, 54)	6.3 (5.4, 7.3)	11 (8.8, 14)
20–39 y	47 (44, 50)	2.6 (2.2, 3.1)	6.7 (5.7, 8.0)	46 (43, 48)	7.0 (6.0, 8.0)	12 (11, 14)
40–59 y	40 (37, 43)	2.4 (2.0, 3.0)	4.9 (4.0, 5.8)	35 (32, 39)	4.5 (3.9, 5.2)	8.7 (7.1, 11)
≥60 y	23 (21, 26)	1.2 (0.9, 1.5)	2.5 (1.9, 3.3)	24 (22, 26)	2.8 (2.4, 3.4)	4.6 (3.7, 5.5)
Sex						
Male	38 (35, 41)	2.0 (1.7, 2.4)	4.7 (3.9, 5.5)	37 (35, 39)	4.9 (4.2, 5.6)	8.9 (7.8, 10)
Female	33 (31, 36)	1.7 (1.4, 2.0)	3.7 (3.2, 4.3)	37 (34, 40)	4.7 (4.3, 5.2)	8.2 (7.3, 9.3)
Race-ethnicity						
Mexican American	41 (37, 44)	1.6 (1.2, 2.2)	5.4 (4.5, 6.5)	41 (36, 47)	5.0 (4.0, 6.2)	10 (8.2, 13)
Non-Hispanic Black	46 (44, 48)	2.9 (2.3, 3.5)	7.7 (6.6, 9.1)	60 (58, 62)	13 (12, 15)	18 (16, 20)
Non-Hispanic White	34 (31, 37)	1.6 (1.4, 1.9)	3.4 (2.8, 4.3)	33 (31, 36)	3.1 (2.6, 3.7)	6.1 (5.1, 7.3)
Supplement use						
Yes	39 (36, 42)	0.8 (0.6, 1.0)	2.0 (1.6, 2.6)	37 (33, 42)	2.2 (1.8, 2.6)	4.0 (3.5, 4.6)
No	34 (31, 37)	2.9 (2.6, 3.3)	6.0 (5.2, 6.9)	37 (34, 40)	7.3 (6.6, 8.0)	12 (11, 14)

[1] Based on rising homocysteine as metabolic indicator; estimates are percentages (95% CI); data for 1988–2006 generated with BioRad radioproteinbindingassay (RPBA); data for 2007–2010 generated with CDC microbiologic assay (MBA-3; chloramphenicol-resistant strain and 5-methyltetrahydrofolate calibration); RBC, red blood cell

[2] BioRad RPBA cutpoint used as is

[3] BioRad RPBA cutpoints of 10 nmol/L (serum folate) and 340 nmol/L (RBC folate) adjusted to MBA-3 units (7)

[†] Estimate suppressed, relative standard error ≥40%

Table 7

Prevalence for risk of folate insufficiency by population subgroup using assay-matched cutpoints and original data for women aged 12–49 y in the post-fortification (2007–2010) NHANES[1]

	RBC folate cutpoint and time period
	<748 nmol/L[2]
	2007–2010
Race-ethnicity	
Mexican American	21 (17, 25)
Non-Hispanic Black	38 (35, 41)
Non-Hispanic White	18 (16, 21)
Supplement use	
Yes	12 (10, 14)
No	30 (27, 33)

[1]Based on elevated neural tube defects risk; estimates are percentages (95% CI); data for 2007–2010 generated with CDC microbiologic assay (MBA-3; chloramphenicol-resistant strain and 5-methyltetrahydrofolate calibration); RBC, red blood cell

[2]Microbiologic assay (MBA-2; chloramphenicol-resistant strain and folic acid calibration) cutpoint of 906 nmol/L adjusted to MBA-3 units (17,18)