Supplemental Table 1. Measurement of serum and RBC folate in the pre-fortification (1988–1994) and post-fortification (1999–2010) NHANES[1]

Survey date	Matrix	Original assay used in NHANES	Target assay	How to adjust data to target assay[2]
1988–1991	Serum	BioRad RPBA QP I[3]	MBA-3 or LC-MS/MS	Fractional polynomial regression applied to BioRad RPBA data (*7*)
	WB lysate	BioRad RPBA QP I[3]	MBA-3	Linear regression applied to BioRad RPBA data (*7*)
1991–1994	Serum	BioRad RPBA QP II	MBA-3 or LC-MS/MS	Fractional polynomial regression applied to BioRad RPBA data (*7*)
	WB lysate	BioRad RPBA QP II	MBA-3	Linear regression applied to BioRad RPBA data (*7*)
1999–2006	Serum	BioRad RPBA QP II	MBA-3 or LC-MS/MS	Fractional polynomial regression applied to BioRad RPBA data (*7*)
	WB lysate	BioRad RPBA QP II	MBA-3	Linear regression applied to BioRad RPBA data (*7*)
2007–2010	Serum	MBA-3	LC-MS/MS	No adjustment needed (*29*)
	WB lysate	MBA-3	None identified	

[1] BioRad RPBA QP I or II, BioRad radioproteinbindingassay Quantaphase I or II; LC-MS/MS, liquid chromatography coupled to tandem mass spectrometry; MBA-3, microbiologic assay using chloramphenicol-resistant strain and 5-methyltetrahydrofolate calibrator; WB, whole blood

[2] Reference that contains information about adjustment equation or need for adjustment is provided in parenthesis

[3] Assay adjustments applied to 1988–1991 folate data before public release to account for method differences between BioRad RPBA QP I and QP II (*14*)

Supplemental Table 2. Unweighted sample sizes for participants aged ≥4 y with serum and RBC folate results in the pre-fortification (1988–1994) and post-fortification (1999–2010) NHANES[1]

	Serum folate in persons ≥4 y			RBC folate in persons ≥4 y			RBC folate in women 12–49 y[2]		
	1988–1994	**1999–2006**	**2007–2010**	**1988–1994**	**1999–2006**	**2007–2010**	**1988–1994**	**1999–2006**	**2007–2010**
Original data[3]									
Total[4]	23705	30984	15889	23404	31278	15950	6391	9348	3977
Age group									
4–11 y	4627	4807	2572	4660	4924	2609	n/a	n/a	n/a
12–19 y	2957	8360	2254	2955	8412	2252	n/a	n/a	n/a
20–39 y	6473	6426	3618	6461	6457	3626	n/a	n/a	n/a
40–59 y	4273	5323	3643	4259	5358	3649	n/a	n/a	n/a
≥60 y	5375	6068	3802	5069	6127	3814	n/a	n/a	n/a
Sex									
Male	11269	15103	7918	11145	15224	7943	n/a	n/a	n/a
Female	12436	15881	7971	12259	16054	8007	n/a	n/a	n/a
Race-ethnicity									
MA	7053	8477	3345	6957	8542	3363	1983	2773	872
NHB	6853	7639	3083	6869	7766	3095	2164	2404	788
NHW	8795	12397	6844	8575	12467	6867	1943	3342	1586
Supplement use									
Yes	7754	12237	6429	7696	12316	6442	1938	3589	1402
No	15907	18711	9451	15663	18925	9499	4440	5749	2574
Adjusted data[5]									
Total[4]	23703	30984	*	22846	30809	*	6273	9236	*
Age group									
4–11 y	4627	4807	*	4498	4771	*	n/a	n/a	*
12–19 y	2956	8360	*	2887	8322	*	n/a	n/a	*
20–39 y	6473	6426	*	6335	6391	*	n/a	n/a	*
40–59 y	4272	5323	*	4179	5296	*	n/a	n/a	*
≥60 y	5375	6068	*	4947	6029	*	n/a	n/a	*

Sex									
Male	11269	15103	*	10878	15023	*	n/a	n/a	*
Female	12434	15881	*	11968	15786	*	n/a	n/a	*
Race-ethnicity									
MA	7053	8477	*	6821	8438	*	1961	2750	*
NHB	6853	7639	*	6695	7608	*	2121	2365	*
NHW	8794	12397	*	8344	12304	*	1893	3304	*
Supplement use									
Yes	7754	12237	*	7398	12153	*	1870	3552	*
No	15905	18711	*	15406	18620	*	4390	5675	*

[1] MA, Mexican American; NHB, non-Hispanic Black; NHW, non-Hispanic White; RBC, red blood cell

[2] No sample sizes shown by age groups or sex (n/a)

[3] 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)

[4] Sample size for "other" race-ethnicity group (persons with multi-ethnic background) not shown but included in total estimates

[5] BioRad RPBA data for 1988–2006 adjusted to MBA-3 (*7*); we excluded 2 participants in 1988–1994 because their unadjusted serum folate concentrations were <1 nmol/L and the adjustment formula requires logarithmic transformation (which produces a negative number) and then calculating the square root; also, we excluded 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 one of these tests was missing

* No sample sizes shown for adjusted data for 2007–2010 because we did not adjust the MBA-3 data

Supplemental Table 3. Prevalence for risk of folate deficiency by population subgroup using assay-mismatched 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		
	<7 nmol/L[2]	**<7 nmol/L[2]**	**<5 nmol/L[3]**	**<305 nmol/L[2]**	**<305 nmol/L[2]**	**<215 nmol/L[3]**
	1988–1994	**1999–2006**	**2007–2010**	**1988–1994**	**1999–2006**	**2007–2010**
Age group						
4–11 y	1.3 (0.8, 2.0)	<1[†]	<1[†]	12 (10, 14)	0.4 (0.2, 0.6)	<1[†]
12–19 y	17 (14, 21)	<1[†]	<1[†]	39 (36, 43)	3.1 (2.5, 3.8)	<1[†]
20–39 y	23 (21, 26)	0.5 (0.3, 0.8)	<1[†]	35 (32, 37)	3.5 (3.0, 4.2)	<1[†]
40–59 y	19 (17, 21)	0.7 (0.5, 1.0)	<1[†]	27 (24, 30)	2.4 (2.0, 2.8)	<1[†]
≥60 y	9.2 (7.8, 11)	0.1 (0.1, 0.3)*	<1[†]	17 (15, 19)	1.6 (1.3, 2.0)	<1[†]
Sex						
Male	18 (16, 20)	0.4 (0.3, 0.6)	<1[†]	28 (26, 30)	2.4 (2.0, 2.8)	<1[†]
Female	15 (14, 17)	0.4 (0.3, 0.6)	<1[†]	28 (25, 30)	2.6 (2.2, 3.0)	<1[†]
Race-ethnicity						
Mexican American	18 (16, 20)	0.3 (0.2, 0.5)	<1[†]	30 (26, 35)	2.6 (2.0, 3.4)	<1[†]
Non-Hispanic Black	23 (22, 25)	0.4 (0.3, 0.7)	<1[†]	49 (47, 51)	7.5 (6.7, 8.3)	<1[†]
Non-Hispanic White	16 (14, 18)	0.4 (0.3, 0.6)	<1[†]	25 (22, 27)	1.6 (1.3, 2.0)	<1[†]
Supplement use						
Yes	18 (16, 21)	0.2 (0.1, 0.4)	<1[†]	27 (24, 32)	1.2 (0.9, 1.5)	<1[†]
No	16 (14, 17)	0.6 (0.4, 0.8)	<1[†]	28 (26, 31)	3.7 (3.3, 4.2)	<1[†]

[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) cutpoint used as is

[3] MBA-1 cutpoint of 7 nmol/L (serum folate) and 305 nmol/L (RBC folate) adjusted to BioRad RPBA units (*14*)

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

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

Supplemental Table 4. Prevalence for risk of possible folate deficiency by population subgroup using assay-mismatched 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		
	<14 nmol/L[2]	**<14 nmol/L[2]**	**<10 nmol/L[3]**	**<624 nmol/L[2]**	**<624 nmol/L[2]**	**<340 nmol/L[3]**
	1988–1994	**1999–2006**	**2007–2010**	**1988–1994**	**1999–2006**	**2007–2010**
Age group						
4–11 y	24 (21, 27)	0.3 (0.1, 0.6)*	<1†	83 (80, 86)	54 (51, 56)	<1†
12–19 y	59 (55, 63)	6.1 (5.3, 6.9)	<1†	92 (90, 94)	72 (70, 74)	<1†
20–39 y	68 (65, 71)	10 (9.2, 11)	1.3 (0.9, 1.9)	89 (88, 91)	63 (61, 65)	0.3 (0.1, 0.5)*
40–59 y	60 (56, 64)	8.1 (7.3, 9.1)	0.6 (0.4, 1.1)	80 (78, 82)	48 (46, 50)	<1†
≥60 y	42 (40, 45)	3.9 (3.4, 4.6)	0.4 (0.2, 0.7)	70 (67, 72)	32 (30, 34)	<1†
Sex						
Male	58 (56, 61)	7.8 (7.1, 8.6)	0.7 (0.5, 1.0)	86 (84, 87)	57 (55, 59)	0.2 (0.1, 0.3)*
Female	53 (50, 55)	6.2 (5.6, 6.9)	0.7 (0.5, 1.0)	82 (80, 83)	50 (48, 51)	0.3 (0.2, 0.5)
Race-ethnicity						
Mexican American	62 (58, 67)	7.5 (6.4, 8.6)	1.1 (0.7, 1.6)	90 (87, 92)	64 (62, 66)	<1†
Non-Hispanic Black	68 (66, 70)	12 (10, 13)	1.4 (0.8, 2.3)	95 (94, 96)	77 (76, 79)	<1†
Non-Hispanic White	53 (50, 56)	6.0 (5.4, 6.7)	0.5 (0.4, 0.7)	81 (79, 83)	46 (44, 49)	0.2 (0.1, 0.3)*
Supplement use						
Yes	60 (36, 42)	3.2 (2.8, 3.6)	0.3 (0.2, 0.6)	85 (83, 87)	37 (35, 38)	<1†
No	54 (31, 37)	11 (9.8, 11)	1.0 (0.7, 1.4)	83 (81, 85)	68 (67, 70)	0.3 (0.2, 0.5)

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

[3] BioRad RPBA cutpoint used as is

* 30% ≤ relative standard error <40%

† Estimate suppressed, relative standard error ≥40%

Supplemental Table 5. Prevalence for risk of folate insufficiency by population subgroup using assay-mismatched cutpoints and original data for women aged 12–49 y in the pre-fortification (1988–1994) and post-fortification (1999–2010) NHANES[1]

	RBC folate cutpoint and time period		
	<906 nmol/L[2]	**<906 nmol/L[2]**	**<906 nmol/L[2]**
	1988–1994	**1999–2006**	**2007–2010**
Race-ethnicity			
Mexican American	77 (74, 80)	33 (30, 35)	41 (35, 46)
Non-Hispanic Black	89 (87, 91)	56 (53, 59)	58 (54, 62)
Non-Hispanic White	69 (65, 72)	25 (22, 27)	33 (29, 37)
Supplement use			
Yes	76 (72, 80)	18 (16, 20)	24 (21, 27)
No	72 (68, 75)	42 (40, 45)	49 (46, 53)

[1] Based on elevated neural tube defects risk; 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] MBA-2 (chloramphenicol-resistant strain and folic acid calibrator) cutpoint used as is

Supplemental Table 6. Prevalence for risk of folate deficiency by population subgroup using assay-matched cutpoints and assay-adjusted data from participants aged ≥4 y in the pre-fortification (1988–1994) and post-fortification (1999–2006) NHANES[1]

	Serum folate <7 nmol/L[2]		RBC folate <305 nmol/L[2]	
	1988–1994	**1999–2006**	**1988–1994**	**1999–2006**
Age group				
4–11 y	1.1 (0.7, 1.7)	<1†	0.4 (0.2, 0.7)*	<1†
12–19 y	16 (13, 19)	<1†	2.5 (1.8, 3.3)	<1†
20–39 y	22 (20, 24)	0.4 (0.3, 0.6)	2.3 (1.7, 3.1)	<1†
40–59 y	18 (16, 20)	0.6 (0.4, 1.0)	2.5 (1.8, 3.4)	<1†
≥60 y	8.3 (7.0, 9.9)	<1†	1.2 (0.9, 1.8)	<1†
Sex				
Male	17 (15, 19)	0.3 (0.2, 0.5)	1.8 (1.3, 2.3)	0.1 (0.0, 0.1)
Female	14 (13, 16)	0.3 (0.2, 0.5)	2.2 (1.8, 2.6)	<1†
Race-ethnicity				
Mexican American	17 (15, 18)	0.2 (0.1, 0.4)*	1.8 (1.3, 2.4)	<1†
Non-Hispanic Black	22 (21, 24)	0.4 (0.2, 0.6)	6.6 (5.6, 7.6)	0.2 (0.1, 0.4)*
Non-Hispanic White	15 (13, 17)	0.4 (0.2, 0.5)	1.4 (1.1, 1.9)	<1†
Supplement use				
Yes	17 (15, 20)	0.2 (0.1, 0.4)	1.9 (1.3, 2.7)	<1†
No	15 (13, 16)	0.4 (0.3, 0.6)	2.0 (1.6, 2.6)	0.1 (0.1, 0.2)*

[1] Based on megaloblastic anemia as hematologic indicator; estimates are percentages (95% CI); data for 1988–2006 generated with BioRad radioproteinbindingassay (RPBA) and adjusted to CDC microbiologic assay (MBA-3; chloramphenicol-resistant strain and 5-methyltetrahydrofolate calibration) (*7*); RBC, red blood cell

[2] Microbiologic assay (MBA-1; wild-type microorganism and folic acid calibrator) cutpoint used as is (assumption that MBA-1 and MBA-3 produce similar results)

* 30% ≤ relative standard error <40%

† Estimate suppressed, relative standard error ≥40%

Supplemental Table 7. Prevalence for risk of possible folate deficiency by population subgroup using assay-matched cutpoints and assay-adjusted data from participants aged ≥4 y in the pre-fortification (1988–1994) and post-fortification (1999–2006) NHANES[1]

	Serum folate <14 nmol/L[2]		RBC folate <624 nmol/L[2]	
	1988–1994	**1999–2006**	**1988–1994**	**1999–2006**
Age group				
4–11 y	8.7 (7.2, 10)	<1[†]	19 (16, 23)	0.9 (0.6, 1.2)
12–19 y	40 (36, 44)	1.5 (1.1, 1.9)	49 (45, 54)	6.2 (5.3, 7.2)
20–39 y	48 (45, 52)	2.9 (2.5, 3.4)	46 (43, 48)	6.9 (6.0, 8.0)
40–59 y	42 (39, 45)	2.9 (2.4, 3.5)	35 (32, 38)	4.3 (3.7, 5.0)
≥60 y	25 (22, 27)	1.3 (1.0, 1.6)	24 (22, 27)	2.8 (2.4, 3.3)
Sex				
Male	40 (37, 42)	2.5 (2.1, 2.9)	37 (35, 39)	4.8 (4.1, 5.6)
Female	35 (32, 37)	1.8 (1.6, 2.2)	37 (34, 40)	4.6 (4.2, 5.1)
Race-ethnicity				
Mexican American	42 (39, 46)	1.8 (1.3, 2.4)	41 (36, 47)	4.9 (4.0, 6.1)
Non-Hispanic Black	48 (46, 51)	3.2 (2.6, 3.9)	60 (57, 62)	13 (12, 14)
Non-Hispanic White	35 (32, 38)	1.9 (1.6, 2.2)	33 (30, 36)	3.1 (2.6, 3.7)
Supplement use				
Yes	41 (37, 44)	0.9 (0.7, 1.2)	37 (33, 42)	2.1 (1.7, 2.6)
No	35 (33, 38)	3.3 (2.9, 3.7)	37 (34, 40)	7.1 (6.5, 7.9)

[1] Based on rising homocysteine as metabolic indicator; estimates are percentages (95% CI); data for 1988–2006 generated with BioRad radioproteinbindingassay (RPBA) and adjusted to CDC microbiologic assay (MBA-3; chloramphenicol-resistant strain and 5-methyltetrahydrofolate calibration) (*7*); RBC, red blood cell

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

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

Supplemental Table 8. Prevalence for risk of folate insufficiency by population subgroup using assay-matched cutpoints and assay-adjusted data for women aged 12–49 y in the pre-fortification (1988–1994) and post-fortification (1999–2006) NHANES[1]

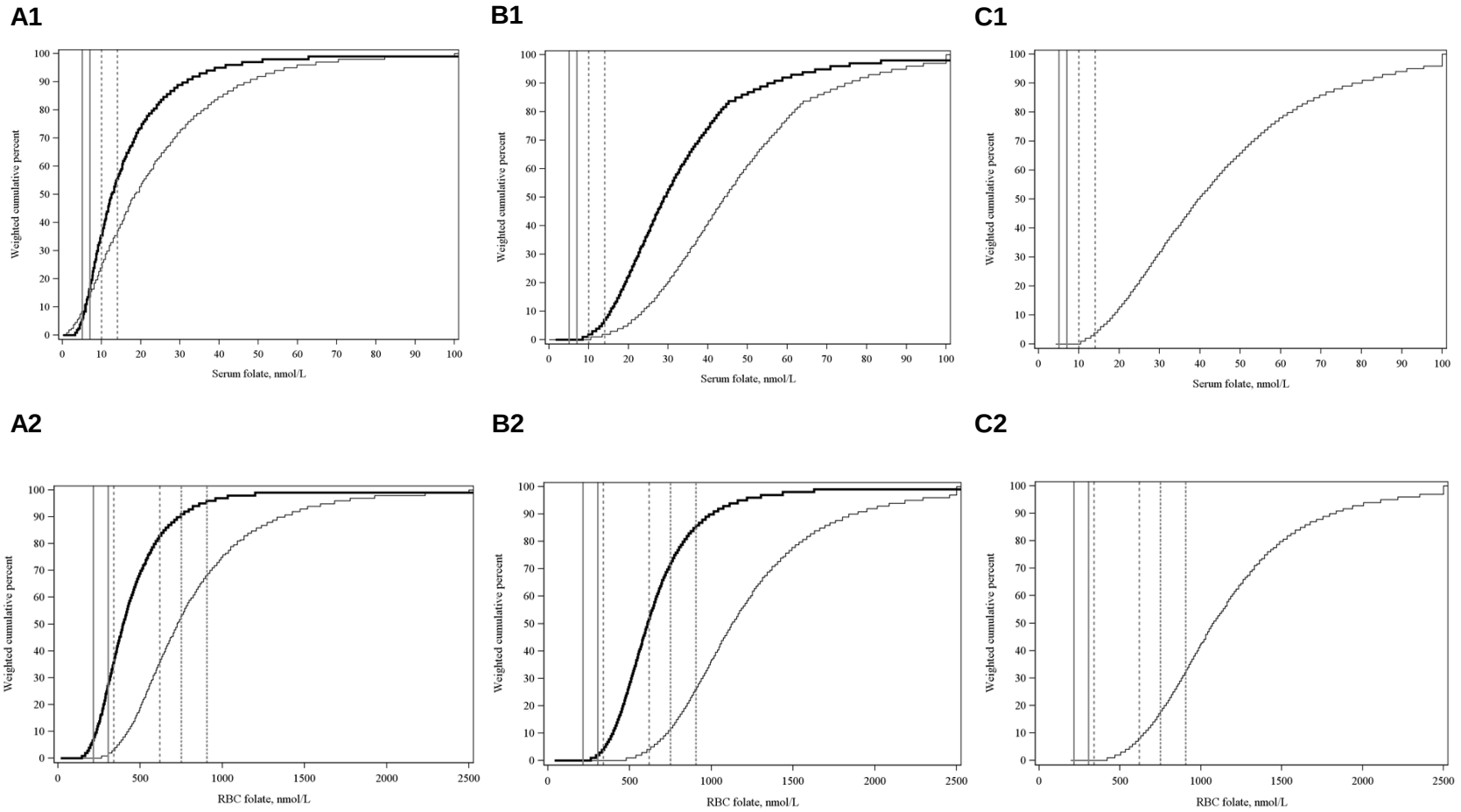
	RBC folate <748 nmol/L[2]	
	1988–1994	**1999–2006**
Race-ethnicity		
Mexican American	64 (60, 68)	15 (13, 17)
Non-Hispanic Black	80 (77, 82)	32 (30, 35)
Non-Hispanic White	55 (51, 59)	11 (9.5, 13)
Supplement use		
Yes	61 (56, 66)	8.0 (6.7, 9.4)
No	58 (54, 63)	21 (20, 23)

[1] Based on elevated neural tube defects risk; estimates are percentages (95% CI); data for 1988–2006 generated with BioRad radioproteinbindingassay (RPBA) and adjusted to CDC microbiologic assay (MBA-3; chloramphenicol-resistant strain and 5-methyltetrahydrofolate calibration) (*7*); 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*)

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

Supplemental Figure 1. Cumulative frequency distributions of original and "forward" adjusted serum and RBC folate concentrations in participants aged ≥4 y in the pre-fortification (1988–1994) and post-fortification (1999–2010) NHANES



A1 (serum) and A2 (RBC) – 1988–1994 pre-folic acid fortification; B1 (serum) and B2 (RBC) – 1999–2006 post-fortification; C1 (serum) and C2 (RBC) – 2007–2010 post-fortification. Hcy, homocysteine; NTD, neural tube defect; RBC, red blood cell.
Heavy distribution curve: BioRad radioproteinbindingassay data.
Light distribution curve: microbiologic assay data (MBA-3; chloramphenicol-resistant strain and 5-methyltetrahydrofolate as calibrator).
Solid vertical lines: cutpoints for risk of deficiency based on megaloblastic anemia (5 and 7 nmol/L for serum folate; 215 and 305 nmol/L for RBC folate).
Dashed vertical lines: cutpoints for risk of possible deficiency based on rising Hcy (10 and 14 nmol/L for serum folate; 340 and 624 nmol/L for RBC folate).
Small dashed vertical lines: cutpoints for risk of insufficiency based on elevated NTD risk (748 and 906 nmol/L for RBC folate).

Supplemental Text 1. Relationship between 3 microbiologic assays used presently

As part of NHANES 2007–2008, CDC assessed the comparability of 3 main types of microbiologic assays (MBA) using 2645 serum and 2613 whole blood lysate samples:

- MBA-2: Molloy assay uses a chloramphenicol-resistant strain of *L. rhamnosus* and folic acid as calibrator (*13*),
- MBA-3: CDC assay uses a chloramphenicol-resistant strain of *L. rhamnosus* and 5-methyltetrahydrofolate as calibrator (*15*), and
- MBA-1_{var}: Tamura assay uses a wild-type microorganism and 5-formyltetrahydrofolate as calibrator (*19*) and is a variation of the "traditional" microbiologic assay (MBA-1).

The findings of that study including the relationships between these assays as described by Deming regression equations were published earlier (*17*).

More recently we investigated 2 alternative approaches, *weighted Deming regression* and *Deming regression using log transformed data*, to generate "prediction" equations that could be used to adjust data in the literature to make it comparable. Both approaches produced comparable and acceptable model characteristics based on residuals (constant variance with increasing concentration), while the earlier Deming regression showed increasing variance with increasing concentration. We chose weighted Deming regression over Deming regression using log transformed data because the coefficients (slope and intercept) are more easily interpretable. Because Deming is an orthogonal regression, reversing the roles of x and y does not change the sum of squares being minimized, as long as we assume the ratio of variances for the 2 assays to be 1 (which is the case because these microbiologic assays have similar variance). In ordinary linear squares regression x and y are not treated symmetrically; this means that the estimators for 1 line using 1 direction vs. the other direction will not be the same, nor will any predictions or inferences. By using Deming regression, we can provide 1 regression equation for each assay pair and users can solve for x if they are interested in the other direction.

Weighted Deming regression relating 3 main types of microbiologic assays for serum and RBC folate used presently

	MBA-2 (y) vs. MBA-3 (x)	**MBA-1_{var} (y) vs. MBA-3 (x)**	**MBA-1_{var} (y) vs. MBA-2 (x)**
Serum folate			
Pearson correlation	0.9510	0.8009	0.8176
Slope (95% CI)	1.2739 (1.26, 1.29)	1.6328 (1.60, 1.67)	1.2732 (1.24, 1.30)
Intercept (95% CI)	-2.3542 (-2.82, -1.89)	-5.5477 (-6.57, -4.53)	-1.8091 (-2.81, -0.80)
RBC folate			
Pearson correlation	0.9166	0.6507	0.6922
Slope (95% CI)	1.2697 (1.25, 1.29)	1.928 (1.82, 2.04)	1.3705 (1.31, 1.43)
Intercept (95% CI)	-43.5239 (-66.1, -21.0)	-770.7 (-887, -654)	-482.714 (-564, -401)