Supplementary Figure 1. Flowchart with number of US persons who were screened, interviewed, examined, for whom RBF concentrations were measured, and who were excluded due to age, NHANES 1988–2010

	Females			**Overall**
	1988-1994	**1999-2006**	**2007-2010**	**2007-2010**
Screened Interviewed Examined	20529 17699 16037	25898 21210 20076	13091 10365 10010	26215 20686 20015
RBF measured	12259	16773	8516	17007
Age exclusion*	*5868*	*7425*	*4539*	*0*
RBF for age range in study	6391	9348	3977	17007
Analytical sample &	**6275**	**9236**	**3951**	**16815**

* For females, we excluded females <12 y and >49 y; for "overall" group, there were no exclusions (persons ≥1 y).
& Analytical sample was limited to participants with complete biomarker data for serum folate, RBF, hematocrit, and hemoglobin.
RBF, red blood cell folate.

On-line Supplementary Material
Population RBC folate concentrations can be accurately estimated from measured whole blood folate,
measured hemoglobin, and predicted serum folate; *Zhang et al.*

Supplementary Figure 2. Bland-Altman plot showing the unweighted relative difference between predicted and measured SFOL (panels A-C) and estimated and measured Hct (panels D-F) in women 12–49 y using 3 different time periods, NHANES 1988–2010. The solid line represents the median difference, while the dashed lines represent the non-parametric "agreement limits" capturing 95% of the data. Panel A & D: 1988-1994; panel B & E: 1999-2006; panel C & F: 2007-2010. We omitted 3 data points to allow use of the same axis scaling: panel A (6, 6140); panel B (856, -90) and (94, 1464). SFOL, serum folate in nmol/L; Hct, hematocrit in %.
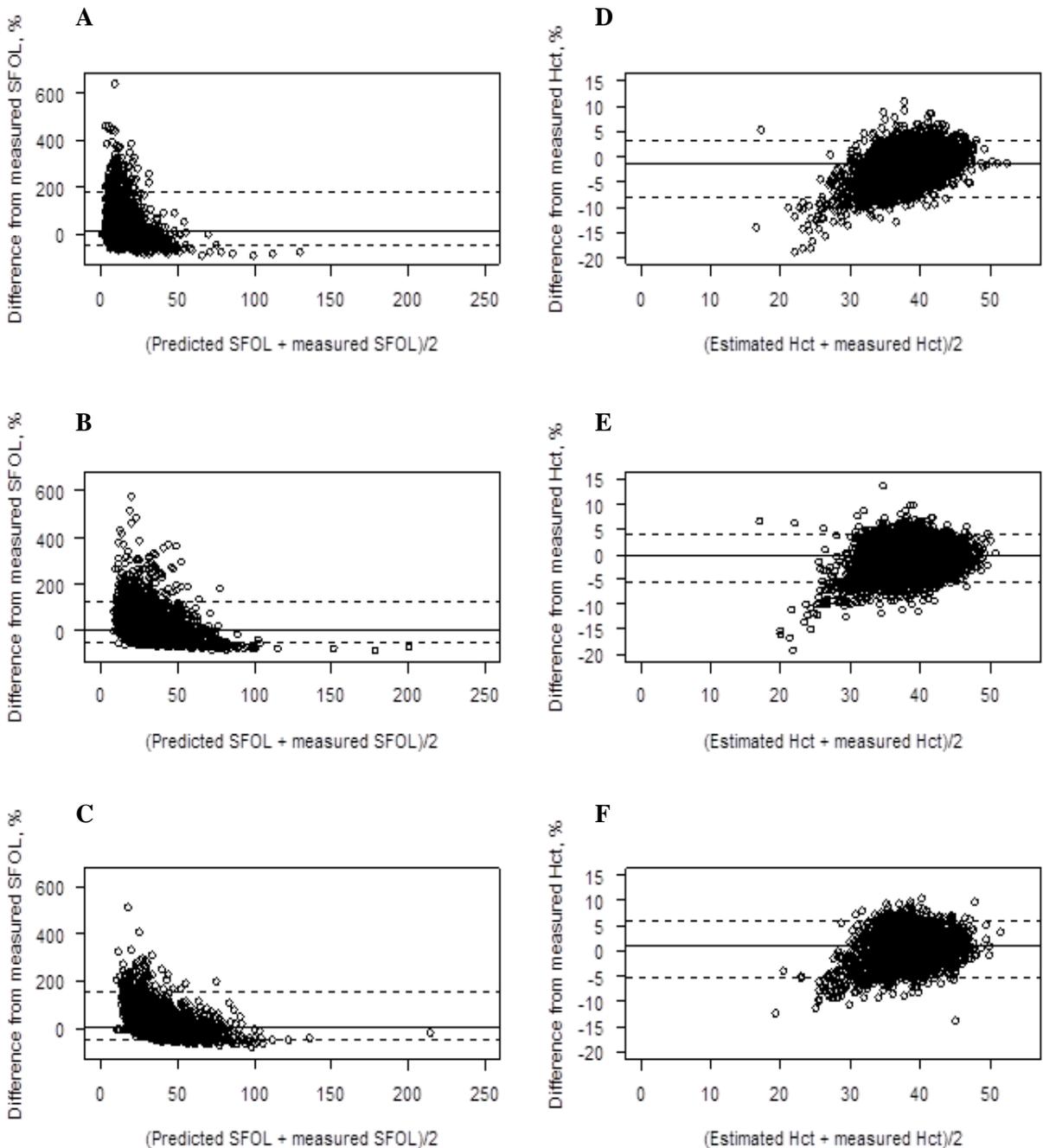
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Supplementary Table 1. Regression equations to predict serum folate from whole blood folate[1]

Survey	Group	Equation	R^2	MSE
1988–1994	Women 12–49 y	Predicted SFOL=1.35023+0.07943*WBF	37.3	7.8
1999–2006	Women 12–49 y	Predicted SFOL=5.52173+0.09046*WBF	42.5	9.5
2007–2010	Women 12–49 y	Predicted SFOL=10.59233+0.06712*WBF	37.1	14.5
2007–2010	Persons ≥1 y	Predicted SFOL=9.58876+0.06463*WBF	34.3	17.5

[1] Unweighted simple linear regression equation based on a simple random sample from NHANES of either 150 women 12–49 y or 150 persons ≥1 y, respectively. MSE, mean square error; SFOL, serum folate; WBF, whole blood folate. R^2 is the coefficient of determination in percent.

On-line Supplementary Material
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Supplementary Table 2. Central 95% reference intervals for estimated and "true" RBF by demographic group, women 12–49 y, NHANES 2007–2010[1]

Group	"True" RBF[2]	Estimated RBF by approach	
		#5: Assume SFOL=0 & Estimate Hct[3]	#6: Predict SFOL & Estimate Hct[4]
Overall	467 – 2210	491 – 2310	454 – 2200
Age, y			
12–19	486 – 1750	518 – 1830	479 – 1740
20–39	465 – 2230	485 – 2310	448 – 2210
40–49	462 – 2280	478 – 2350	442 – 2240
Race-Hispanic origin			
Mexican-American	496 – 1960	534 – 2060	498 – 1950
Non-Hispanic Black	425 – 1840	462 – 2030	419 – 1920
Non-Hispanic White	474 – 2290	494 – 2370	459 – 2250

[1] Values represent 2.5[th] – 97.5[th] percentile in nmol/L; for sample sizes, see Table 1; Hct, hematocrit; MCHC, mean corpuscular hemoglobin concentration; RBF, red blood cell folate; SFOL, serum folate; WBF, whole blood folate.

[2] RBF calculated using measured WBF, SFOL, and Hct: RBF=[WBF–SFOL*(1–Hct)]/Hct.

[3] RBF estimated using measured WBF and estimated Hct without correcting for measured SFOL: RBF=WBF/estimated Hct; Estimated Hct=measured Hb/imputed MCHC using MCHC=340 g/L.

[4] RBF estimated using measured WBF, predicted SFOL, and estimated Hct: RBF=[WBF–predicted SFOL*(1–estimated Hct)]/estimated Hct; SFOL predicted using unweighted simple linear regression derived from measured WBF in a simple random sample of women 12–49 y (*n*=150) as x-variable and measured SFOL as y-variable from NHANES 2007–2010 (predicted SFOL=10.59233+0.06712*WBF); Estimated Hct=measured Hb/imputed MCHC using MCHC=340 g/L.

Supplementary Table 3. Measured Hct and SFOL, "true" RBF, and relative difference between estimated and "true" RBF by fasting status and supplement use, women 12–49 y, NHANES 2007–2010[1]

Group	Sample size	Hct	SFOL	"True" RBF[2]	Difference to "true" RBF by approach	
					#5: Assume SFOL=0 & Estimate Hct[3]	#6: Predict SFOL & Estimate Hct[4]
	n	*%*	*nmol/L*		*%*	
Fasting status, h						
<3	1320	38.7 (38.3, 39.0)	39.0 (37.2, 40.5)	1040 (1010, 1090)	4.9 (4.3, 5.4)	-1.2 (-1.6, -0.73)
3–8	768	38.6 (38.3, 38.9)	34.0 (31.5, 36.2)	966 (906, 1000)	5.0 (4.6, 5.5)	-1.1 (-1.5, -0.71)
>8	1863	39.1 (38.8, 39.5)	37.0 (35.6, 39.2)	1000 (977, 1030)	5.3 (4.8, 5.9)	-0.78 (-1.3, -0.27)
Supplement use						
Yes	957	38.8 (38.4, 39.3)	50.2 (47.1, 53.0)	1270 (1210, 1310)	5.5 (4.9, 6.1)	-0.33 (-0.87, 0.21)
No	293	38.7 (38.1, 39.5)	37.3 (34.7, 39.6)	1020 (954, 1120)	5.1 (4.4, 5.7)	-0.93 (-1.5, -0.37)

[1] Values represent sample sizes, median (95% CI) concentrations, or mean percent difference (95% CI); Hct, hematocrit; MCHC, mean corpuscular hemoglobin concentration; RBF, red blood cell folate; SFOL, serum folate; WBF, whole blood folate.

[2] Calculated using measured WBF, SFOL, and Hct: RBF=[WBF–SFOL*(1–Hct)]/Hct.

[3] RBF estimated using measured WBF and estimated Hct without correcting for measured SFOL: RBF=WBF/estimated Hct; Estimated Hct=measured Hb/imputed MCHC using MCHC=340 g/L.

[4] RBF estimated using measured WBF, predicted SFOL, and estimated Hct: RBF=[WBF–predicted SFOL(1–estimated Hct)]/estimated Hct; SFOL predicted using unweighted simple linear regression derived from measured WBF in a simple random sample of women 12–49 y (*n*=150) as x-variable and measured SFOL as y-variable from NHANES 2007–2010 (predicted SFOL=10.59233+0.06712*WBF); Estimated Hct=measured Hb/imputed MCHC using MCHC=340 g/L.

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Supplementary Table 4. Folate concentrations by demographic group, persons ≥1 y, NHANES 2007–2010[1]

Group	Sample size	RBF[2]	WBF	SFOL	Predicted SFOL[3]
	n		*nmol/L*		
Overall	16815	1070 (1040, 1100)	465 (452, 477)	39.8 (38.7, 40.9)	39.6 (38.8, 40.4)
Age, y					
1–5	1593	1050 (1020, 1070)	416 (406, 424)	53.1 (51.3, 54.9)	36.5 (36.0, 37.1)
6–11	1990	1090 (1060, 1120)	455 (443, 469)	56.1 (54.9, 57.2)	39.1 (38.3, 40.0)
12–19	2239	940 (909, 977)	409 (393, 424)	39.4 (37.6, 41.0)	36.0 (35.0, 36.9)
20–39	3605	978 (951, 1000)	429 (417, 441)	32.5 (31.4, 33.5)	37.3 (36.5, 38.1)
40–59	3621	1110 (1060, 1160)	487 (470, 510)	37.1 (35.3, 38.8)	41.1 (39.9, 42.5)
≥60	3767	1340 (1300, 1380)	583 (563, 600)	50.0 (48.1, 52.0)	47.3 (46.0, 48.3)
Sex					
Male	8394	1050 (1010, 1080)	475 (460, 491)	37.4 (36.1, 38.5)	40.3 (39.3, 41.3)
Female	8421	1090 (1070, 1120)	451 (443, 463)	42.8 (41.8, 43.8)	38.7 (38.2, 39.5)
Race-Hispanic origin					
MA	3634	983 (935, 1030)	418 (404, 435)	36.5 (35.2, 38.4)	36.6 (35.7, 37.7)
NHB	3273	875 (847, 908)	364 (353, 375)	32.0 (31.0, 33.1)	33.1 (32.4, 33.8)
NHW	7124	1150 (1100, 1180)	500 (484, 519)	42.5 (40.8, 43.8)	41.9 (40.8, 43.1)

[1] Values represent sample sizes and median (95% CI) concentrations measured by the microbiologic assay; Hct, hematocrit; MCHC, mean corpuscular hemoglobin concentration; MA, Mexican American; NHB, non-Hispanic Black; NHW, non-Hispanic White; RBF, red blood cell folate; SFOL, serum folate; WBF, whole blood folate.

[2] Calculated using measured WBF, SFOL, and Hct: RBF=[WBF–SFOL*(1–Hct)]/Hct.

[3] SFOL predicted using unweighted simple linear regression derived from measured WBF in a simple random sample of persons ≥1 y (n=150) as x-variable and measured SFOL as y-variable from NHANES 2007–2010 (predicted SFOL=9.58876+0.06463*WBF); Estimated Hct=measured Hb/imputed MCHC using MCHC=340 g/L.

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Supplementary Table 5. Hematologic variables by demographic group, persons ≥1 y, NHANES 2007–2010[1]

Group	Hct	Hb	MCHC	Estimated Hct[2]
	%	*g/L*	*g/L*	*%*
Overall	40.8 (40.5, 41.1)	140 (139, 141)	344 (342, 345)	41.3 (40.9, 41.5)
Age, y				
1–5	36.2 (36.0, 36.5)	125 (124, 126)	347 (345, 348)	36.8 (36.6, 37.0)
6–11	38.2 (38.0, 38.5)	133 (131, 134)	346 (345, 347)	39.0 (38.5, 39.3)
12–19	40.8 (40.4, 41.3)	140 (138, 142)	343 (341, 345)	41.2 (40.7, 41.8)
20–39	41.9 (41.6, 42.1)	144 (143, 145)	345 (344, 346)	42.3 (42.0, 42.6)
40–59	41.8 (41.4, 42.2)	143 (142, 145)	344 (342, 345)	42.2 (41.8, 42.6)
≥60	40.9 (40.4, 41.3)	140 (138, 141)	342 (340, 344)	41.2 (40.7, 41.6)
Sex				
Male	43.7 (43.4, 43.9)	150 (149, 150)	345 (343, 346)	44.0 (43.8, 44.2)
Female	38.9 (38.7, 39.2)	134 (133, 135)	343 (342, 345)	39.4 (39.2, 39.7)
Race-Hispanic origin				
MA	40.3 (39.8, 40.9)	139 (137, 140)	344 (343, 346)	40.7 (40.4, 41.3)
NHB	39.0 (38.7, 39.4)	132 (131, 133)	338 (336, 341)	39.0 (38.6, 39.2)
NHW	41.2 (40.9, 41.6)	142 (141, 143)	345 (343, 347)	41.8 (41.4, 42.1)

[1] Values represent median (95% CI); for sample sizes, see Supplemental Table 4; Hb, hemoglobin; Hct, hematocrit; MA, Mexican American; MCHC, mean corpuscular hemoglobin content; NHB, non-Hispanic Black; NHW, non-Hispanic White.

[2] Calculated as measured Hb/imputed MCHC using MCHC=340 g/L.

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Supplementary Table 6. Relative difference between estimated and "true" RBF by demographic group using different approximation approaches, persons ≥1 y, NHANES 2007–2010[1]

Group	RBF estimation approach					
	#1: Assume SFOL=0[2]	#2: Impute SFOL[3]	#3: Impute Hct[4]	#4: Estimate Hct[5]	#5: Assume SFOL=0 & Estimate Hct[6]	#6: Predict SFOL & Estimate Hct[7]
Overall	5.9 (5.8, 6.0)	0.01 (-0.12, 0.14)	2.4 (1.7, 3.1)	-1.2 (-1.5, -0.8)	4.6 (4.2, 5.0)	-0.72 (-1.10, -0.35)
Age, y						
1–5	9.6 (9.3, 9.9)	2.4 (2.2, 2.7)	-8.9 (-9.6, -8.2)	-1.7 (-2.1, -1.3)	7.6 (7.0, 8.2)	1.5 (0.98, 2.1)
6–11	8.8 (8.6, 9.0)	2.6 (2.4, 2.8)	-3.8 (-4.5, -3.2)	-1.7 (-2.1, -1.3)	6.8 (6.3, 7.4)	1.2 (0.69, 1.6)
12–19	6.4 (6.2, 6.6)	-0.23 (-0.47, 0.01)	2.5 (1.6, 3.5)	-1.0 (-1.4, -0.67)	5.2 (4.8, 5.6)	-0.34 (-0.72, 0.05)
20–39	5.1 (4.9, 5.2)	-1.2 (-1.3, -0.99)	4.2 (3.6, 4.7)	-1.3 (-1.7, -0.97)	3.6 (3.2, 4.1)	-1.7 (-2.1, -1.3)
40–59	5.2 (5.0, 5.3)	-0.48 (-0.70, -0.25)	4.1 (3.3, 5.0)	-1.1 (-1.5, -0.74)	3.9 (3.5, 4.4)	-1.3 (-1.6, -0.88)
≥60	5.9 (5.8, 6.1)	1.0 (0.91, 1.2)	2.2 (1.1, 3.2)	-0.64 (-1.1, -0.21)	5.2 (4.6, 5.8)	0.05 (-0.44, 0.53)
Sex						
Male	5.1 (5.1, 5.2)	-0.30 (-0.44, -0.15)	7.6 (7.0, 8.3)	-1.4 (-1.7, -1.0)	3.6 (3.2, 4.0)	-1.4 (-1.8, -1.0)
Female	6.6 (6.5, 6.7)	0.30 (0.17, 0.44)	-2.6 (-3.3, -2.0)	-0.94 (-1.3, -0.56)	5.6 (5.1, 6.0)	-0.08 (-0.49, 0.33)
Race-Hispanic origin						
MA	6.0 (5.8, 6.3)	-0.37 (-0.62, -0.12)	1.8 (0.86, 2.7)	-1.3 (-1.6, -0.94)	4.7 (4.3 - 5.0)	-0.83 (-1.1, -0.52)
NHB	6.3 (6.1, 6.5)	-1.3 (-1.5, -1.1)	-1.6 (-2.3, -1.0)	0.41 (-0.13, 0.94)	6.7 (6.1, 7.4)	0.70 (0.08, 1.3)
NHW	5.7 (5.6, 5.8)	0.33 (0.16, 0.50)	3.3 (2.4, 4.2)	-1.4 (-1.8, -1.0)	4.1 (3.7, 4.6)	-1.0 (-1.4, -0.61)

On-line Supplementary Material
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[1] Values represent mean percent difference (95% CI) between each RBF estimation approach and the "true" RBF measured by microbiologic assay; for sample sizes, see Supplemental Table 4; Hb, hemoglobin; Hct, hematocrit; MA, Mexican American; NHB, non-Hispanic Black; NHW, non-Hispanic White; RBF, red blood cell folate; SFOL, serum folate; WBF, whole blood folate.

[2] RBF estimated using measured WBF and Hct without correcting for measured SFOL: RBF=WBF/Hct.

[3] RBF estimated using measured WBF and Hct and an imputed SFOL of 40 nmol/L: RBF=[WBF–40*(1–Hct)]/Hct.

[4] RBF estimated using measured WBF and SFOL and an imputed Hct of 40%: RBF=[WBF–SFOL*(1– 0.4)]/0.4.

[5] RBF estimated using measured WBF and SFOL and an estimated Hct: RBF=[WBF–SFOL*(1–Estimated Hct)]/Estimated Hct; Estimated Hct=measured Hb/imputed MCHC using MCHC=340 g/L.

[6] RBF estimated using measured WBF and an estimated Hct without correcting for measured SFOL: RBF=WBF/Estimated Hct; Estimated Hct = measured Hb/imputed MCHC using MCHC=340 g/L.

[7] RBF estimated using measured WBF, predicted SFOL, and estimated Hct: RBF=[WBF–Predicted SFOL*(1–Estimated Hct)]/Estimated Hct; SFOL predicted using unweighted simple linear regression derived from measured WBF in a simple random sample of persons ≥1 y (n=150) as x-variable and measured SFOL as y-variable from NHANES 2007–2010; Estimated Hct=measured Hb/imputed MCHC using MCHC=340 g/L.