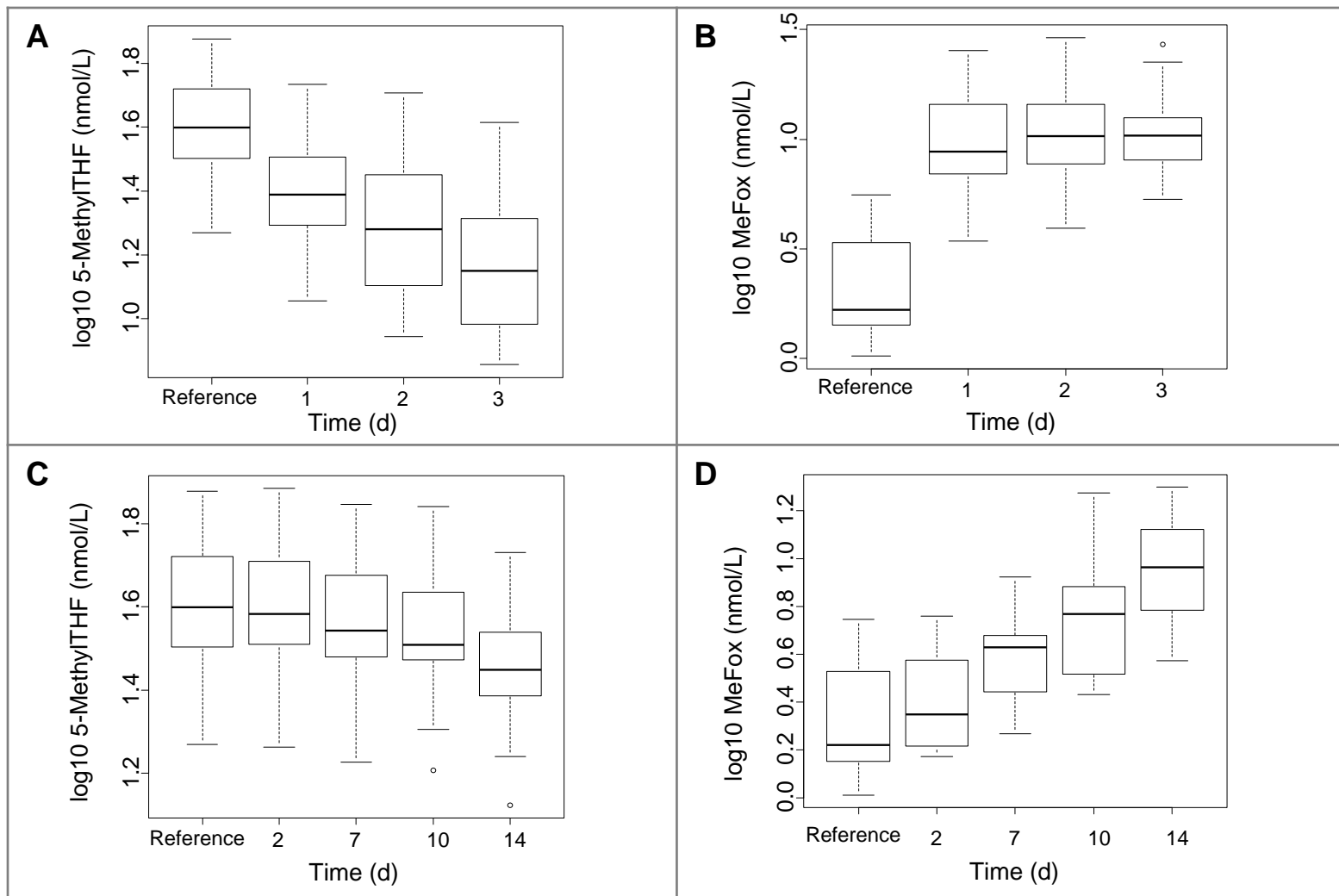**Supplemental Figure 1.** Box and whisker graphs for 5-methylTHF and MeFox geometric mean concentrations as a result of delayed processing of whole blood stored at 32ºC for ≤3 d (A and B) or delayed freezing of serum stored at 11ºC for ≤14 d (C and D)

Box represents interquartile range, horizontal line in box represents median, and whiskers represent 2.5th and 97.5th percentiles.
5-MethylTHF, 5-methyltetrahydrofolate; MeFox, pyrazino-s-triazine derivative of 4α-hydroxy-5-methyltetrahydrofolate.

Supplemental Table 1. Effect of short-term (≤24 h) exposure of serum QC pools to elevated temperature (room temperature and 37°C) on serum folate concentrations[1]

Analyte QC pool	Serum folate concentration[2]					Relative change[3]			
	Reference	2 h	4 h	6 h	24 h	2 h	4 h	6 h	24 h
	nmol/L					*%*			
Serum stored at room temperature									
tFOL						0.46 (-3.24, 4.30)	0.56 (-3.15, 4.40)	1.01 (-2.71, 4.48)	4.00* (0.16, 7.97)
Low	16.6	16.1	16.8	16.3	17.0				
Medium	36.3	37.3	37.9	37.9	38.5				
High	60.7	59.7	58.8	59.1	60.9				
5-MethylTHF						0.54 (-2.92, 4.13)	0.12 (-3.33, 3.69)	0.67 (-2.80, 4.26)	-2.32 (-5.69, 1.16)
Low	14.9	14.6	15.0	14.7	14.4				
Medium	31.5	32.5	32.7	32.9	31.6				
High	48.4	47.7	47.0	46.9	46.2				
Folic acid						-4.06 (-10.1, 2.35)	-3.83 (-9.86, 2.60)	-2.64 (-8.74, 3.88)	5.00 (-1.59, 12.0)
Low	0.68	0.64	0.64	0.61	0.68				
Medium	3.69	3.60	3.68	3.65	3.78				
High	9.23	8.87	8.57	8.51	9.06				
MeFox									
Low	1.02	0.85	1.06	1.02	1.85	-16.6* (-29.3, -1.57)	4.02 (-11.8, 22.7)	-0.54 (-15.7, 17.3)	81.2** (53.6, 114)
Medium	1.11	1.27	1.54	1.28	3.12	14.6 (-2.87, 35.1)	39.2** (18.1, 64.2)	15.4 (-2.16, 36.1)	182.4** (139, 233)
High	3.07	3.17	3.27	3.67	5.63	3.18 (-12.5, 21.7)	6.52 (-9.69, 25.6)	19.5* (1.29, 40.9)	83.3** (55.4, 116)

Serum stored at 37°C

tFOL						5.45* (2.05, 8.96)	3.63* (0.29, 7.08)	5.73* (2.33, 9.25)	0.40 (-2.83, 3.74)
Low	16.4	16.8	17.0	16.8	16.7				
Medium	37.0	38.8	38.2	38.6	36.8				
High	58.8	60.5	60.4	60.9	58.3				
5-MethylTHF						4.11 (-4.62, 13.6)	1.00 (-7.47, 10.2)	1.79 (-6.75, 11.1)	-33.2** (-38.8, -27.1)
Low	14.8	15.0	15.0	14.6	10.6				
Medium	32.1	33.3	32.2	32.2	21.3				
High	46.9	47.7	46.7	46.2	29.9				
Folic acid						1.34 (-4.72, 7.78)	-3.71 (-9.47, 2.41)	0.37 (-5.63, 6.75)	-1.17 (-7.08, 5.11)
Low	0.72	0.70	0.70	0.71	0.69				
Medium	3.82	3.82	3.82	3.83	3.89				
High	9.06	8.99	9.07	9.25	9.34				
MeFox									
Low	0.84	1.12	1.29	1.46	5.41	32.6** (16.7, 50.7)	52.3** (34.0, 73.1)	72.8** (52.1, 96.4)	541** (464, 628)
Medium	1.09	1.68	2.14	2.60	11.6	54.4** (35.9, 75.5)	96.8** (73.2, 124)	139** (110, 172)	966** (838, 1111)
High	2.84	3.85	4.67	5.43	19.1	35.4** (19.1, 53.8)	64.2** (44.5, 86.6)	90.9** (68.0, 117)	571** (491, 663)

[1] Serum QC pools stored at -70°C were used as reference; statistical analysis by 2-way ANOVA

[2] Values are geometric means from 4 replicates/condition

[3] Values are percent change (95% Cl) compared to reference across the QC pools except for MeFox where a significant interaction between time and pool (P-interaction = 0.0072 at room temperature and P-interaction = 0.0013 at 37°C) necessitated providing separate estimates for each pool

* $0.0001 \geq P < 0.05$; ** $P < 0.0001$

Supplemental Table 2. Effect of long-term storage at suboptimal temperature (5ºC) for up to 12 mo on serum folate concentrations[1]

Analyte	Serum folate concentration[2]			Relative change[3]
QC pool	Reference	6 mo	12 mo	6 mo
		nmol/L		%
tFOL				-49.6** (-52.3, -46.8)
Low	17.5	8.68	5.47	
Medium	40.2	19.6	13.4	
High	63.7	33.6	22.9	
5-MethylTHF				**-**74.0** (-76.6, -71.1)
Low	14.8	4.09	0.97	
Medium	32.6	7.97	1.92	
High	48.2	12.5	2.53	
Folic acid				-5.42 (-11.1, 0.59)
Low	0.84	0.78	0.77	
Medium	3.88	3.66	3.69	
High	9.16	8.81	8.54	
MeFox				362** (294, 443)
Low	0.62	2.51	2.52	
Medium	0.86	5.16	4.99	
High	1.90	7.64	7.28	

[1] Serum QC pools stored at -70ºC were used as reference; statistical analysis by 2-way ANOVA

[2] Values are geometric means from 6 replicates/condition

[3] Values are percent change (95% Cl) compared to reference across the QC pools (no significant interaction between time and pool)

** *P* <0.0001