**Supplemental Method:**

The concentrations of MTX PG2-5 in erythrocytes were measured using a validated ion pairing liquid chromatography method with tandem mass spectrometric detection. First, MTX PGs were extracted as follows: erythrocyte samples (200 µL) were lysed in an internal standard solution containing deuterated MTX PGs (210 µL) followed by precipitation with perchloric acid (40 µL). The samples were centrifuged at 20,000 x g for 5 minutes, and the supernatant filtered prior to transfer onto an Atlantis dc18 column (3 μm, 1 x 50 mm, Waters, Milford, MA). MTX PGs were eluted using a gradient from 10% to 25% mobile phase B, followed by a wash up to 90% mobile phase B, with mobile phase A composition at 10 mM ammonium bicarbonate and 5 mM dimethylhexylamine (DMHA) in water pH ~7.4 with formic acid, and mobile phase B composition at 5mM DMHA and 0.1% formic acid in acetonitrile. Detection of the MTX PGs and the internal standards was performed by positive electrospray ionization on an API-4000 Triple Quad mass spectrometer (AB Sciex LLC, Framingham, MA). The calibration curve for the MTX PGs (2 through 5) ranged from 1 to 100 nM each. The inter-run mean precision values at the lower limit of quantitation (LLOQ) were ≤ 7.5% and mean bias was between –8.2 and –2.2% across all MTX PGs. The inter-run mean bias values for the QCs of MTX PGs were between –5.4% and 1.8% and the inter-run precision values were ≤ 7.7%. MTX-(PG1) was measured separately using a validated liquid chromatography method with tandem mass spectrometric detection. After extraction from erythrocytes, using a method similar to that described above, the filtered supernatant was transferred onto an XBridge BEH Shield RP18 column (2.5 μm, 2.1 x 50 mm, Waters, Milford, MA). MTX was eluted using a gradient from 0% to 70% mobile phase B, with mobile phase A composition at 10mM ammonium bicarbonate and 0.1% ammonium hydroxide in 5/95 (v/v) methanol/water, and mobile phase B composition at 5mM ammonium bicarbonate and 0.05% ammonium hydroxide in 50/50 (v/v) methanol/water. Detection of MTX and the internal standards was performed using electrospray ionization on an API-4000 Triple Quad mass spectrometer (AB Sciex LLC, Framingham, MA). The calibration curve for MTX ranged from 1.05 to 198 nM. The inter-run mean bias and precision values for LLOQ of MTX were 0.0% and 1.5%, respectively. The inter-run mean bias values for the QCs of MTX were between -2.0% and 2.1%. The inter-run precision values for the QCs of MTX were ≤ 2.7%.