Comparison of Five Automated Serum and Whole Blood Folate Assays

William E. Owen, MT(ASCP),[1] *and William L. Roberts, MD, PhD*[2]

Key Words: Hemolysate; Imprecision; Linearity; Method comparison

DOI: 10.1309/L2U6HH5KAYG48L40

Abstract

Serum and whole blood folate measurements are used to establish folate deficiency. Most methods used in clinical laboratories are automated, nonisotopic methods that use folate-binding protein.

Linearity, imprecision, and method comparison studies, including serum and whole blood hemolysates, were performed with the Access, Advia Centaur, ARCHITECT i2000, Elecsys 2010, and IMMULITE 2000 methods. The QuantaPhase II radioassay served as the comparison method. (Proprietary information is given in the text.)

The Access and IMMULITE 2000 methods had higher systematic errors in linearity studies than the other 3 methods. The imprecision of all methods was acceptable (coefficient of variation, <10%) even at low folate concentrations with the exception of the Elecsys 2010 (coefficient of variation, 16%). Method comparison studies using serum samples revealed calibration differences between the Access and Elecsys 2010 methods and the comparison method. Method comparison studies using whole blood samples showed poorer agreement between each of the automated methods and the comparison method than was seen with serum samples.

The ARCHITECT i2000 folate assay demonstrated the best analytic performance. The poor agreement seen with whole blood hemolysates likely is due to calibration differences and differences in hemolysate preparation conditions.

Folic acid is a water-soluble vitamin that serves as a cofactor in 1-carbon transfers required for DNA replication. Its deficiency is associated with a megaloblastic anemia. More recently, it has been of interest for the prevention of neural tube defects and in homocysteine metabolism in the prevention of cardiovascular disease.[1-4] Measurement of the serum or plasma folate concentration reflects recent dietary intake, while measurement of the erythrocyte folate concentration is a better indicator of body stores.[5] A number of methods can be used to quantify folate, including microbiologic methods, assays using folate-binding protein, and various chromatographic techniques.[6-9] Most current commercial assays use folate-binding protein with chemiluminescence detection.[10] The National Health and Nutrition Examination Survey, which was conducted from 1988 to 1994, provides information on the health and nutritional status of a wide cross-section of the US civilian population.[11] Samples included 23,378 serum folate determinations and 23,082 RBC folate determinations using the QuantaPhase II kit (Bio-Rad, Hercules, CA), a radioassay, at the Centers for Disease Control and Prevention. The purpose of the present study was to evaluate how current automated serum and whole blood folate assays perform and specifically to determine how they compare with the QuantaPhase II method.

Materials and Methods

Surplus serum and whole blood samples submitted for folate testing were obtained from the clinical laboratory after clinical testing was complete. Both serum and whole blood samples were stored for up to 2 weeks at –20°C. All patient

identifiers were removed, samples were divided into multiple aliquots, and aliquots were stored at –70°C until analysis. All aliquots were subjected to exactly the same handling procedures before analysis by each method. All studies using samples from human subjects were approved by the institutional review board of the University of Utah Health Sciences Center (Salt Lake City).

The following automated methods and instruments were evaluated: the Access (Beckman Coulter, Brea, CA), the Advia Centaur (Folate BA method, Bayer Diagnostics, Tarrytown, NY), the ARCHITECT i2000 (Abbott Diagnostics, Abbott Park, IL), the Elecsys 2010 (Roche Diagnostics, Indianapolis, IN), and the IMMULITE 2000 (Diagnostics Products, Los Angeles, CA). The QuantaPhase II method was selected as the comparison method. All methods examined in the study are competitive, use folate-binding protein, were used according to their manufacturers' instructions, and required manual preparation of hemolysates for whole blood folate determinations. The hemolysate for the Access whole blood folate method was prepared by making a 1:21 dilution of whole blood with 0.2% ascorbic acid. For the Advia Centaur method, a 1:21 dilution of whole blood was made with 1.0% ascorbic acid. For the ARCHITECT i2000 method, a 1:11 dilution of whole blood was made with a solution containing 0.4% ascorbic acid and guanidine hydrochloride. A second 1:2 dilution with citric acid and guanidine hydrochloride was made before analysis. For the Elecsys 2010 method, a 1:31 dilution of whole blood was made with 0.2% ascorbic acid. For the IMMULITE 2000 method, a 1:5 dilution of whole blood was made with 0.1% ascorbic acid. For the QuantaPhase II method, a 1:11 dilution of whole blood was made with 0.4% ascorbic acid, and a second 1:2 dilution was made with assay diluent before analysis.

Linearity was assessed by preparing 2 serum pools by combining multiple patient samples, one with a low concentration of folate and the other with a high concentration. The high pool was diluted with the low pool to obtain final concentrations of the high pool of 0%, 10%, 20%, 40%, 60%, 80%, and 100% of the original. Each sample used to assess linearity was assayed in duplicate by each automated method. Assay imprecision was assessed by using 3 concentrations of Lyphocheck quality control material (Bio-Rad Laboratories), which was reconstituted, multiple bottles were pooled, and aliquots for daily use were prepared and stored frozen at –70°C until used. Duplicate analyses were performed for each run, and 2 runs per day were performed on 5 days. EP Evaluator Release 4 software (David G. Rhoads Associates, Kennett Square, PA) was used for complex imprecision calculations, Deming regression analysis, calculation of r and $S_{y/x}$, and linearity assessment. For Deming regression analysis, the imprecision of each method, including the comparison method, was taken to be the same.

Results

The linearity of each method was evaluated by using pooled patient serum samples as described **Figure 1**. The slopes of the regression lines differed substantially between methods. The Access method had the lowest slope (13.9), and the Advia Centaur method had the highest slope (30.6). The ARCHITECT i2000 and Elecsys 2010 methods had similar slopes, while the slope of the IMMULITE 2000 was slightly higher than these two. The target value for each linearity sample was calculated individually for each method by using the measured concentrations of the "0%" pool and the highest linearity sample that fell within the analytic measuring range. The Advia Centaur had a maximum average deviation from the target recovery of 11.4%; the IMMULITE 2000, 16.6%; and the Access, 22.1%. However, if an arbitrary 10% deviation limit from the target values was used, only the ARCHITECT i2000 (maximum deviation from the target value, 4.6%) and Elecsys 2010 (maximum deviation, 9.6%) methods would be deemed acceptable. The imprecision of each assay was assessed with commercial quality control materials **Table 1**. All methods except the Elecsys 2010 demonstrated total imprecision of less than 10%, even at the lowest folate concentration examined, which fell in the folate-deficient range for serum.

Method comparison studies were performed using serum samples **Figure 2** and **Table 2**. These demonstrated varying degrees of agreement with the comparison method, with slopes ranging from 0.60 to 0.99 and correlation coefficients of 0.83 to 0.95. The differences in slope may be due to calibration issues. The Access method had a slope of 0.60 compared with the QuantaPhase II comparison method. It also demonstrated 1 point that was a statistical outlier even after repeated testing. The reason for this discordant result that was unique to the Access method is unknown. The Elecsys 2010 method had a slope of 0.76 relative to the comparison method. This could be due to a calibration difference; however, the linearity study shown in Figure 1D does not support this hypothesis. It also is noteworthy that the dispersion of data around the regression line as reflected in either the correlation coefficient or the SE of the estimate is less than desirable. The ARCHITECT i2000 method demonstrated the best overall agreement with the comparison method.

Method comparison studies were performed using whole blood samples **Figure 3** (Table 2) and, in general, showed poorer agreement with the comparison method than was seen with serum samples. The calibration difference seen with the Access method for serum was not confirmed with whole blood samples, which gave a slope of 1.34. The Advia Centaur method had a slope of 1.20 compared with the comparison method. The ARCHITECT i2000 method

DOI: 10.1309/L2U6HH5KAYG48L40

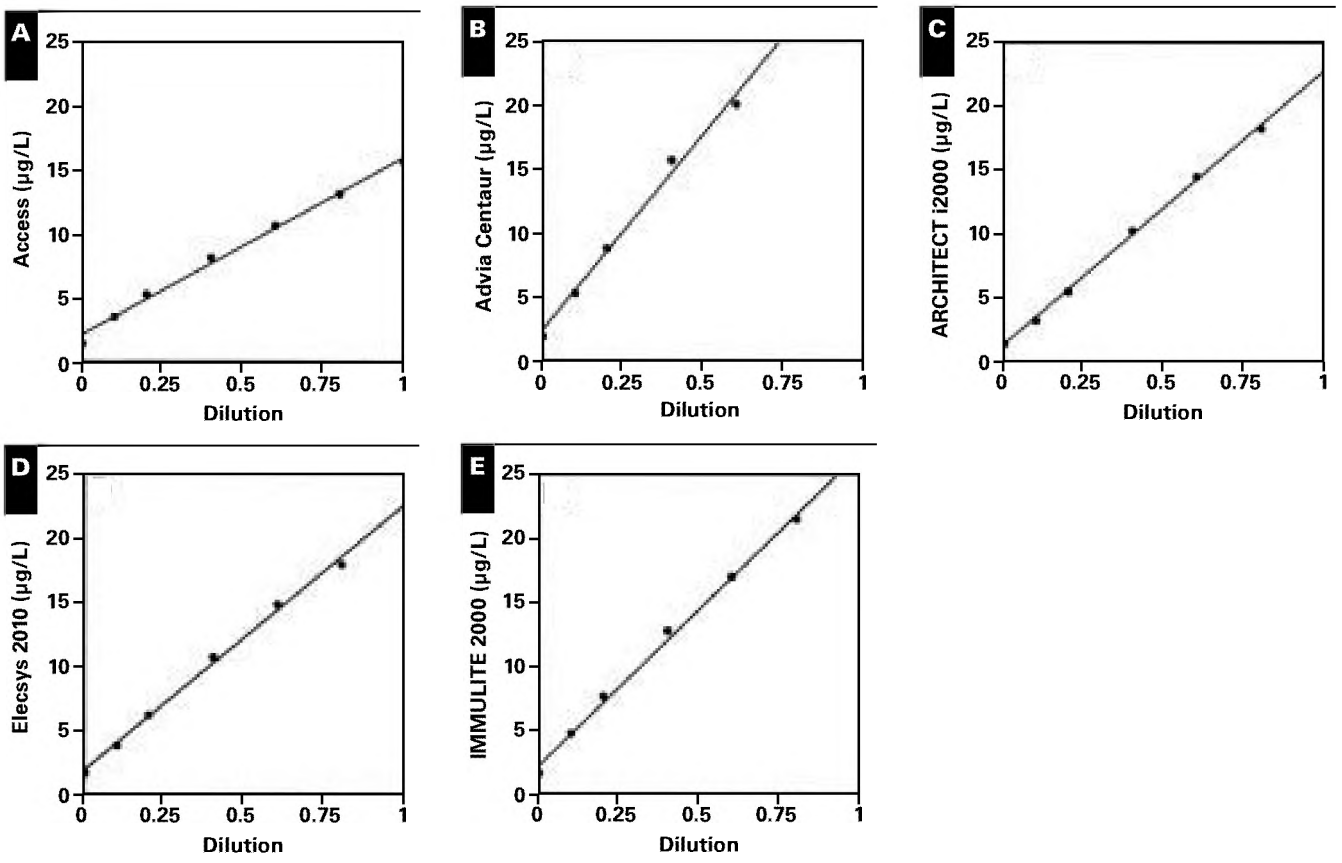

Figure 1 Linearity of automated serum folate methods. Aliquots of the same serum samples were used for each method. The solid lines are linear regression analysis. **A**, Evaluation of the Access method. Linear regression analysis gave a slope of 13.9, an intercept of 2.13 µg/L, and $r = 0.994$. **B**, Evaluation of the Advia Centaur method. Linear regression analysis gave a slope of 30.6, an intercept of 2.41 µg/L, and $r = 0.992$. **C**, Evaluation of the ARCHITECT i2000 method. Linear regression analysis gave a slope of 21.5, an intercept of 1.38 µg/L, and $r = 0.999$. **D**, Evaluation of the Elecsys 2010 method. Linear regression analysis gave a slope of 20.8, an intercept of 1.92 µg/L, and $r = 0.996$. **E**, Evaluation of the IMMULITE 2000 method. Linear regression analysis gave a slope of 24.5, an intercept of 2.37 µg/L, and $r = 0.996$. For proprietary information, see the text.

also had a negative intercept (–39) but a slope of 1.06. The Elecsys 2010 method had a slope of 1.88 and a negative intercept (–27). The IMMULITE 2000 had a slope of 2.99 but a large negative intercept (–141). Correlation coefficients for all methods were higher than for serum samples. The ARCHITECT i2000 method performed the best of the automated methods evaluated in this study for whole blood folate determinations.

Discussion

Of the 5 automated folate methods evaluated in this study, the ARCHITECT i2000 and Elecsys 2010 demonstrated the best linearity with recoveries for each point falling within ±10% of the target value. The linearity of the Advia Centaur method was slightly worse. The Access and IMMULITE 2000 methods had higher deviations from ideal recoveries than the other 3 methods. The lack of linearity exhibited by the Access method, although similar in some respects, is much less pronounced than what has been published for the AxSYM folate method.[12,13] The imprecision of all methods was acceptable with coefficients of variation of less than 10%, even at low folate concentrations, with the exception of the Elecsys 2010 method. It had an overall imprecision of 16% at the lowest concentration of folate examined. Method comparison studies using serum samples revealed substantial calibration differences between some of the automated methods and the QuantaPhase II comparison method. The Access method yielded lower results for patient serum samples compared with the QuantaPhase II method. This presumed difference in calibration is reflected in the linearity samples (Figure 1A) but not in the means of repeated analysis of quality control material (Table 1). This may reflect a matrix effect with the control materials. The Elecsys 2010 method also yielded lower results compared

DOI: 10.1309/L2U6HH5KAYG48L40

Table 1
Summary of Imprecision Data

Method*	Level†	Folate Concentration (µg/L)		Coefficient of Variation (%)	
		Mean	Target Mean‡	Within Run	Total
Access	1	2.2	2.2	7.7	9.9
	2	6.0	5.8	1.6	3.8
	3	11.7	11.1	2.4	5.9
Advia Centaur	1	2.4	2.3	5.4	8.0
	2	5.5	5.7	2.5	6.4
	3	9.3	9.9	4.4	8.0
ARCHITECT i2000	1	2.1	2.1	8.2	8.4
	2	4.6	5.0	2.8	4.2
	3	9.9	11.1	1.0	2.3
Elecsys 2010	1	1.7	2.2	9.9	16.0
	2	3.8	4.4	3.6	11.6
	3	6.6	7.3	2.9	7.0
IMMULITE 2000	1	2.0	2.0	4.5	8.8
	2	6.5	5.9	3.3	7.5
	3	12.3	11.1	2.0	4.8

* For proprietary information, see the text.
† Level 1 corresponded to lot number 40121, level 2 to lot number 40122, and level 3 to lot number 40123. Together these constitute lot 40120.
‡ The target mean folate concentration was the cumulative mean from the Unity Worldwide Report for Immunoassay Plus provided by Bio-Rad Laboratories (Hercules, CA) for November 2002 for lot 40120. This information serves to confirm that each of the methods was calibrated in a manner consistent with what would be expected based on data from multiple clinical laboratories.

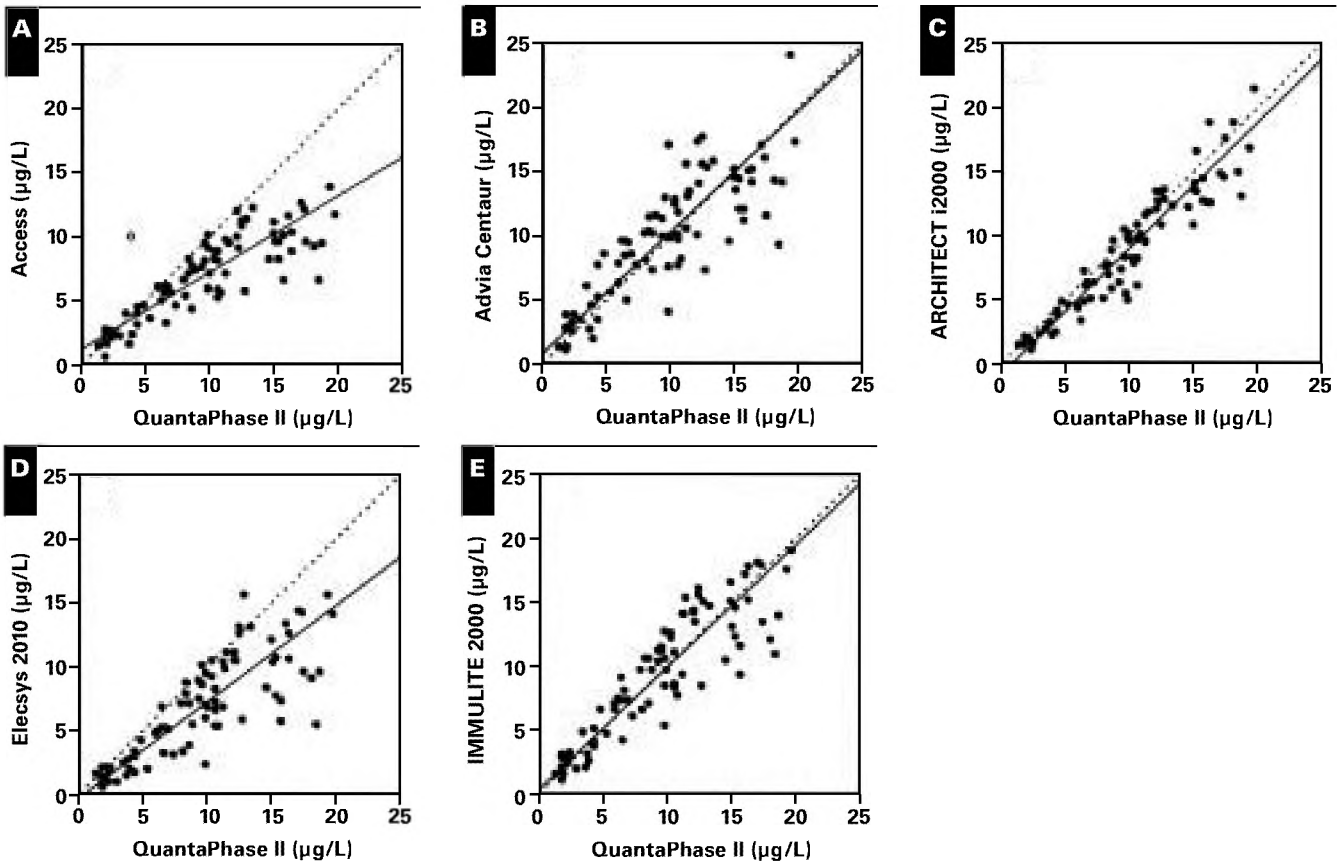

Figure 2 Comparison of automated serum folate methods (**A**, Access; **B**, Advia Centaur; **C**, ARCHITECT i2000; **D**, Elecsys 2010; **E**, IMMULITE 2000) with the QuantaPhase II comparison method. The solid lines are from Deming regression, and the dashed lines are x = y. Unless otherwise indicated, 83 samples were included in the analysis. In the comparison of the Access method (**A**), 1 outlier, indicated by an open circle, was excluded from further analysis. The regression statistics are given in Table 2. For proprietary information, see the text.

DOI: 10.1309/L2U6HH5KAYG48L40

Table 2
Summary of Deming Regression Statistics for Serum and Whole Blood Samples

Method	Sample Type	Slope	Y Intercept (µg/L)	SE of the Estimate (µg/L)	*r*
Access	Serum	0.60 ± 0.04	1.17 ± 0.40	1.68	0.858
	Whole blood	1.34 ± 0.08	17 ± 16	40	0.940
ADVIA Centaur	Serum	0.95 ± 0.06	0.83 ± 0.66	2.78	0.833
	Whole blood	1.20 ± 0.15	79 ± 29	76	0.725
ARCHITECT i2000	Serum	0.99 ± 0.03	−0.94 ± 0.37	1.58	0.950
	Whole blood	1.06 ± 0.06	−39 ± 11	28	0.954
Elecsys 2010	Serum	0.76 ± 0.05	−0.42 ± 0.54	2.27	0.835
	Whole blood	1.88 ± 0.11	−27 ± 21	54	0.944
IMMULITE 2000	Serum	0.96 ± 0.05	0.32 ± 0.55	2.32	0.887
	Whole blood	2.99 ± 0.20	−141 ± 40	103	0.919

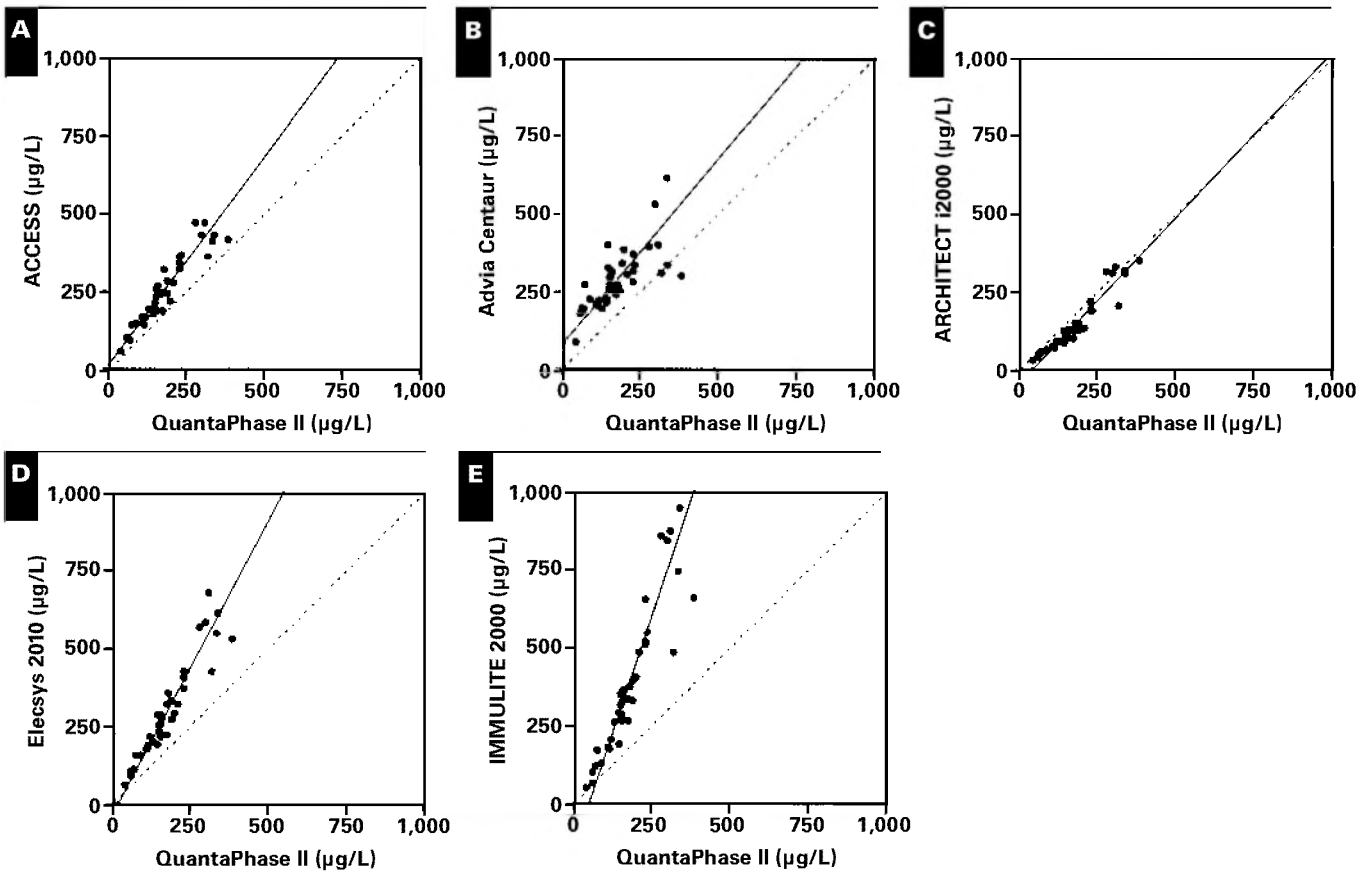

Figure 3 Comparison of automated whole blood folate methods (**A**, Access; **B**, Advia Centaur; **C**, ARCHITECT i2000; **D**, Elecsys 2010; **E**, IMMULITE 2000) with the QuantaPhase II comparison method. The solid lines are from Deming regression, and the dashed lines are x = y. A total of 37 samples were included in the analysis. The regression statistics are given in Table 2. For proprietary information, see the text.

with the QuantaPhase II method. The results of repeated analysis of quality control material (Table 1) reflect this presumed calibration difference, while the linearity sample (Figure 1D) does not. The basis for these differences is uncertain. The agreement between these 2 methods was better than that between either one and the comparison method (data not shown). Additional calibration standardization efforts seem to be required for some of these methods.

Previous studies examining folate assay performance found higher overall variability for whole blood results compared with results for serum samples.[7] Some possible reasons for the generally poorer folate method comparisons seen with whole blood samples have been described previously. Folate can be trapped irreversibly by oxyhemoglobin.[14] It has been suggested that hemoglobin can irreversibly denature at low pH (<5.0) and trap folate, although

DOI: 10.1309/L2U6HH5KAYG48L40

this is controversial.[15,16] Furthermore, incomplete lysis of RBCs also can contribute to underrecovery of folate. More complete hemolysis can be obtained by the addition of saponin or Triton X-100 nonionic detergent.[15,16] A careful review of the manufacturer-specified conditions used to prepare whole blood hemolysates for each of the methods showed that a range of ascorbic acid concentrations and dilution factors were recommended. The whole blood folate method comparison between the Elecsys 2010 and IMMULITE 2000 methods and QuantaPhase II comparison method demonstrated substantially higher results for both, while with serum samples the 2 methods were comparable. The reason for this apparent overrecovery with whole blood samples is unclear. The ARCHITECT i2000 method demonstrated the best agreement with the comparison method. Both this method and the QuantaPhase II comparison method required a 1:11 dilution with 0.4% ascorbic acid for initial hemolysis preparation followed by a second 1:2 dilution. This may, at least in part, explain the good agreement seen for these 2 methods. It is noteworthy that hemolysate for the ARCHITECT i2000 method also contained guanidine hydrochloride and citric acid. The role of these agents is unknown.

From the [1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT; and the [2]Department of Pathology, University of Utah Health Sciences Center, Salt Lake City.

Supported by Abbott Diagnostics and by the ARUP Institute for Clinical and Experimental Pathology.

Address reprint requests to Dr Roberts: c/o ARUP Laboratories, 500 Chipeta Way, Salt Lake City, UT 84108.

References

1. MRC Vitamin Study Research Group. Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. *Lancet*. 1991;338:131-137.
2. Czeizel AE, Dudas I, Metneki J. Pregnancy outcomes in a randomized controlled clinical trial of periconceptional multivitamin supplementation; final report. *Arch Gynecol Obstet*. 1994;255:131-139.
3. Green R, Jacobsen DW. Clinical implications of hyperhomocysteinemia. In: Bailey LB, ed. *Folate in Health and Disease*. New York, NY: Marcel Dekker; 1995:99-102.
4. Selhub J, Jacques PF, Wilson PWF, et al. Vitamin status and intake as primary determinants of homocysteine in an elderly population. *JAMA*. 1993;270:2693-2698.
5. Sauberlich HE. Folate status of US population groups. In: Bailey LB, ed. *Folate in Health and Disease*. New York, NY: Marcel Dekker; 1995:171-194.
6. van den Berg H, Finglas PM, Bates C. FLAIR intercomparisons on serum and red cell folate. *Int J Vitam Nutr Res*. 1994;64:288-293.
7. Gunter EW, Bowman BA, Caudill SP, et al. Results of an international round robin for serum and whole-blood folate. *Clin Chem*. 1996;42:1689-1694.
8. Deuker SR, Lin Y, Jones AD, et al. Determination of blood folate using acid extraction and internally standardized gas chromatography–mass spectrometry detection. *Anal Biochem*. 2000;283:266-275.
9. Fazili Z, Pfeiffer CM. Stable-isotope-labeled LC/MS/MS method on Sciex API3000 for folate measurements in serum [abstract]. *Clin Chem*. 2002:48:A72.
10. College of American Pathologists. *Chemistry Survey Series K, Set B*. Northfield, IL: College of American Pathologists; 2002.
11. Wright JD, Bialostosky K, Gunter EW, et al. Blood folate and vitamin B12: United States, 1988-94. *Vital Health Stat 11*. 1998;243:1-78.
12. Billen J, Zaman Z, Claeys G, et al. Limited dynamic range of a new assay for serum folate. *Clin Chem*. 1999;45:581-582.
13. Manzella S, Gronowski A, Ladenson J, et al. Limited linear range of the Abbott AxSYM serum and erythrocyte folate methods. *Clin Chem*. 1999;45:582-583.
14. Wright AJA, Finglas PM, Southon S. Erythrocyte folate analysis: a cause for concern? *Clin Chem*. 1998;44:1886-1891.
15. Wright AJA, Finglas PM, Southon S. Erythrocyte folate analysis: saponin added during lysis of whole blood can increase apparent folate concentrations, depending on hemolysate pH. *Clin Chem*. 2000;46:1978-1986.
16. O'Broin S, Kelleher B. Optimization of erythrocyte folate extraction [letter]. *Clin Chem*. 2001:47;2181-2182.

DOI: 10.1309/L2U6HH5KAYG48L40