Limited Dynamic Range of a New Assay for Serum Folate

To the Editor:

Recently, several new nonisotopic protein-binding folate assays have become available. We have studied the AxSYM folate assay (Abbott Laboratories, Abbott Park, IL), in which folate is quantified by measurement of unoccupied binding sites on the folate-binding protein (1). The calibrators for the assay cover the range 0–45 nmol/L.

In the absence of an internationally accepted reference method or material (2), we have compared the performance of the AxSYM method with two methods commonly used nationally and internationally, namely the SimulTrac-SNB (ICN Fig. 1. Comparison of results for 100 serum folate samples. (A), AxSYM folate assay vs Quantaphase II folate assay ($y = 0.63x + 2.8$ nmol/L; $r = 0.824$; $n = 100$). (B), difference of AxSYM and Quantaphase II folate plotted against the average of AxSYM and Quantaphase II folate; horizontal lines are the mean bias ($\ldots$) and $\pm 2$ SD values ($\ldots$). (C), AxSYM folate assay vs SimulTrac folate assay ($y = 0.58x + 2.7$ nmol/L; $r = 0.823$; $n = 100$). (D), difference of AxSYM and SimulTrac folate plotted against the average of AxSYM and SimulTrac folate; 1, patients with renal failure. (E), linearity study; (---), least-squares linear regression line for the points at 10–30% high sample.
Pharmaceuticals Inc.) and the Quantaphase II (Bio-Rad Laboratories). Both of these are competitive RIAs.

We selected 100 patient samples from among serum specimens received in the laboratory for determination of serum folate to obtain a concentration range up to 68 nmol/L (30 μg/L) as measured by the SimulTrac-SNB assay. The sera were frozen at -20°C until analysis. After thawing, all three comparison assays were performed on the same day. Sample collection procedures were in agreement with the guidelines of the hospital's Ethical Commission.

Results by the two comparison methods agreed well, with Passing–Bablok (3) analysis yielding the following regression equation: y (Quantaphase II) = 0.93x (SimulTrac) – 0.23 nmol/L; r = 0.942; n = 100. In comparisons of the AxSYM assay with the two isotopic methods, the regression plots (Fig. 1, A and C) and the Bland–Altman (4) bias plots (Fig. 1, B and D) suggested regression lines with three different slopes. Above 23 nmol/L (10 μg/L) as measured by the comparison methods, the AxSYM method produced a flat response. Linearity of the assays was tested by mixing a very high (45 nmol/L) and a very low sample (1 nmol/L) to obtain increasing concentrations in steps of 10%. Each mixture was tested in duplicate, and statistical evaluations were made by linear regression analysis. The AxSYM method showed significant nonlinearity (Fig. 1E). The analogous plots for SimulTrac and Quantaphase II were linear between 10% and 100% of high sample, with r² = 0.996 and r² = 0.999, respectively. The measured/expected concentration was between 99% and 101% at the highest values.

Although the manufacturer’s product insert states that “some samples in the upper region of the dynamic range may read lower in the AxSYM assay when compared with some other commercial assays”, our data suggest that samples >23 nmol/L (the middle of the dynamic range) routinely read lower in the AxSYM assay than in either of the two widely used methods. In agreement with the manufacturer’s warning that “serum and plasma specimens from patients with renal impairment may exhibit varying degrees of falsely depressed values”, marked disagreement was observed between the AxSYM and the comparison methods for the serum specimens of chronic renal failure patients (indicated by the numeral 1 in Fig. 1).

Similar apparent limitations in the dynamic range of the AxSYM method appeared to be present when folate was measured in erythrocyte hemolysates. At concentrations >906 nmol/L (>400 μg/L) with the SimulTrac method and >453 nmol/L (>200 μg/L) with the Quantaphase II method, the regression lines were found to bend. We saw no erythrocyte folate results >1360 nmol/L (>600 μg/L) by the AxSYM method, even in patients known to be taking folate supplements regularly for a protracted period of time.

We conclude that the dynamic range of the AxSYM folate assay does not extend above 23 nmol/L (10 μg/L) for serum folate and not above 1360 nmol/L (600 μg/L) for erythrocyte folate. This is only half the dynamic range of other protein-binding folate assays.

References

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Editor’s Note: A representative of the manufacturer declined the opportunity to reply.

Limited Linear Range of the Abbott AxSYM Serum and Erythrocyte Folate Methods

To the Editor:
Recent studies have clearly demonstrated that plasma homocysteine is an independent risk factor for atherosclerosis (1, 2). Although increased serum homocysteine is genetically determined, increasing dietary folate (3) can significantly lower the values. Indeed, the US Food and Drug Administration has recently increased the daily dietary guidelines for folate intake, and since January 1998 wheat flour-based foods have been supplemented with folate (4, 5). Decreased folate during pregnancy is also a strong risk factor for neurological birth defects (6). Historically, plasma and red blood cell (RBC) folate determinations have been performed to assess folate deficiencies in investigations of macrocytic anemias, and little or no clinical information was ascribed to normal or increased folate values. However, the recent goals of reducing serum homocysteine and maintaining high folate concentrations during pregnancy suggest that examination of serum and RBC folate concentrations with an interest in higher values may become common. Indeed, it has been recommended by some that the lower limit of the reference range for plasma folate be raised by 250% (7). Thus, it is likely that folate concentrations will be increased in the population and that accurate determination of high as well.