Letters to the Editor should be typed double-spaced (including references) with conventional margins. The overall length is limited to five manuscript pages, including not more than one figure or one table.

Determination of Citrate In Urine by Simple Direct Photometry

To the Editor:

I attempted to perform the urinary citrate method of Millán et al. (1), as corrected (2). However, I was unable to confirm the claimed sensitivity.

The method is based on the formation of a yellow ferric citrate complex measured photometrically at 390 nm. There has been some confusion as to the exact procedure to follow. To avoid adding further to that confusion, the procedure that I chose is as follows:

1. To 4 mL of sample add 0.1 mL of NH₄OH (30%) and 0.9 mL of MgCl₂ solution (0.2 mol/L). Filter (or centrifuge) to obtain the phosphate-free urine. Adjust pH to 2 with 0.1 mL of 10 mol/L HCl.
2. To 0.25 mL of urine (phosphate free) add 4.5 mL of HCl (pH 2), then add 0.25 mL of the ferric chloride reagent (FeCl₃·6H₂O, 18 mmol/L in HCl, 1 mol/L). Read against a reagent blank of 0.25 mL of ferric reagent in 4.75 mL of HCl (pH 2).
3. Prepare a urine blank, 0.25 mL of urine (phosphate free) plus 4.75 mL of HCl. Read against pH 2 HCl.
4. Subtract the absorbance of the urine blank from the absorbance of the urine sample. Obtain the citrate concentration from a calibration curve. (Treat standard citrate solutions exactly as urine specimens.) I read absorbances with a Uvikon 680 spectrophotometer.

The color of the ferric chloride reagent is yellow. The color of the ferric citrate product is also yellow. The mean absorbance value of the reagent blank was 0.145. The range of the urine blank absorbance values was 0.45-0.120. At citrate concentrations <2.5 mmol/L, the final absorbance change in the reaction, after subtracting reagent and urine blank values, was <0.020. In an attempt to improve the sensitivity, I doubled the sample volume (0.5 mL); however, the absorbance change for citrate concentrations <2.5 mmol/L remained <0.020. Interference from the urine blank, however, increased with the sample volume.

The method was linear for citrate concentrations between 2.5 and 12.5 mmol/L (n = 5). Millán et al. claim linearity from 52.9 to 696.6 µmol/L. Taking into account the sample dilution, I presume this is equivalent to urinary concentrations of citrate from 1 to 14 mmol/L, although this is not clear from the Technical Brief.

At concentrations of citrate <2.5 mmol/L, I was unable to accurately detect the ferric citrate produced, owing to the large reagent and urine blank absorbances. This method, therefore, lacks the sensitivity to accurately identify low-citrate excreters.

References

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Accuracy of Reflotron Cholesterol Assays Evaluated

To the Editor:

In an interesting paper, Bachorik et al. (1) report on cholesterol method-comparison studies in four Lipid Research Clinics. Their approach to calculating the inaccuracy of the Reflotron total cholesterol assay is not flawless, for two reasons:

(a) Recommended guidelines for management of cardiovascular risk indicators have been published in the medical literature in recent years. Such consensus reports have appeared in several countries, beginning in the U.S.A. (2–5). Although, unfortunately, in these documents sometimes the term "blood cholesterol" is used, there is no doubt that the proposed cutpoints to distinguish normal risk from increased risk are values for serum cholesterol. When EDTA is used as an anticoagulant, it is known to cause a slight osmotic water shift from cells to plasma. This dilution lowers the obtained cholesterol values by 3% (6). Varying amounts of EDTA and the use of different brands of tubes may cause this difference to vary somewhat. The often-quoted 3% difference was found when 1 mg of EDTA was used per milliliter of blood (6), whereas Bachorik et al. (1) took 1.5 mg/mL, which may have caused a slightly larger than 3% dilution. Cholesterol standardization is highly recommended (2–5) in connection with consensus guidelines, and it has become our goal to reduce analytical bias in cholesterol assays (including those performed in screening procedures) to <3% within the next two years. We therefore cannot allow a difference of the same magnitude between plasma and serum—without correction. A screening method for whole-blood cholesterol should be made comparable to serum cholesterol assays. My rough estimate is that the bias of −0.8% to 7.8% found in this study of the Reflotron assay would have been −4% to +3% in a serum comparison.

(b) The authors discuss the differences they have found in some detail. This is done on the basis of linear-regression calculations. Although the results of linear-regression analysis vs orthogonal regression may be comparable in some data, it is certainly not ideal to use the first when the coefficient of correlation (r) is low (<0.97). It is better to carry out an orthogonal or a Passing & Bablok regression (7) in circumstances like those in Table 7 of reference 1. Otherwise, it is almost certain that the calculated intercepts are overestimations.

Further: it is surprising that two of the Lipid Research Clinics did not receive correctly calibrated Reflotron reagents at the start of the study (supposing the work began around mid-1988). After reports in the literature had pointed to a negative analytical bias in the test strips, Boehringer Mannheim Co. contacted the Centers for Disease Control and our Lipid Reference Laboratory in Rotterdam to assist in improving the calibration. Since early 1988, the Rotterdam Reference Laboratory, an international collaborating member of the U.S.A. National Reference Method Laboratory Network, being close to Mannheim (F.R.G.), has provided the values...