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Automated Analysis for Urinary Protein by Pyrogallol
Red-Molybdate Method

To the Editor:

Recently, considerable interest has arisen concerning the pyrogallol red-molybdate method for determining urinary protein. This procedure, originally described by Fujita et al. (1), measures the blue color formed when pyrogallol red-molybdate (IV) complex binds basic amino acid groups (2). Protein in urine can be quantified by this method with good sensitivity, linearity, and reproducibility. Addition of sodium dodecyl sulfate to the reaction mixture equalizes the response of the reagent for human gamma globulins and albumin (2).

This assay has been adapted to various automated analyzers, such as the Hitachi 726 (3) and the Cobas Bio (2). Because the reagent reportedly does not adsorb onto the wall of cuvettes, we decided to implement this method on an SMA-I continuous-flow analyzer (CFA); for method evaluation, we used a Mark-II CFA (both from Technicon Instruments Corp., Tarrytown, NY).

Initially, good results were obtained with use of the following conditions: sample time 40 s, wash 10 s, predilution 1:10, reagent 0.2 ml/min, measurement 5 min after mixing. The reagent was used exactly as described by Watanabe et al. (3), with addition of Brj-35 surfactant, 1 ml/L. Linearity, reproducibility, and sensitivity were comparable with or superior to the manual method. However, although we confirm that the reagent itself does not stick to the wall of cuvettes, routine use of the method was limited by adsorption of the blue product onto the wall of the cuvette, producing an unacceptable baseline drift, when samples containing high concentrations of protein (>5 g/L) were analyzed. Use of Tween as surfactant or greater predilution (1:20) of the samples did not solve this problem.

In our view, application of the pyrogallol red-molybdate method for determining urinary protein with a CFA is not possible in a clinical setting in which samples containing high protein concentrations are to be analyzed. Automation of this method may be limited to instruments with single-use cuvettes.

References

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Measurement of Zinc Protoporphyrin with the ProtoFluor-Z System

To the Editor:

In their discussion, Stanton et al. (I) compared the measurement of zinc protoporphyrin (ZPP) by the recently introduced ProtoFluor-Z System (Helena Laboratories, Inc., Beaumont, TX) with that by established hematofluorometers. They cite several problems encountered with hematofluorometry and conclude that "the ProtoFluor-Z is no different from any other model of hematofluorometer," referring to plasma interference. Having participated in the development of the ProtoFluor-Z System, we take issue with this conclusion. Although some interference from plasma components is common to hematofluorometry, the major problems of hemoglobin oxygenation and instrument calibration have been resolved. The ProtoFluor-Z System effectively and totally eliminates the oxygenation issue, which can give false-negative results, and allows regular calibration by the user.