Use of an Enzymatic Kit Method for Cholesterol, Designed for Continuous-Flow Instrumentation, with Discrete Bichromatic and Centrifugal Analyzers

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I examined a cholesterol-assay kit ("Autoflo"; Biodynamics/bmc) designed for use with continuous-flow instrumentation for its use with some discrete analyzers. At pH 7.2, color develops within 3 min upon exposure to cholesterol, then begins to fade. At pH 8, the color develops more slowly but remains stable for at least 15 min after reaching a maximum. At pH 8.8, the color develops still more slowly and standards' sera behave differently. Reagent at pH 7.2 is preferred because assays can be completed most rapidly, but pH 8 reagent must be used with instruments requiring longer periods of incubation. Cholesterol assays can be performed accurately, rapidly, reproducibly, and at low cost with an Abbott ABA-50 and pH 7.2 reagent or with a Gemeni Centrifugal Analyzer and the pH 8 reagent.

Total cholesterol in serum is routinely determined by using cholesterol esterase (EC 3.1.1.13) to hydrolyze cholesterol esters, followed by cholesterol oxidase (EC 1.1.3.6) to oxidize cholesterol to cholest-4-en-3-one and hydrogen peroxide. The hydrogen peroxide is then coupled via phenol with 4-amino-phenazone to form a red dye (7). A kit containing these compounds and designed for cholesterol determinations on continuous-flow instrumentation has been described (2).

I show here how this same cholesterol kit can be used with discrete analyzers. The preparation offers several advantages over the reconstituted powdered preparations commonly recommended for use by the manufacturers of the discrete instrumentation: ease of preparation, greater stability of the liquid reagents as compared to powders, and lower cost per test.

Materials and Methods

Equipment

For cholesterol determinations, I used an ABA-50 Bichromatic Analyzer (Abbott Laboratories, South Pasadena, Calif. 91030) and a Gemeni Centrifugal Analyzer (Electro-Nucleonics Inc., Fairfield, N.J. 07006).

For kinetic monitoring studies, I used a Model 55 spectrophotometer (Perkin-Elmer Corp., Oak Brook, Ill. 60521) fitted with a 250-µl glass-jacketed flow cell and coupled to a Perkin-Elmer calculator (Coleman 5-100) and printer (Coleman 47).

All determinations were made at 37 °C.

Reagents

The commercially available kit for analysis of cholesterol ("Autoflo") was obtained from Biodynamics/bmc, Indianapolis, Ind. 46250. I used the buffer, enzymes, and chromogen from the kits, only adjusting the pH of the buffer with 1 mol/liter sodium hydroxide, then combining reagents in essentially the same proportions as indicated by the manufacturer for continuous-flow analysis. The liquid stock reagents are stable for nine months. Working reagents are prepared as follows: Soln. 1, 100 ml, pH 7.4 (ABA-50); Soln. 1, 100 ml, pH 8.2 (Gemeni); Soln. 2, 1.6 ml; Soln. 3, 1.6 ml; Soln. 4, 2.0 ml; water, to 200 ml.

The working solution is stable for at least two months when stored at 4–10 °C.

For reference reagent I used "A-Gent" cholesterol reagent (3) according to the instructions of the manufacturer, Abbott Laboratories.

As standards, I used "Preciset" cholesterol standards (Biodynamics/bmc), 1.00, 2.00, 3.00, and 4.00 g of cholesterol per liter.

Procedure

Procedure for the Gemeni is as follows: Pipet the equivalent of 5 µl of sample into appropriate sample wells of the disc. Pipet 0.7 ml of working reagent into each reagent well on the disc. A 1.00 or 1.50 g/liter standard, placed in well number three, is used to standardize the instrument. The cholesterol computer card directs a rate-ratiometric analysis at 500 nm over a period of 10 min.

Settings and procedure for the ABA-50 are as follows: filter, 500/600; decimal, 0000; coarse scaling, 1; test/end point, depressed; incubator, 37 °C. Using the Abbott microdilutor with a 1:100 plate, pipet 5 µl of sample and 0.5 ml of working reagent into each cuvet. Into cuvet 0, pipet a water sample for the zero setting. Into cuvets 1 and 2, pipet 1.00 or 1.50 g/liter standard to standardize the instrument. Pipet patients' sera, controls, and reference standards into the other cuvets. Wait 4 min. Adjust the 0 cuvet to read zero with the appropriate knob. Adjust the standards in cuvets 1 and 2 appropriately with the scaling fine adjust. Then, at 5 min immediately begin reading and recording the values of each sample.

Results

Figures 1, 2, and 3 describe the change in absorbance with time at pH 7.2, 8.0, and 8.9, respectively, for four cholesterol standards and sera containing, according to the reference method, 1.00, 2.00, 3.00, and 4.00 g of cholesterol per liter.

The reaction proceeds most rapidly at pH 7.2, reaching a
At pH 8, maximum color intensity is reached somewhat more slowly; all standards and sera reach a plateau by 9 min, whereupon the intensities of all samples are linearly proportional.

At pH 8.9, by 15 min neither the standards nor the sera reached a plateau that was linearly related in intensity, and sera developed color more slowly than the standards. At intermediate pH's the color develops over a continuum of rates between those seen in the figures.

The relationship between cholesterol concentrations and print out on the Gemeni is directly proportional up to 5.00 g/liter when the assay is performed with pH 8.0 reagent. It is not proportional above 2.00 g/liter when pH 7.2 reagent is used.

The correlation coefficient between 29 patients' sera assayed on the ABA-50 with use of the reference reagent and the same sera on the Gemeni with pH 7.2 reagent was $r = 0.991$. The linear relationship is given by the equation $y = 1.072x - 17.271$. The correlation coefficient between patients' sera assayed on the ABA-50 with pH 7.2 reagent and on the Gemeni with pH 8.0 reagent was $r = 0.976$. The linear relationship is given by the line $y = 0.933x + 9.835$. Coefficients of variation for control sera with values near 1.5 and 3.0 g/liter, assayed hundreds of times on the ABA-50 during two years, have never exceeded 3.7 and 3.5%, respectively. Coefficients of variation for control sera of 1.5 and 2.89 g/liter, assayed once each day for 30 consecutive days on the Gemeni, were 4 and 3.2%, respectively.

**Discussion**

This cholesterol preparation, designed for use on continuous-flow instrumentation, can be used to assay cholesterol rapidly, accurately, and reproducibly at 37 °C in an end-point mode on discrete analyzers.

The reagent at pH 7.2 is preferred because cholesterol can be assayed more rapidly at this pH. This pH is appropriate for instruments that can record the color between 5 and 7 min,
Urinalysis by Use of Multi-test Reagent Strips: Two Dipsticks Compared

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We compared Ames' "N-Multistix" and Boehringer's "Combur-8" ("Chemstrip-8") multi-test urine reagent strips by analysis of contrived urine specimens, testing accuracy, precision, specificity, and limits of detection of both products. Relative cost and ease of use were also examined. Each brand of urinary dipstick had specific advantages but it is unlikely that patient care would be adversely affected by preferential use of either product.

As new dipstick-type tests have been developed for urinalysis they have commonly been included in increasingly larger multi-test products. The advantages claimed for combination dipsticks include the additional relevant data obtained, the little skill required, time and cost savings, and reliability as compared to rarely used single-test reagent strips of limited storage life.

Boehringer Mannheim Corporation has recently developed an eight-test urinalysis reagent dipstick similar to Ames' "N-Multistix." Among the advantages claimed for this new product are larger test-pad areas, closer color matching, greater analytical sensitivity, greater specificity and stability of color reactions, superior construction, and an ability to differentiate between hemoglobin and intact erythrocytes. It is also claimed that the end points of the color reactions are not time dependent if the dipsticks are visually assessed between 30 and 60 s.

Some single urinary dipstick tests have been assessed previously (1), but in the present study we used multi-test strips, because these now are the most frequently and widely used in Australia. Analytical aspects of the performance of both test strips have been evaluated and compared, the samples consisting of artificially supplemented ("contrived") urine, intended to simulate pathological specimens.

Methods

N-Multistix (A; Ames, Division Miles Laboratories, Australia Pty. Ltd., Mulgrave, Vic. 3170) and Combur-8, which is marketed in the United States under the name "Chemstrip-8" (B; Boehringer Mannheim, Mount Waverley, Vic. 3149, Australia) were kindly donated for this study. Two different batches of each product were used throughout.

Urinary pH, protein, glucose, ketones, bilirubin, blood, and nitrite tests were assessed in the present study. Urobilinogen was not studied, owing to the nonavailability of satisfactory reference materials.

Contrived urines were prepared by adding analytical-grade chemicals to urine obtained from apparently healthy laboratory personnel. All such urine had a pH of 5–7, all tests were negative with both dipsticks A and B, the relative density (sp. gravity) was between 1.010 and 1.025, and the ascorbic acid concentration was less than 50 mg/liter. The concentrations of analytes in the contrived urines were verified by quantitative methods in routine laboratory use. Barnett (2) has stated that differences in results obtained with aqueous solutions and solutions of the same concentration prepared in biological fluids may indicate the presence of interfering substances, and we used this approach in delineating substances interfering with the glucose chemistry. Results of the analysis for urinary nitrite were compared with results of microscopic and microbiological examination.

Certain substances that cause positive and negative inter-