New Test for Urinary Glucose (BM33071) Evaluated

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Results for urinary glucose by the Boehringer Mannheim BM33071 test pad and a hexokinase-based method agree well. The new test, which involves the glucose oxidase/peroxidase reaction, measures as little as 260 mg of glucose per liter. Acetoacetate, β-hydroxybutyrate, and human hemoglobin do not interfere.

Additional Keyphrases: dipsticks · enzyme methods · urine

Blood glucose is widely measured in monitoring diabetic control and to screen for undiagnosed diabetics. We have evaluated the performance of a recently introduced test strip for glucose, comparing results with those by the hexokinase/glucose-6-phosphate dehydrogenase (EC 2.7.1.1/1.1.1.49) procedure (1) and assessing its sensitivity and liability to interference.

Materials and Methods

We evaluated the performance of the glucose pad "BM33071" (Bio-Dynamics, a Boehringer Mannheim Co., Indianapolis, IN 46250) by comparison with Hema-Combistix® (Ames Co., Division of Miles Laboratories, Inc., Elk hart, IN 46514). The reaction principle of BM33071 is the glucose oxidase/peroxidase (EC 1.1.3.4/1.11.17) reaction with tetramethylbenzidine as redox indicator; the reaction color changes from yellow to green with increasing glucose concentrations. The label for BM33071 has comparison colors for glucose concentrations of 0, 0.5, 1.0, 3.0, and 10.0 g/L. The Hema-Combistix glucose test has comparison colors for 0, 1.0, 2.5, 5.0, 10.0, and 20.0 g/L. According to the manufacturer, the Hema-Combistix glucose test is based on a double sequential enzyme reaction similar to that of the BM33071. However, the peroxidase catalyzes the reaction of hydrogen peroxide with a potassium iodide chromogen to oxidize a chromogen to color changes from green to brown with increasing glucose concentrations. For a quantitative comparison method, we used a hexokinase/glucose-6-phosphate dehydrogenase procedure (1) with a centrifugal analyzer (Multistat III), and reagents from Instrumentation Laboratory, Lexington, MA 02173. The accuracy of this method has previously been established (2); however, we also included aqueous glucose controls (0.5 and 1.5 g/L) with each batch of urine samples tested.

For method correlation we used 500 freshly collected patients' urines, selected to provide a range of glucose concentrations, refrigerating them at 4°C until analysis (not more than 5 h). We tested each urine with the BM33071 strips, reading the color reaction 1 min after immersion, and with Hema-Combistix, reading the color reaction at 30 s after immersion, both as recommended by the manufacturers. The time between dipstick analysis and the hexokinase comparison method never exceeded 30 min.

To establish the practical level of sensitivity for the glucose test on the BM33071, we used the procedure suggested by Kutter (3). The practical sensitivity, which allows the quantitative assessment of the analytical detection limit for diagnostic tests, has a yes/no answer, is expressed as that specific concentration of an analyte in a body fluid that causes a positive result in 90% of all tested samples. We tested 20 fresh urine samples that were separated into six portions and had glucose added to give a final concentration of 0, 200, 300, 400, 500, and 600 mg/L. The urines were arranged in random order and the BM33071 glucose reaction was read on each by three separate technologists. Every reaction color clearly distinguishable from the negative label was considered as positive.

After addition of glucose to fresh urine samples, separated into four portions each. To all portions we added glucose to give a final concentration of 1.0 g/L; to one, acetoacetic acid (final concentration 5.0 g/L); to another, β-hydroxybutyrate (final concentration 10.0 g/L); and to a third, human hemoglobin (final concen-
tation 0.5 g/L). All portions were tested with both the BM33071 and the Hema-Combistix glucose tests.

Because ascorbic acid has been shown to interfere with other tests on urine chemistry strips (4), we also evaluated the effect of ascorbic acid on the glucose pad of BM33071. To 10 fresh urines with a negative ascorbic acid test result (by C-Stix from Ames) and a relative density of 1.020 or greater, we added glucose to give a final concentration of 1.0 or 5.0 g/L. Each urine was divided into four portions and ascorbic acid added to give a final concentration of 0, 100, 200, or 400 mg/L. All portions were then tested with both strips.

Results and Discussion

Method correlation: Figure 1 shows the correlation between results by the BM33071 and the hexokinase/glucose-6-phosphate dehydrogenase procedures. Aqueous controls used in the hexokinase procedure had a mean of all analyses of 0.4971 (SD 1.219) g/L for the 0.5 g/L control (CV 2.45%). The 1.5 g/L control had a mean of all analyses of 1.5006 (SD 2.159) and a CV of 1.44%. To clearly demonstrate the method correlation, we assigned the comparison method values to five categories, half-way between the correspond-

![Figure 1](image1)

**Fig. 1. Method comparison with patients' urine samples: hexokinase method vs BM33071 (upper) or vs Hema-Combistix glucose (lower)**

![Figure 2](image2)

**Fig. 2. Practical sensitivity curves for glucose measured with the BM33071**

ing BM33071 label color codes as done previously by others (5).

Figure 1 also shows the association between the Hema-Combistix glucose test and the hexokinase/glucose-6-phosphate dehydrogenase procedure. This time we assigned the comparison method values into six categories, halfway between the corresponding label color codes for the Hema-Combistix glucose test.

We used the index gamma (G) of Goodman and Kruskal (6) to measure the degree of association between the quantitative hexokinase method and each of the test products. For perfect positive association, linear or curvilinear, G = 1.0. Calculated values of G, and the corresponding 95% confidence intervals, for BM33071 were 0.996 (0.994–0.998) and for Hema-Combistix glucose were 0.978 (0.968–0.988). As shown by the non-overlapping confidence intervals, results by the BM33071 method displayed stronger association with those by the hexokinase method than did those by the Hema-Combistix glucose test.

As indicated in Figure 1, glucose concentrations in 73.4% of the urines estimated by BM33071 agreed with the hexokinase method results, 2.6% were lower and 24% were higher; however, the deviation never exceeded one color block on the label. Hema-Combistix results agreed with the hexokinase method results in 68.8% of the urine samples, 5% were lower, and 26.2% were higher; values for six urines (1.2%) deviated by more than one comparison color.

Practical sensitivity: Figure 2 shows the practical sensitivity curves for the BM33071. The average level of sensitivity was 260 mg/L at the 90% positive level, as described by Kutter (3). Although the lowest concentration color coded for BM33071 is 0.5 g/L, these strips uniformly detected glucose concentrations of 0.4 g/L 100% of the time, and 97% of the time detected glucose at 0.3 g/L.

Interference study: Acetoacetate (5 g/L), β-hydroxybutyrate (10.0 g/L), or hemoglobin (0.5 g/L) did not interfere with glucose results by the BM33071. However, the acetoacetate totally inhibited the glucose reaction by the Hema-Combistix. Further evaluation showed that acetoacetate as low as 0.75 g/L interfered with the glucose reaction on the Hema-Combistix. Ascorbic acid (0.4 g/L) interfered with BM33071 glucose measurements in 20% of the urines containing 1.0 g of glucose per liter but not with urines containing 5.0 g of glucose per liter. With the Hema-Combistix this concentration of ascorbic acid produced negative reactions in 90% of the urines containing glucose at 1.0 g/L, and 80% of the
urines containing the 5.0 g/L concentrations of glucose produced readings of 2.5 g/L. Neither method showed interference from 0.1 or 0.2 g of ascorbic acid per liter.

On the basis of these interference data, we predict that the BM33071 would not present false-negative results under the conditions studied. The effect of ascorbic acid should be minimal, given the normal excreted concentration of ascorbic acid of usually less than 0.25 g/L.

References


Indican Interference with Six Commercial Procedures for Measuring Total Bilirubin

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We have studied the effect of indican on six commercial procedures for the measurement of total bilirubin in serum. Total bilirubin measured by the Bilirubin A-Gent™ (Abbott) 2,4-dichlorophenyl diazonium procedure increased by 50 mg/L for each 1 mmol/L of added indican. Similarly, total bilirubin measured by the Bilirubin C-System™ (Boehringer Mannheim) 2,5-dichlorophenyl diazonium procedure increased by 33 mg/L per mmol/L of indican. Indican also interfered with the Micro Bilirubin Reagent Set™ (Harleco) Malloy–Evelyn procedure, but to a much lesser extent. The Jendrassik Bilirubin Reagent System™ (American Monitor) and a modified Jendrassik–Grof procedure (Hoffmann-LaRoche) adapted to the Cobas Bio analyzer were unaffected by the presence of indican. The amount of interference with the 2,5-dichlorophenyl diazonium procedure increased significantly with color development time and was twice the initial amount after 30 min. Concentrations of indican as high as 0.38 mmol/L have been found in sera of patients with renal failure, which would increase total bilirubin values measured by the first two procedures above by 19 and 12 mg/L, respectively. Users of these procedures should therefore be suspicious of unexpectedly high bilirubin values obtained with sera from patients with chronic renal disease.

Additional Keyphrases: renal disease · analytical error

Fifty-six years ago, Harrison and Bromfield (1) reported that indican (indol-3-yl sulfate), a natural metabolite that accumulates in the sera of patients with chronic renal failure (2, 3), interfered with the colorimetric determination of total bilirubin. However, except for one report by Ertingshausen et al. (4), that the determination of total bilirubin by a procedure involving 2,4-dichlorophenyl diazonium (2,4-DCPD) gave falsely increased results in the presence of indican, no attention has since been paid to this interferent.

We have studied the effect of indican on six commercial colorimetric procedures for the measurement of total bilirubin in serum and report our findings.

Materials and Methods

Indican was obtained from Sigma Chemical Co., St. Louis, MO 63178; reference grade bilirubin from Pfanzleih Laboratories Inc., Waukegan, IL 60085; bovine serum albumin from Armour Pharmaceutical Co., Kankakee, IL 60901; and 2,4-dichloroaniline and 2,5-dichloroaniline from Aldrich Chemical Co., Milwaukee, WI 53201. All other chemicals used were reagent grade, obtained from Fisher Scientific Co., Ottawa, Ontario, K2E 7L6.

We prepared samples with indican concentrations between 0 and 0.92 mmol/L by adding appropriate volumes of a 47 mmol/L solution of indican to aliquots of pooled human serum. The manual and automated colorimetric bilirubin measurement procedures we studied are described in Table 1. The kit manufacturers' directions for use were strictly followed. Where calibrators were not provided, we used bilirubin standards in bovine serum albumin, 40 g/L, prepared as described by Perry et al. (5).

Stabilizer-free 2,4-DCPD reagent was prepared at room temperature by adding sodium nitrite (final concentration, 10 μmol/L) to a solution containing 2 mmol of 2,4-dichloroaniline and 69 mmol of sulfamic acid per liter of water/methanol (1/1, by vol). We used the reagent 2 min after adding the sodium nitrite.

To measure absorbance, we used a Beckman DU-8B spectrophotometer (Beckman Instruments Inc., Fullerton, CA 92634); difference spectra were obtained with a Cary 219 spectrophotometer (Varian Associates Inc., Palo Alto, CA 94303).

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