Four Commercial Urease Reagents and a Laboratory-Prepared Reagent Compared for Analysis of Blood Urea Nitrogen with the Beckman Analyzer

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Three laboratory-prepared urease reagents were compared with a commercial preparation supplied for routine use on the Beckman Blood Urea Nitrogen Analyzer. There were discrepancies in results for urea nitrogen among the four urease reagents when matching serum and the corresponding oxalate/fluoride treated plasma were compared as measured with the Beckman Analyzer and continuous-flow (AutoAnalyzer) method. All four urease preparations were affected by fluoride, but to different extents. We believe that an effective laboratory reagent can be prepared in the laboratory at significantly lower cost.

Additional Keyphrase: effect of fluoride on urea nitrogen values (by urease)

In the Beckman BUN Analyzer a conductivity rate method is used to determine urea nitrogen (1):

\[ \text{Urea} + \text{H}_2\text{O} \xrightarrow{\text{urease}} 2\text{NH}_4^+ + \text{HCO}_3^- \]

The formation of ammonium and bicarbonate ions results in an increased conductivity of the reaction mixture, which is monitored by a conductivity electrode. The rate of ammonium bicarbonate formation is directly proportional to the urea concentration of the sample.

Use of commercially available reagents usually presents a significant savings relative to the preparation of such a reagent. This note describes our attempts to prepare a laboratory reagent that provides results equivalent to the commercial product, but at a smaller cost.

Materials and Methods

Urea nitrogen was assayed with a Beckman BUN Analyzer (Beckman Instruments, Inc., Fullerton, Calif. 92634) and the AutoAnalyzer II System (AA-II) for BUN and glucose (Technicon Instruments Corp., Tarrytown, N. Y. 10591). In the AA-II system a modification of the diacetylmonoxime method as described by Marsh et al. (2) is used.

A commercially available urease (EC 3.5.1.5) reagent, Beckman BUN Reagent Pack (Beckman Instruments) was compared to three laboratory-prepared reagents. The commercial reagent requires mixing two separately included reagents, urease and a tri(hydroxymethyl)aminomethane buffer, which results in a final urease activity of 35–45 kU/liter. The laboratory-prepared reagents were prepared from three different sources of jackbean-derived urease: (a) 3.4 kU/g (Sigma Chemical Co., St. Louis, Mo. 63178), (b) 120 kU/g

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Fig. 2. Comparison of results for urea nitrogen in plasma as measured with the Beckman BUN Analyzer and an AutoAnalyzer method, with use of four commercial urease preparations. n = 27 for all except Worthington, for which n = 26

(Miles Labs., Elkhart, Ind. 46514), and (c) 120 kU/g (Worthington Biochemical Corp., Freehold, N.J. 07728). All were prepared by dissolving 100 mg of urease in 5 ml of 0.124 mol/liter tris(hydroxymethyl)aminomethane, pH 7.3 (pH-adjusted with glacial acetic acid/water, 30/70 by vol) and diluting this mixture to a final volume of 250 ml with a resulting urease activity of approximately 48 U/ml for the Miles and Worthington enzyme. However, attempts to increase the final urease activity of the Sigma product to more than 2 U/ml resulted in a reagent that was incompatible with the Beckman BUN Analyzer. This appeared to be related to the initial conductivity, which was several orders of magnitude greater than was so for the other urease preparations. A final Sigma urease activity of about 1.4 U/ml, consistent with the reagent preparation described above, was found to be compatible with the Beckman BUN Analyzer.

Blood samples were collected in red-stoppered Vacutainer tubes with no additive (cat. No. 4670-M3200), and gray-stoppered Vacutainer tubes containing 14 mg of potassium oxalate and 17.5 mg of sodium fluoride (cat. No. 4500-M3204PS), both from Becton, Dickinson and Co., Rutherford, N.J. 07709.

Results and Discussion

Figure 1 demonstrates the results observed when we compared matching plasma and serum samples from patients on the Beckman BUN Analyzer. Worthington urease demonstrated the best correlation, followed by Miles, Beckman, and Sigma, which all demonstrated higher values for serum urea nitrogen than for plasma urea nitrogen. With the Sigma urease, plasma values for urea nitrogen were only 85% of serum values.

Figure 2 compares plasma urea nitrogen as assayed with the Beckman BUN Analyzer and the AA-II system. Results with Miles urease correlated the poorest of the four tested, the Beckman BUN Analyzer results being about 22% higher than AA-II values. The Beckman reagent and Worthington urease correlated best.

Figure 3 demonstrates the similarly poor results for the Miles urease when serum urea nitrogen values (Beckman BUN Analyzer) were compared to plasma values (AA-II). Worthington, Sigma, and Beckman reagents showed about 8% higher values for serum.

All of the urease reagents were inhibited by fluoride (Figure 4), the Sigma enzyme being the most sensitive, which explains the lower values for plasma urea nitrogen (assayed on the Beckman BUN Analyzer) observed in Figures 1 and 2. The Miles enzyme showed the least effect of fluoride inhibition, but correlation was poor with the AA-II system (Figures 2 and 3). The Worthington enzyme and Beckman reagent exhibit
Use of Blood Specimens Collected on Filter Paper in Screening for Abnormal Hemoglobin

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Both cellulose acetate electrophoresis and citrate agar electrophoresis were performed on 834 blood samples collected on filter paper in Jamaica and shipped for testing to the National Hemoglobinopathy Standardization Laboratory at the U.S. National Center for Disease Control. Additionally, 30 blood samples collected locally were stored on filter paper, in microhematocrit capillary tubes, and as whole blood specimens; at selected times the samples were tested for stability to determine the best sample-collection technique for hemoglobin electrophoresis. Results were most nearly accurate when both cellulose acetate electrophoresis and citrate agar testing were used. The methods are easy to perform, but results are unreliable if the blood samples on filter paper are stored at 4 °C for longer than two weeks before they are tested.

Additional Keyphrases: hemoglobinopathies • sickle cell anemia • electrophoresis

Several recent reports have suggested that blood specimens collected on filter paper for hemoglobinopathy testing are quite suitable for mass-screening programs (1–3). In addition, Garrick et al. (4) suggested that specimens collected on filter paper can be used to detect sickle cell anemia and to identify other hemoglobinopathies in newborns. Although the work of these investigators indicated that this is a reliable, efficient method of detecting abnormal hemoglobins, their results were not definitive because their 2435 cord-blood specimens included no FS phenotypes and only 14 FAS phenotypes. If blood samples collected on filter paper from a fingerprick can be stored and shipped to a central laboratory, this method of collection would be particularly useful for hemoglobinopathy screening programs. Our study was designed to investigate whether accurate results can be obtained with such samples.

Materials and Methods

Cord-blood specimens from 834 consecutive deliveries at the Royal Victoria Jubilee Hospital in Kingston, Jamaica, were collected in heparinized tubes and on filter paper. Staff of the Medical Research Council Epidemiology Unit, University of the West Indies, screened the blood specimens collected in tubes for abnormal hemoglobins by the methods described by Serjeant et al. (5). The corresponding filter paper samples were stored at 4 °C for two weeks during the entire collection/screening procedure and then were air-mailed in refrigerated containers to the National Hemoglobinopathy Standardization Laboratory located at the Center for Disease Control.

When the specimens arrived at the Atlanta laboratory, 0.6-cm (diameter) discs of filter paper containing the blood spots were punched out and placed in labeled test tubes. About 0.2 ml of a tetrasodium ethylenediaminetetraacetate (EDTA) solution, 2.5 g/liter, was added to each tube to elute the hemoglobin from the paper discs. The tubes were left overnight at 4 °C. The next day, cellulose acetate electrophoresis of the eluted hemoglobin was performed as described previously (6). Subsequently, citrate agar electrophoresis was also done on the eluted hemoglobin samples by the method of Milner and Gooden (7). All specimens were tested in Atlanta no longer than four weeks after the initial testing in Jamaica.

References


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The use of trade names is for identification only and does not constitute endorsement by the PHS or the U. S. Dept. of HEW.

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