Determination of Serum or Plasma Glucose on the “AutoAnalyzer II” by Use of the Hexokinase Reaction

G. M. Widdowson and J. R. Penton

An automated method is described for determination of glucose in serum or plasma by use of the hexokinase reaction. The method has good precision and the results correlate well with those from a manual method in which the same reaction is used.

Additional Keyphrases automated glucose determination • hexokinase method • single-reagent procedure

Use of the following reactions for determination of glucose in serum, plasma, or blood has been described by Steil (1), Magee and Farese (2), and Wright et al. (3):

\[ \text{Glucose} + \text{ATP} \xrightarrow{\text{Hexokinase}} \text{G-6-P} + \text{ADP} \]

\[ \text{G-6-P} + \text{NADP} \xrightarrow{\text{GPD}} \text{NADPH} + 6\text{-Phosphogluconate} \]

Manual procedures based on these reactions and involving the direct addition of serum to a single reagent are commonly used as emergency procedures in routine clinical chemistry laboratories of hospitals.

We have adapted one of these methods, “Glucose 500-Pack” (Calbiochem, P.O. Box 12087, San Diego, Calif. 92177) to an automated method on the “AutoAnalyzer II” (Technicon Instruments Corp., Tarrytown, N.Y. 10591).

The AutoAnalyzer II is well suited to automation of this method because the sample blank can be measured simultaneously. In addition, smaller manifold pump tubes can be used with the AutoAnalyzer II colorimeter than were necessary in the AutoAnalyzer “N” methodologies, thus permitting use of about half the reagent volume per sample suggested in the reagent manufacturer’s instructions.

Materials and Methods

Equipment

An AutoAnalyzer Sampler II, Pump III, AutoAnalyzer II colorimeter (Technicon), and Model A-25 chart recorder (Varian Aerograph, 2700 Mitchell Drive, Walnut Creek, Calif. 94598) were used. One sample and one blank channel on the colorimeter are required for this determination. The recorder was set at 100 mV full scale and at a chart speed of 1/4 inch per minute.

Reagents

Glucose 500-Pack. Reconstitute two vials, “A” (glucose reagent) and “B” (NADP), exactly as described in the manufacturer’s instructions to give a final volume of 155 ml.

According to the manufacturer, the concentrations of the Glucose 500-Pack constituents are Tris buffer (pH 7.5) 50 mmol/liter, ATP 0.5 mmol/liter, NADP 0.45 mmol/liter, magnesium 17 mmol/liter, hexokinase 666 U/liter, and GPD 333 U/liter.

Sodium chloride. Dissolve 9 g of sodium chloride in distilled water and dilute to 1 liter.

Benzoic acid solution. Dissolve 2.5 g of benzoic acid in distilled water and dilute to 1 liter. Filter the solution immediately before use.

Stock glucose standard (10 g/liter). Dissolve 1 g of D-glucose (National Bureau of Standards Reference Material No. 917) in saturated benzoic acid and dilute to 100 ml with benzoic acid solution.

Fig. 1. Manifold diagram for glucose determination (40 samples per hour, 2:1 sample-to-wash ratio)

From the Research Institute of Laboratory Medicine, Institute of Medical Sciences, 2200 Webster St., San Francisco, Calif. 94115.

1 Nonstandard abbreviations used: G-6-P, glucose-6-phosphate; GPD, glucose-6-phosphate dehydrogenase (p-glucose-6-phosphate:NADP oxidoreductase, EC 1.1.1.49).

2 This abbreviation GPD is one of those recently recommended by IUB [see J. Clin. Pathol. 24, 656 (1971)]. This journal will attempt to cooperate with these recommendations—Editor.

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Working standards. Make glucose standards containing 50, 100, 150, 200, 250, and 300 mg/100 ml by appropriate dilution of the stock standard with benzoic acid solution.

Figure 1 shows the manifold design; numbered fittings were standard AutoAnalyzer parts. The sampling rate was 40 samples per hour with a 2:1 sample-to-wash ratio. On an earlier manifold, the resampling technique shown was not used; however, to obtain a suitable sample-to-reagent ratio, a 15 μl/min sample pump tube was required, which led to frequent blockages. Glass tubing, 1.8 mm i.d., was used to connect the two 20-turn coils to the colorimeter inlets; the length of this tubing may have to be adjusted slightly in one of the two channels so that test and blank reaction mixtures from a given sample arrive at the two colorimeter flow cells in phase. It is important to keep the two diluted sample paths from the C5 debubbler through the P7 stream splitter to the connector assemblies as short as possible. In those portions of the manifold, the sample stream was not segmented by air bubbles and so was subject to undesired mixing between specimens. The delay time for the reaction mixture to reach the colorimeter was about 4.25 min.

Results

A typical run at 40 samples per hour with 2:1 sample-to-wash ratio is shown in Figure 2. It was possible to run at 60 samples per hour with 2:1 sample-to-wash ratio, but precision was decreased.

Linearity. Peak height is linearly related to concentration from 0 to 300 mg/100 ml.

Interaction between samples. Interaction from a high (281 mg/100 ml) to a low (48 mg/100 ml) sample, measured according to the method of Broughton et al. (4), was 0.017 (1.7%).

Recovery. When human serum pools with glucose concentrations of 52 mg/100 ml and 47 mg/100 ml were enriched to 102 mg/100 ml and 147 mg/100 ml, respectively, the observed recoveries were 97.0% and 100.0%.

Precision. The within-batch precision of the method was measured for three lots of pooled human sera having different glucose concentrations. Twenty specimens of each lot were analyzed consecutively in each case, with respective means, standard deviations (mg/100 ml), and coefficients of variation of 64, ±0.6, 1.0%; 105, ±0.2, 0.2%; and 213, ±0.3, and 0.2%.

Day-to-day precision was determined by analyzing aliquots of two different control sera over periods of 29 and 28 days, respectively, with the control sera being placed in random positions on a routine run: (a) N = 29, mean = 65 mg/100 ml, SD = ±1.2, CV = 1.8%; (b) N = 28, mean = 87 mg/100 ml, SD = ±1.3, CV = 1.5%.

Comparison of AutoAnalyzer II and manual methods. Thirty sera were analyzed in duplicate by the AutoAnalyzer II method described, and manually according to the instructions supplied by the reagent manufacturer. The same glucose standards were used for both methods. A paired t test revealed no statistical significance of difference between the two means (t = 1.682; critical t = 2.042, 95% confidence limits).

References