Evaluation of the BMC Glucose Oxidase/Peroxidase-4-Aminophenazone-Phenol Procedure for Glucose as Adapted to the Technicon SMAC

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We evaluated the analytical performance of Trinder's glucose oxidase (EC 1.1.3.4)/peroxidase (EC 1.11.1.7) 4-aminophenazone-phenol method for the quantification of serum glucose as adapted to the Technicon SMAC. Our results correlated well with those by the routine SMAC glucose oxidase/peroxidase 3-methyl-2-benzothiazolinone hydrazone-N,N-dimethylaniline method (y = 1.02x - 49.4; r = 0.99) and the glucose oxidase oxygen-rate method (y = 0.99x + 14; r = 0.99) with the Beckman Glucose Analyzer. Sample-to-sample interaction was <1%. Ascorbic acid or uric acid in concentrations as high as 200 mg/L were without demonstrable effect on results for glucose. Intra- and inter-assay precisions (CV) were 1.6 and 2.3%, respectively. The upper limit of linearity was about 5 g/L.

Adaptation of the Trinder method for glucose to the SMAC is simple and provides an analytically acceptable and economical alternative to the methods ordinarily used with the SMAC.

Additional Keyphrases: continuous-flow analysis, intermethod comparison, normal range

The original method developed for the measurement of glucose with the SMAC analyzer (Technicon Instruments Corp., Tarrytown, NY 10591) was the glucose oxidase/peroxidase 3-methyl-2-benzothiazolinone hydrazone (MBTH)-N,N-dimethylaniline (DMA) procedure (1), as modified by Technicon (2). Schwartz et al. (3) and Westgard et al. (4) evaluated the method as part of an overall evaluation of the SMAC and determined the analytical performance to be acceptable.

Subsequently, Technicon (5) developed a co-immobilized coil hexokinase (EC 2.7.1.1)/glucose-6-phosphate dehydrogenase (EC 1.1.1.49) method for glucose analysis with the SMAC. Garber et al. (6) evaluated the analytical performances of both of these methods and determined that either gave accurate results, relative to the National Glucose Reference Method (7).

Here, we present the results of studies conducted to evaluate the analytical performance of the glucose oxidase/per-

oxidase 4-aminophenazone-phenol method (8), as adapted to the SMAC. The method was compared to the routine SMAC glucose oxidase/peroxidase-MBTH-DMA method and the glucose oxidase oxygen-rate method (9) with the Beckman Glucose Analyzer.

We also present data on the linearity, effects of high concentrations of ascorbic acid or uric acid, intra- and inter-assay variations, sample-to-sample interaction, and the normal range for samples from fasting individuals.

Materials and Methods

Glucose oxidase/peroxidase 4-aminophenazone-phenol method. The method adapted to the SMAC is a modification of the original work of Trinder (8). Figure 1 illustrates the flow diagram for the method, as adapted for the SMAC. The reagents used were purchased in prepackaged kit form from Bio-Dynamics/bmc, Indianapolis, IN 46250 ("GOD-PAP Trinder," cat. no. 124001). The pre-weighed enzymes and substrate were dissolved in de-ionized water and diluted to 1 L. According to the manufacturer, the resulting solution contained the following per liter: 0.1 mol of phosphate buffer (pH 7.0), 1.48 nmol of 4-aminophenazone, ≥12 kU of glucose oxidase, and ≥1.2 kU of peroxidase. A 0.56 mol/L solution of phenol was also included with the kit. The method was calibrated with the same SMAC calibration material (SMAC Reference I, Technicon) used to calibrate the SMAC glucose oxidase/peroxidase-MBTH-DMA method. The glucose concentration of the SMAC Reference I was verified by assaying the material by a continuous-flow (AutoAnalyzer II) method adapted with the "GOD-PAP Trinder" method (10) and standardized with aqueous glucose standards (500, 1000, 1500, 2000, 2500, and 3000 mg/L). In these studies we used version 7.6 of the SMAC computer software.

Glucose oxidase/peroxidase-MBTH-DMA method. This method is the glucose oxidase method (1) as modified by Technicon (2) for the SMAC. The method was calibrated with the same SMAC Reference I described for the "GOD-PAP Trinder" method.

Glucose oxidase oxygen-rate method. We used the Glucose Analyzer (Beckman Instruments Corp., Fullerton, CA 92634) to measure glucose in serum samples by the oxygen-rate method. The instrument and all reagents were purchased from Beckman and used according to the manufacturer's instructions.

Results

Comparison with other glucose methods. We compared the "GOD-PAP Trinder" method as adapted to the SMAC (y) with the glucose oxidase/peroxidase-MBTH-DMA method (x) as modified for the SMAC by Technicon (2) (x). Linear regression analysis of the data obtained when 147 specimens were analyzed by the two methods yielded the following: r = 0.99, y =
1.02x - 49.4. The mean glucose concentrations were 1436 and 1460 mg/L, respectively, for the "GOD-PAP Trinder" and the MBTH-DMA methods. Results by the two methods correlated well over the range 600 to 3500 mg/L.

In addition, we compared the "GOD-PAP Trinder" (y) method with the glucose oxidase oxygen-rate method (x) as adapted to the Beckman Glucose Analyzer (9). We analyzed 20 specimens by the two methods. Linear regression analysis of the data yielded the following: \( r = 0.99; y = 0.99x + 14 \). The mean glucose concentrations were 1585 and 1565 mg/L, respectively, for the "GOD-PAP Trinder" and the oxygen-rate technique. The two methods compared well over the range 780 to 3000 mg/L.

**Linearity, interferences, and precision.** The linearity of the "GOD-PAP Trinder" method as adapted to the SMAC extended to approximately 5000 mg/L, as determined by diluting (with 0.15 mol/L NaCl) and analyzing three serum specimens with glucose concentrations >8000 mg/L and one serum specimen with a glucose concentration of 4900 mg/L.

We evaluated the interferences from ascorbic acid and uric acid that ordinarily are observed with glucose oxidase/peroxidase methods by adding various amounts of these two analytes, separately, to aqueous glucose standards of 500 and 1000 mg/L. Such addition did not affect the assayed values of the glucose standards at ascorbic acid or uric acid concentrations up to 200 mg/L.

**Intra-assay variation** was evaluated by assaying 35 quality-control pools with normal and above-normal concentrations of glucose on the same day. The following data were obtained: normal, \( \bar{x} = 860 \pm 14 \text{ mg/L}, \text{CV} = 1.6\%; \) and above-normal, \( \bar{x} = 2150 \pm 23 \text{ mg/L}, \text{CV} = 1.1\% \).

**Inter-assay variation** was evaluated by assaying the same quality-control pools over a period of 63 days. The following data were obtained: normal, \( \bar{x} = 860 \pm 20 \text{ mg/L}, \text{CV} = 2.3\%; \) and above-normal, \( \bar{x} = 2110 \pm 40 \text{ mg/L}, \text{CV} = 1.8\% \).

**Carryover and normal range.** Carryover (sample-to-sample interaction), determined according to the method of Broughton et al. (11), was calculated to be <1%.

The normal range was determined by collecting serum samples from 121 apparently normal, healthy adult volunteers (41 men and 80 women) between the ages of 18 and 54 years, who had fasted for at least 12 h. The sera were collected with the "Corvac" (Corning Glass Works, Corning, NY 14830) collection vessel, mixed by gentle inversion, and centrifuged within 1 h of the venipuncture. [Stability studies conducted by our laboratory, with the fluoridated "Vacutainer" (Becton-Dickinson, Rutherford, NJ 07070) as a control, demonstrated that glucose was stable in serum under these conditions.] We found a mean value of 860 mg/L, with a range from 700 to 1050 mg/L.

**Discussion**

Adaptation of the method to the SMAC was simplified by the similarity of the computer programming of version 7.6 to the SMAC software between the standard MBTH-DMA method and the "GOD-PAP Trinder" method.

Our observation regarding the lack of interference in the "GOD-PAP Trinder" method at ascorbic acid or uric acid concentrations up to 200 mg/L has been substantiated by others (8, 12, 13). The observation by Lott and Turner (14) that an ascorbic acid concentration of 200 mg/L decreased the apparent glucose concentration of a serum pool from 1300 to 790 mg/L was not demonstrable with the "GOD-PAP Trinder" method as adapted to the SMAC.

The inter-assay precision of the "GOD-PAP Trinder" method as adapted to the SMAC that we found compares well
to the data of Garber et al. (6), who reported that both the MBTH-DMA and the hexokinase/glucose-6-phosphate dehydrogenase co-immobilized coil methods demonstrated coefficients of variation of 2.4 and 1.6% at serum glucose concentrations of 960 and 2150 mg/L, respectively.

The fasting-normal range (700-1050 mg/L) established with the "GOD-PAP Trinder" method adapted to the SMAC, is similar to the range (700-1070 mg/L) reported for the MBTH-DMA method (1) and the range (670-1010 mg/L) reported by Lott and Turner (14) with the method of Trinder (8).

The "GOD-PAP Trinder" method is simple to adapt to the SMAC. Results compare well to those by other standard procedures for serum glucose. Further, it provides an economic alternative to other, more expensive methods.

References
2. Technicon SMAC Methodology Files, Method No. SG4-0036PC6, Technicon Instruments Corp., Tarrytown, NY 10591.