AN EVALUATION OF THE BECKMAN GLUCOSE ANALYSER 2

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INTRODUCTION

THE Beckman Glucose Analyser 2 (Beckman Instruments, Inc. Fullerton, California) incorporates automatic pumps for filling and draining its reaction chamber, as against its earlier, less sophisticated version. This analyser offers a convenient and rapid means of processing routine glucose requests and also those that come in at odd times. The analyser is easy to operate, requiring only manual delivery of a sample into the reaction chamber, after initial calibration with an aqueous glucose standard. With the result obtained under 40 seconds after the introduction of a 10 ul sample, the analyser becomes very suitable for handling urgent requests and pediatric specimens.

The analyser utilises an oxygen-sensitive electrode to determine the rate of oxygen consumption, which is proportional to the glucose concentration in the sample, in the following reactions:

$$\beta\text{-D-Glucose} + 0_2 \xrightarrow{\text{glucose oxidase}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{ethanol} \xrightarrow{\text{catalase}} \text{acetaldehyde} + H_2O_2$$

Iodide and molybdate are included in the glucose oxidase reagent to ensure destruction of H_2O_2, especially with diminished catalase activity on storage. The reactions are claimed to be free from interference from other reducing substances, and the usual blood anticoagulants and glucose preservative. Being a method independent of the optical properties of a solution, it is free from the effects of haemolysis, bilirubinemia and turbidity.

Acquisition of the Beckman Glucose Analyser 2 has removed the tedium of manual glucose assays from our laboratory. However, the o-toluidine method of Hyvarinen and Nikkila (1962), a reliable manual method which we have been using, is retained to serve as a back-up. Also, because our laboratory is the training and reference centre for the country, retention of the manual method is relevant as most peripheral laboratories will have to continue with manual glucose determination.

This paper presents the findings of our evaluation study of the Beckman Glucose Analyser 2. Comparisons were also made against a spectrophotometric hexokinase method carried out on the Kem-O-Mat autoanalyser (Coulter Electronics Ltd., England), and also against the o-toluidine method done manually. The superior modified glucose oxidase reagent of Fischl, *et al.* (1975) is recommended, and recycling of the reagent as proposed by Case and Phillips (1977) was adopted to cut costs.

MATERIALS AND METHODS

Glucose oxidase reagent (modified) by Fischl, et al. (1975)

Place about 1,500 ml deionised water in a 2-litre volumetric flask. Add 2.92 g of sodium chloride, AR, and dissolve. Add 5.68 ml of glacial acetic acid, and adjust the pH to 6.0 with sodium hydroxide. Add 25.0 g of glucose oxidase (EC 1.1.34) powder (Sigma Type II, Cat. No. G 6125) and dissolve. Add 0.5 ml of an aqueous solution of ammonium molybdate (10 g/litre), 2 ml of an ethanolic solution of iodine (10 g/litre), 200.0 ml of 96% ethanol and 4.0 mg of mercuric iodide powder. Shake vigorously for about 5 minutes (to prevent foaming, add a few drops of octanol at this stage). Pass the mixture through sintered glass

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filter, add 200.0 ml of glycerol, and 5.0 ml of 40% formaldehyde. Dilute to 2 litres with water.

After aging the reagent for about 4 to 5 days, during which time the insoluble matter will settle, carefully decant it into clean 250 ml polyethylene bottoles. At this stage, the reagent may be safely used for determination of glucose. The reagent is stable for over one year at 2 — 8 C, and at least 6 months at room temperature. Do not freeze.

Recyling glucose oxidase reagent according to Case and Phillips (1977)

Collect the used glucose oxidase reagent into a waste reservoir. Aerate by forcing air through the solution for about an hour. Filter through sintered glass filter before use.

According to Case and Phillips (1977), the glucose oxidase reagent can be recycled up to at least 12 times.

Glucose standard, 150 mg/dl

Dissolve 1.50 g D-glucose, AR, in 0.1% (w/v) benzoic acid, and make up to 1 litre with the benzoic acid solution. Keep at about 4°C.

Beckman Glucose Analyser 2 (Beckman Instruments Inc., Fullerton, California, U.S.A.), with the Beckman Blue-Tip Pipettor of 10 ul capacity iwth disposable tips

Plasma from patients' blood in fluoride-oxalate (2 mg sodium fluoride and 2 mg potassium oxalate per ml blood)

Lyophilised sera were reconstituted, and stood at room temperature for at least 1 hour to enable mutarotational equilibrium to be reached between the α and β forms of D-glucose.

(The Beckman Glucose Analyser 2 can also measure cerebrospinal fluid and urine glucose)

RESULTS

Each of 4 sera of glucose concentrations between 63 and 387 mg/dl was assayed 20 times in a single run. The "within-batch" coefficients of variation did not exceed 1.2% (Table I). For "between-batch" precision, duplicate analyses were performed for 10 consecutive days on 3 sera with 66, 200 and 397 mg glucose/dl. The coefficients of variation were not more than 1.7% (Table II).

Table I
Within-Batch Precision from replicate analyses, in a single run, of sera at 4 levels of glucose concentration

Serum	1	2	3	4
Number of analyses	20	20	20	20
Mean, mg glucose/dl	63	100	195	387
Standard deviation	0.74	0.74	2.00	4.47
Coefficient of variation	1.2%	0.7%	1.0%	1.2%

Table II
Between-Batch Precision from analyses in duplicate, over a period of 10 days, of sera at 3 levels of glucose concentration

Serum	1	2	3
Number of analyses	10 x 2 (10 days)	10 x 2 (10 days)	10 x 2 (10 days)
Mean, mg glucose/dl	66	200	397
Standard deviation	1.14	2.85	5.24
Coefficient of variation	1.7%	1.4%	1.3%

Several commercial control sera were assayed. The values obtained were compared, respectively, against the weighed-in glucose value in Versatol (General diagnostics, Warner-Lambert, U.S.A.), the glucose concentration quoted for Beckman Glucose Analyser in Monitrol (Dade, American Hospital Supply Corpn., U.S.A.) and Hyland (Travenol Laboratories Inc., U.S.A.), and the value stated for glucose oxidase methods in Wellcomtrol (Wellcome Reagents Ltd., England). The values specified for the control sera were returned to within limits of allowable error (Table III).

Table III
Results of analyses of commercial control sera with known glucose concentrations

Control Serum	Value Obtained mg glucose/dl	Value Quoted mg glucose/dl	% of the Quoted Value
Versatol	200	204	98
Hyland	88	90	98
Hyland	188	196	96
Wellcomtrol	60	61	99
Wellcomtrol	93	90	103
Monitrol	101	100	101

Three sera of known glucose concentrations were mixed to obtain samples with theoretical values from 114 to 324 mg/dl. Each sample was then analysed in triplicate, and the mean value obtained. Recoveries of the theoretical values closely averaged 99% (Table IV).

Table IV
Recovery Study using samples concocted from sera of known glucose values

Sample	Value Obtained (average of triplicates) mg glucose/dl	Theoretical Value mg glucose/dl	Recovery %
1.	135	136	99
2.	235	239	98
3.	303	305	99
4.	322	324	99
5.	181	183	99
6.	112	114	98

While it is recommended that a sample assayed should have a glucose concentration not exceeding 450 mg/dl, we favourably demonstrated linear response extending beyond 500 mg/dl. Also, no drift was recorded up to 50 tests after the initial calibration, although the manufacturer advises re-calibration of the analyser after every 10 samples in a test batch. Carry-over between sera of glucose concentrations 67 mg/dl (low), 200 mg/dl (medium) and 395 mg/dl (high) was negligible.

A comparative study of the Beckman Glucose Analyser 2 method against a spectrophotometric hexokinase method run on Coulter's Kem-O-Mat autoanalyser, involving 36 plasma samples of glucose concentrations up to 350 mg/dl, showed excellent correlation, with the coefficient of correlation, $r = 0.99$ ($p < 0.001$), and the line of regression, $y = 1.04x + 0.11$ indicating that the values from the glucose analyser are 104% that obtained by the hexokinase method. Good correlation was also noted when the manual o-toluidine method was compared against the Beckman analyser method. Based on analyses of 60 plasma samples, from 70 — 350 mg glucose/dl, the coefficient of correlation, $r = 0.96$ (p 0.001), and the line of regression $y = 0.9x + 4.42$.

We established a fasting glucose reference range of 70 — 115 mg/dl for plasma as against 70 — 110 mg/dl quoted by the manufacturer.

DISCUSSION

The Beckman Glucose Analyser 2, which is easy to operate with little maintenance required, was shown to be capable of precise and accurate glucose measurement. The "fast forward five" pipetting technique is easily mastered, and the user has only to recharge the electrode, which simply means changing the Teflon membrance of the oxygen electrode. Recharging is done once every fortnight, but may be of longer intervals if the daily workload is small. Little else is required in the maintenance of the analyser, which, for already more than 9 months since it was put to use, has remained trouble-free. Further, we have not yet had cause for replacing the tubings, although the manufacturer suggests replacement after 2 months.

The modified glucose oxidase reagent of Fischl, *et al.* (1975), which contains glycerol, is superior in that it also has the property of lubricating the tubing walls and the reaction chamber, and preventing rapid drying of the electrode gel. Its cost approximates to just one-fifth that of the Beckman reagent. Cost of running the Beckman analyser can even be further reduced by recycling the used reagent. Case an Phillips (1977) reported that the glucose oxidase reagent can be re-used up to at least 12 times by regeneration of the used reagent through a simple process of aeration. Owing to some loss during collection and recycling of the reagent, we have managed to re-use one batch of reagent 10 times, and have not found the capability of the reagent to be affected. Cost per test worked out to be less than 5 Malaysian cents.

Another glucose analyser currently in the market is the Yellow Springs Glucose Analyser (Yellow Springs Instrument Co., Yellow Springs, Ohio, U.S.A.). Like the Beckman analyser, it returns quick results, requires only microvolume samples for analysis, and is easy to operate; but differs in that it employs a hydrogen peroxide-sensitive electrode and uses "immobilised" glucose oxidase. While the enzyme-impregnated membrance for the Yellow Springs analyser and the free enzyme for the Beckman analyser cost about the same, adoption of the reagent of Fischl, *et al.* (1975) and recycling the used reagent have made it possible for the Beckman analyser to be operated far more economically. Chua and Tan (1978) reported no special advantage in the use of

immobilised enzyme (a relatively new concept) over free enzyme, and observed that the Yellow Springs analyser showed noticeable drift after only 10 consecutive analyses and that the recovery times and working life spans of different membrances vary.

Besides being burdened with urgent glucose requests which come in at odd times, many hospital laboratories have routine requests often exceeding the capacity of a manual method to cope with satisfactorily. A glucose analyser would be a definite asset to these laboratories. An existing manual method, however, need not be discarded as it can then serve as a back-up. The analyser could also be complementary where glucose assays are already automated, because of the convenience and rapidity with which the analyser can handle urgent requests.

SUMMARY

The Beckman Glucose Analyser 2, which employs the "glucose oxidase-oxygen rate" method, offers a convenient and rapid means of measuring glucose in microvolume samples with good precision and accuracy. Results from the Beckman analyser compare well with those from a hexokinase method run on Coulter's Kem-O-Mat autoanalyser, and also with the results from a manual o-toluidine method. The operating cost of the Beckman analyser can be made inexpensive by following the recommendations of Fischl, *et al.* (1975) and Case and Phillips (1977). The analyser should be appropriate for a busy hospital laboratory.

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