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Evaluation of a Micromethod for the Determination of Glucose in Skin-Puncture Blood for the DuPont aca

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Summary: A micromethod is described for the determination of glucose in 20 μl of skin-puncture blood for the DuPont aca. For the determination of glucose we prefer whole blood which is deproteinised with uranyl acetate. Some modification of test conditions are programmed into the computer II. The emergency analysis of supernatant on the aca then gives the same values as the Technicon Autoanalyzer II, using glucose dehydrogenase for routine analyses. The aca readout is linearly related to glucose concentrations up to 60 mmol/l.

Mikromethode zur Bestimmung von Glucose in Kapillarblut mit dem DuPont aca

Zusammenfassung: Eine Mikromethode zur Bestimmung der Glucose in 20 μl Kapillarblut wird für den DuPont aca beschrieben. Wir bestimmen die Glucose in Vollblut nach Enteiweißung mit Uranylacetat. Die Analyse des Überstandes ergibt nach entsprechenden Änderungen, die nur am Computer II auszuführen sind, für Notfalluntersuchungen auf dem aca die gleichen Ergebnisse wie für Routineuntersuchungen mittels Glucosedehydrogenase auf dem Autoanalyzer II. Linearität besteht für den aca bis 60 mmol/l.

Introduction

We claim that the determination of glucose in skin-puncture (capillary) blood is superior to the determination of glucose in serum or plasma; only the former method is physiologically sound and logical (1, 2, 3).

The original aca glucose method is based on the analysis of 40 μl serum using the hexokinase glucose-6-phosphate dehydrogenase-method (4). In order to prevent systematic differences in our results (5, 6) and different ways of taking samples, the aca had to be integrated into our system of sampling. Apparently two different approaches are possible:

1. Deproteinisation of the samples with acidic reagents (e.g. perchloric acid or perchloric acid/perchlorate) or
2. deproteinisation with neutral reagents (e.g. uranyl acetate)

Our group preferred the second way, which appeared easier, while another group independently verified the first modification (7) using 50 μl of whole blood, as we heard later.

Here we describe a method permitting determination of glucose in only 20 μl of whole blood on the DuPont aca.

Materials and Methods

Procedure

20 μl of skin-puncture blood were deproteinised with 500 μl of uranyl acetate solution (1.9 mmol/l) as described elsewhere (3). After centrifugation (7500 *g*, 1 min) the supernatant was ready for analysis, either in the aca or with the glucose dehydrogenase method on the Autoanalyzer II.

Apparatus

An Automatic Clinical Analyzer (aca) with computer II was used (DuPont de Nemours Company, Wilmington, Delaware 19898, USA).

The following modifications were necessary and easy to realize:

1. The volume of diluent 1 was reduced from 4960 μl to 4600 μl according to the manufacturers' manuals (4). The volume of supernatant aspirated by the aca was then 400 μl.
2. The theoretical scale factor (0.0448) was multiplied by our dilution factor 2.6: the new scale factor was 0.1165.
3. The theoretical offset (−17) was corrected to −44. The new starting point was 9956. Count-by and A/D offset remained unchanged.

Readout units: The aca prints out in 1 mg/dl increments. No further variations to the original aca procedure were necessary.

Calibration

Calibration was carried out with protein-containing (60 g/l) glucose standards. We suggest calibration levels of 27.8, 16.7, 5.6 mmol/l glucose. After deproteinisation with uranyl acetate and centrifugation, calibration was performed in the usual manner (4).

Tab. 1. Accuracy, tested with commercially available sera

Serum	Assigned value (mmol/l)	aca value (mmol/l)	No. of analyses	Serum obtained from
Monitrol I (141 A)	4.37 (Hexokinase)	4.33	5	Merz and Dade GmbH, München.
Precinorm U (606)	6.22 (Glucose dehydrogenase)	6.28	12	Boehringer Mannheim GmbH.
Monitrol II (46B)	12.0 (Hexokinase)	11.94	5	Merz and Dade GmbH, München.

Results

Linearity and Accuracy

Linearity exists in the range of 0–60 mmol/l glucose as determined with glucose standards at levels of 0, 1.4, 2.8, 9.7, 16.7, 27.8, 38.9, 55.6 mmol/l. The method yields a correlation coefficient of 0.999 with a regression line of: y (aca) = 0.99x (standard) + 0.06.

Accuracy was tested with commercially available sera. Table 1 lists the sera investigated and shows our results.

Interfering Substances

Experiments have shown that there is less interference caused by hemolytic or icteric specimens in the new method than in the original aca version. Hemolyzed blood shows no significant interference up to 2.0 g/dl hemoglobin per sample. Higher concentrations of hemoglobin may give falsely depressed values. Icteric samples show no significant interference up to 680 µmol/l bilirubin per sample and lipemic samples can be analysed without significant interference up to 46 mmol/l serum triglycerides. Serum protein concentrations from 40 g/l up to 120 g/l show no significant influence on the results. Lower protein concentrations may cause falsely elevated values.

Precision

Table 2 indicates good results for within-run and day-to-day precision. The day-to-day imprecision encloses data from five different lot nos. of test packs and is nearly the same as that of the Autoanalyzer II.

Comparison of Methods

Results obtained by the present method were directly compared with those obtained by the glucose dehydrogenase method on the Technicon Autoanalyzer II (3,8). Figure 1 shows the regression line $y = b_{yx} \cdot x + a_{yx}$ calculated from 61 data pairs. The slope is $b_{yx} = 1.006$ and the y-intercept is $a_{yx} = -0.045$. The coefficient of regression is r = 0.994.

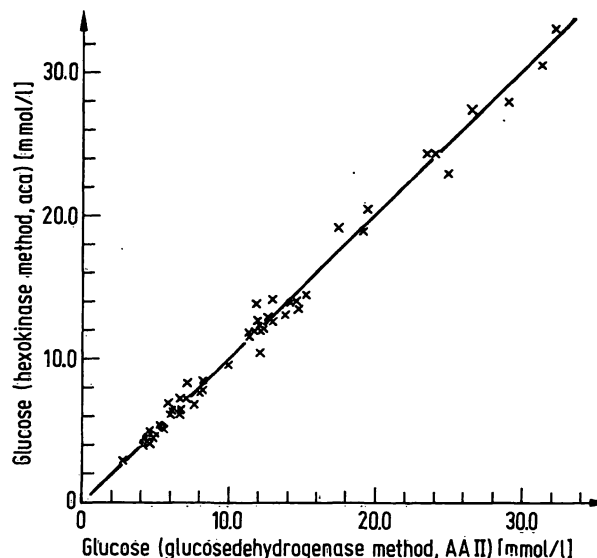

Fig. 1. Comparison for patients' specimens.

The reference method (glucose dehydrogenase on Technicon Autoanalyzer II) is on the x-axis (mmol/l) and the aca method is on the y-axis (mmol/l).

Statistical analysis:
No. of pairs: N = 61. Mean: $\bar{x} = 9.95$, $\bar{y} = 9.96$ mmol/l.
Standard deviation: $s_x = 6.099$, $s_y = 6.175$ mmol/l.
$s_{\bar{x}} = 0.794$, $s_{\bar{y}} = 0.804$ mmol/l.
Bias ($|\bar{y} - \bar{x}|$) = 0.01 mmol/l.
Slope: $b_{yx} = 1.006$, $b_{xy} = 0.982$.
y-intercept: $a_{yx} = -0.045$, $a_{xy} = 0.168$ mmol/l.
Variance: $S_{xy} = 2170.5$ (mmol/l)^2.
Covariance: $s_{xy} = 37.42$ (mmol/l)^2.
Standard deviation of y about x: $s_{yx} = 0.698$ mmol/l.
Correlation coefficient: r = 0.994.
T-test: t = 0.013, critical t value at P = 0.05 is 1.98; at P = 0.01 is 2.36.

Discussion

We have evaluated a microdetermination of glucose for the DuPont aca based on 20 µl of whole, skin-puncture blood. There are considerable practical advantages in our method: The volume of samples is minimal and easy to get, even from newborn children; no specially trained personel are required.

20 µl of sample reduce systematic errors due to deproteinisation (volume displacement effects) to a neglectable amount of 0.3%, thus permitting a cali-

Tab. 2. Precision data.
Within-run precision data from glucose standards:

Mean (mmol/l)	S.D. (mmol/l)	C.V. (%)	N
0.17	0.18		7
1.72	0.09	5.3	15
4.17	0.13	3.2	15
6.17	0.13	2.1	15
12.8	0.12	1.0	10
26.6	0.17	0.6	15

Day-to-day precision data from Monitrol II XPT 62 (Merz and Dade, München):

Mean (mmol/l)	S.D. (mmol/l)	C.V. (%)	N (days)	Method
12.6	0.3	2.2	161	aca (Hexokinase)
12.6	0.2	1.6	164	AA II (Glucose dehydrogenase)

S.D.: Standard deviation
C.V.: Coefficient of variation
N: Number of analyses resp. number of days.
Lot nos. of test packs: I 7094 A, I 7171 A, I 7215 A, I 7227 B, I 7241 A.

bration of the aca with usual protein-containing materials. Thus we can avoid determination of special "aca-values" for reference materials as described in l.c. (7). Such bottle values have to be obtained by split sample comparison with a reference method, and they may cause uncertainties.

In our opinion the choice of uranyl acetate instead of acidic reagents for deproteinisation facilitates the adaption of the aca to the described modification: Less changes in the lacing diagramm of computer II have to be carried out and no special buffer solutions have to be prepared, which may cause complications with new aca tests requiring a diluent 6. Last, but not least, neither acidic nor corrosive reagents are aspirated by the aca in our modification.

The clinical validity of the method is documented by an increased range of linearity compared to (4, 7), good coefficients of variation even at low, pathological levels, less interference due to glycolysis by red and white blood cells, icteric or lipemic samples, and results which show no systematic differences to an Autoanalyzer II method based on glucose dehydrogenase.

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