A micromethod for the determination of the glucose concentration in 20 μl samples with the autoanalyzer

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Introduction

The method described in the present work is simple and
specific and is suitable for the determination of glucose in
serum, plasma, whole blood, cerebrospinal fluid and diluted urine.
The sample is taken in disposable capillaries (1,2) and added to
a hemolyzing solution. With approximately 300 to 400 blood-sugar
determinations per day, the described sampling system with dis-
posable capillaries presented a considerable improvement and time
saving. We have been using this method for 12 months for routine
determinations and for 6 months also for emergency determinations without any major disruptions. It seemed logical to publish this method since it might be of practical use to many laboratories. A more detailed publication is in preparation.

Material and Method

Reagents

Solution No. 1: (0.154 mol/litre sodium chloride & Triton X-100)

9 g sodium chloride, reagent grade, is dissolved in 1 litre twice-distilled water. To this solution is added 1 ml Triton X-100.

Solution No. 2: All reagents from lot No. 15931 of Boehringer Co. Buffer substance (contents of bottle 1) is dissolved in 800 ml twice-distilled water (with new packages the content of both bottles No. 1 should be added).

Content of bottles 2 and 3 (NADP and ATP) is dissolved in approximately 10 ml twice-distilled water each and added to the buffer.

Content of bottle 4 (enzyme mixture) is rinsed into the buffer solution. To this mixture is added 1 ml Triton X-100 which is then topped up to 1 litre with twice-distilled water.

Rinsing Solution

(for washing the equipment): 0.1 mol/litre NaOH

Hemolyzing Solution

0.5 g sodium fluoride + 0.5 g potassium oxalate + 1 g 4-chloro-3-m-cresol dissolved in 1 litre twice-distilled water.

Standard Solution

To precisely 200 ml twice-distilled water is added 2 ml control serum (e.g. Hyland abnormal) and then mixed well.
Equipment

Second Generation Technicon Auto Analyzer, Sampler IV with cam 60 2/1. Dialyzer: 24" Standard Membrane Type C. Reaction spirals: 2 x 20 turns.
Colorimeter: 1.5 cuvettes (10 mm light path) 340 nm filter. Phototube 65 CE, lamp with yellow cables.

Analysis

The sample is introduced into the capillary, added to 2 ml hemolyzing solution and shaken well.\(^1\)

The specimen is sucked up via the sampler, diluted further and segmented with air. The reagent is dialized in a 24" dializer. In this way, high-molecular substances such as protein do not reach the reaction phase. The reaction takes place in several tarrying coils (reaction diagram see l.c. 3,4).

Following the removal of air, the formed NADPH is measured in the flow cell of the colorimeter at 340 nm.
Results

The method was tested for its accuracy, precision, linearity, freedom from problems and practical applicability. Fig. 2 shows the correlation between the method with the auto analyzer and the manual hexokinase method (original method) for 55 values.

![Graph showing correlation between glucose determination methods.](image)

**Fig. 2**
Correlation of method for glucose determination with the auto analyzer and the manual hexokinase method (original method by Boehringer Co.). The values of the manual method are no longer linear above 16.65 mmol/litre (3.00 g/litre).

- Correlation coefficient \( r = 0.9955 \)
- Regression line \( y = 11.2 + 0.9035x \)

The linearity in the range of 0 to 27.75 mmol/litre (5.00 g/litre) is shown in Fig. 3. Accuracy was monitored with the aid of various commercial control serums (Table 1). At the same time, Table 1 lists the day to day precision during the course of one week.
Fig. 3

Linearity of the auto-analyzer method in the range from 0 - 27.75 mmol/litre (0-5.00 g/litre).
* Required value
** Actual value

<table>
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<tr>
<th>Kontroll-serum</th>
<th>Deklaration [mmol/l]</th>
<th>Ergebnis [mmol/l]</th>
<th>VK_A [%]</th>
<th>VK_B [%]</th>
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<td>2,25</td>
<td>4,2</td>
</tr>
</tbody>
</table>

Table 1

Examination of accuracy and precision on a day-to-day basis during the course of one week (VK_A) and during one month (VK_B) (routine conditions).

1. Control serum
2. Declaration
3. Result

The recorder response for a concentration of 27.75 mmol/litre glucose is 100% of the measuring range for 13.875 mmol/litre glucose, thus 50% of the scale.
The signal attains approx. 97% of the steady state signal. The interference by fructose following interaction with hexokinase\(^2\) and phosphoglucone-isomerase\(^3\) of the erythrocytes in the hemolysate is absent due to the fact that the second enzyme does not reach the reagent mixture because of dialysis.

**Literature**


1. This Journal
2. This Journal
3. This Journal

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2) ATP: D-hexose 6-phosphotransferase, EC 2.7.1.1
3) D-Glucose-6-phosphate ketol-isomerase, EC 5.3.1.9