Sarstedt S-Monovette® GlucoEXACT

A blood collection device for stabilizing glucose levels for 96 hours

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Abstract

Analysis of blood glucose levels is an important topic in gestational diabetes as well as in diabetes mellitus. Citric acid acidified fluoride/EDTA is superior in stabilizing blood glucose than non-acidified fluoride/EDTA and is therefore recommended by nearly all clinical organizations related to diabetes. We have developed the S-Monovette® GlucoEXACT which stabilizes blood glucose levels up to 96 hours.

Introduction

Hyperglycemia in different forms (e. g. diabetes mellitus or gestational diabetes) is a disease of the modern civilization with rising numbers of affected people worldwide [1; 2]. As long term effects are severe, early identification of people suffering from hyperglycemia is important [3]. The primary diagnostic step in uncovering hyperglycemia is the measurement of blood glucose levels. Based on fasting blood or in context with oral glucose tolerance tests, the categorization into different kinds and/or stages of hyperglycemic diseases is possible [4; 8]. Consequently, a qualified therapy for the specific patient is applied in order to normalize the blood glucose level [1]. Stabilization of blood glucose in blood samples is essential since the glucose concentration is continuously decreasing when drawn in serum or heparin blood collection devices (-12 %/2 h). Even in fluoride/EDTA (FE) blood collection devices the rate of decrease is about -8.2 %/2 h [5; 6; 7].

For gestational diabetes mellitus (GDM) the German Society of Diabetes (DDG), the German Society for Gynecology and Obstetrics (DGGG), the American Diabetes Association (ADA) as well as the International Association of Diabetes and Pregnancy Study Groups (IADPSG) advise a very strict management of blood glucose stabilization, mainly based on the HAPO study [8]. All associations deprecate the use of capillary blood. Whereas blood glucose stabilization with NaF/EDTA alone is unsuitable the use of citric acid/citrate in combination with NaF/EDTA for stabilization of blood glucose is recommended by most organizations [9].

NaF alone inhibits enolase, an enzyme located distally in the glycolytic pathway. But the enzymes proximal in the glycolytic pathway still metabolize glucose until equilibrium is reached [10]. Only the acidification of blood by citric acid/citrate inhibits two enzymes (hexokinase and phosphofructokinase-1) proximal in the glycolytic pathway, which stops glycolysis immediately [7, 11]. Therefore we developed the S-Monovette® GlucoEXACT, a blood collection device that combines the immediate effect of acidification by citric acid/citrate and the long term glucose stabilization effect achieved by fluoride.

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The aim of this work is to determine the immediate and long term stabilization of glucose levels in the S-Monovette® GlucoEXACT.

Materials & Methods

Analysis of glucose in S-Monovettes GlucoEXACT:

During the procedure of blood collection, glucose levels may vary due to physiologic reactions [13]. To achieve a common glucose level as starting point (t = 0 h) neutral S-Monovettes (Sarstedt, REF.: 02.1726.001; Safety-Multifly®-Needle, 21 G, REF.: 85.1638.205) were filled with blood from a healthy donor and pooled into a 70 mL container (Sarstedt, REF.: 75.9922.744). The blood was immediately pipetted (Brand, HandyStep; Eppendorf, Combitips 50 mL, REF.: 0030089480) into S-Monovettes GlucoEXACT (Sarstedt, REF.: 05.1074.001). Each Monovette® for t = 0 h was directly given on ice, centrifuged (10 min, 2000g) within 30 minutes at 4 °C and analyzed (Analyticon, Biolyzer 300) for blood glucose (Analyticon, Bio Cal E Multi-Calibrator, REF.: 1430; Contronorm Plus, REF.: 1205; Fluitest GLU HK, Glucose Hexokinase, REF.: B5833; Fluitest GLU, Glucose GOD, REF.: B5703) within further 30 minutes. At several additional intervals during storage at 20 °C, a separate S-Monovette® GlucoEXACT was inverted and analyzed. All blood glucose values were measured in triplicate applying the hexokinase and/or the glucoseoxidase method. Finally the median was multiplied with the dilution factor of 1.16 to obtain the correct concentration of glucose in plasma.

Results

Plasma glucose concentrations were determined at different measuring times from nine healthy donors. Additionally, recovery rates were calculated, referring to the glucose concentration at the starting point (table 1). On average, the recovery rates were constant during the first 96 hours with a maximum increase of 0.7 %, applying the hexokinase method. The maximum increase of 2.2 % in a single donor was measured after 96 hours. Additionally, for six donors the plasma glucose concentration was measured using the glucoseoxidase method. Here a maximum increase of 2.0 % was observed after 96 hours (table 2). Interestingly, a slight continuous increase during the 96 hours of storage was observed (figure 2). A maximum increase of 3.7 % was measured after 72 hours in plasma of a single donor. The comparison of measured values from hexokinase and glucoseoxidase revealed that the hexokinase method gives 4.7 % higher values on average.

Table 1: Glucose concentrations of nine different blood donors over time at 20 °C applying the hexokinase method.

Hexokinase	concentration of glucose [mg/dL]						recovery rate [%]					
	0 h	4 h	24 h	48 h	72 h	96 h	0 h	4 h	24 h	48 h	72 h	96 h
Donor 1	107.1	n.d.	107.0	105.7	107.0	107.6	100	n.d.	100.0	98.7	99.9	100.5
Donor 2	101.6	n.d.	101.9	101.1	102.3	102.8	100	n.d.	100.3	99.5	100.7	101.1
Donor 3	117.4	n.d.	118.6	117.1	118.3	119.3	100	n.d.	101.0	99.8	100.7	101.7
Donor 4	104.8	n.d.	104.8	104.9	105.7	105.4	100	n.d.	100.0	100.1	100.8	100.6
Donor 5	101.1	n.d.	101.7	100.3	101.4	102.2	100	n.d.	100.6	99.3	100.3	101.1
Donor 6	114.1	n.d.	115.8	115.0	115.1	116.6	100	n.d.	101.5	100.7	100.9	102.2
Donor 7	100.6	101.8	101.0	100.9	n.d.	100.2	100	101.1	100.3	100.2	n.d.	99.5
Donor 8	104.4	104.7	104.2	103.2	n.d.	104.1	100	100.3	99.9	98.9	n.d.	99.8
Donor 9	95.3	94.9	94.5	95.8	n.d.	95.5	100	99.6	99.2	100.5	n.d.	100.3
Avg.							**100.0**	**100.3**	**100.3**	**99.7**	**100.5**	**100.7**
SD							**0.00**	**0.77**	**0.67**	**0.72**	**0.39**	**0.85**

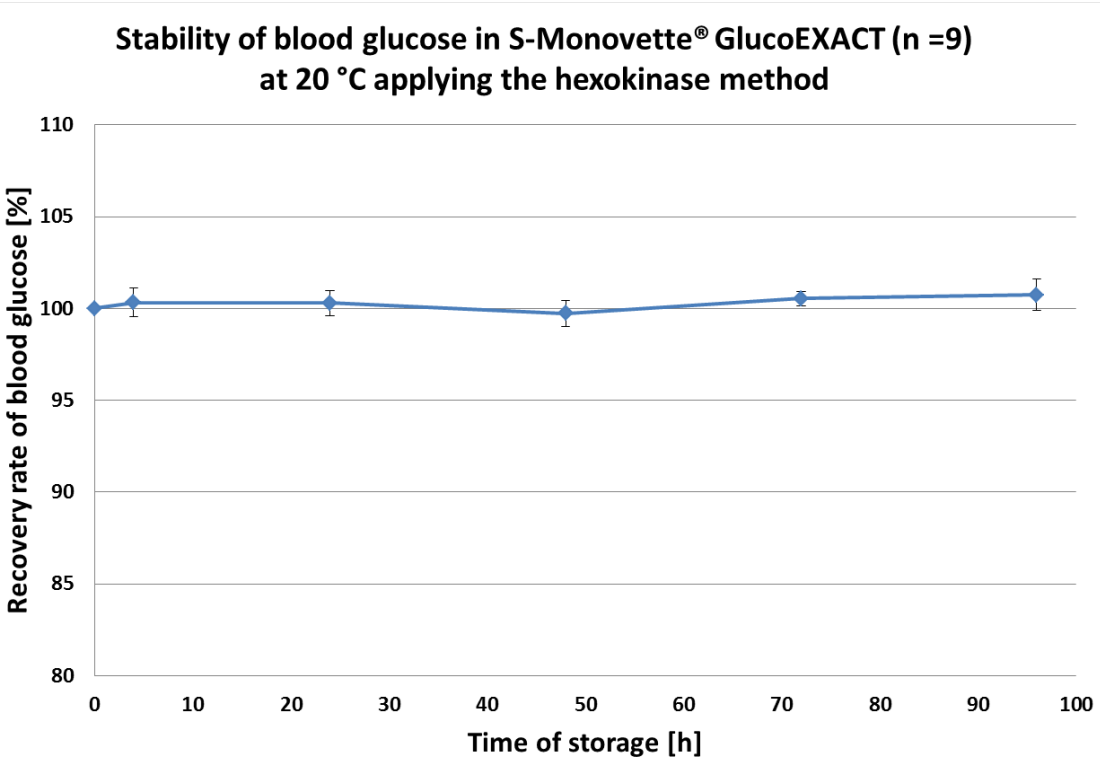

Figure 1: Depicted are the average recovery rates with standard deviations of blood glucose from 9 different donors up to 96 hours applying the hexokinase method.

Table 2: Glucose concentrations of six different blood donors over time at 20 °C applying the glucoseoxidase method.

Glucoseoxidase	concentration of glucose [mg/dL]					recovery rate [%]				
	0 h	24 h	48 h	72 h	96 h	0 h	24 h	48 h	72 h	96 h
Donor 1	104.5	103.5	104.6	105.0	108.0	100	99.0	100.1	100.4	103.3
Donor 2	97.6	99.3	99.4	101.2	100.0	100	101.8	101.9	103.7	102.5
Donor 3	114.1	115.4	115.2	114.6	116.6	100	101.1	100.9	100.4	102.1
Donor 4	103.0	103.2	104.2	104.1	104.1	100	100.2	101.1	101.0	101.0
Donor 5	98.8	97.4	99.9	98.5	100.8	100	98.6	101.1	99.6	102.0
Donor 6	113.9	113.7	114.3	114.6	114.8	100	99.8	100.3	100.6	100.8
Avg.					·	**100.0**	**100.1**	**100.9**	**101.0**	**102.0**
SD						**0.00**	**1.22**	**0.64**	**1.40**	**0.94**

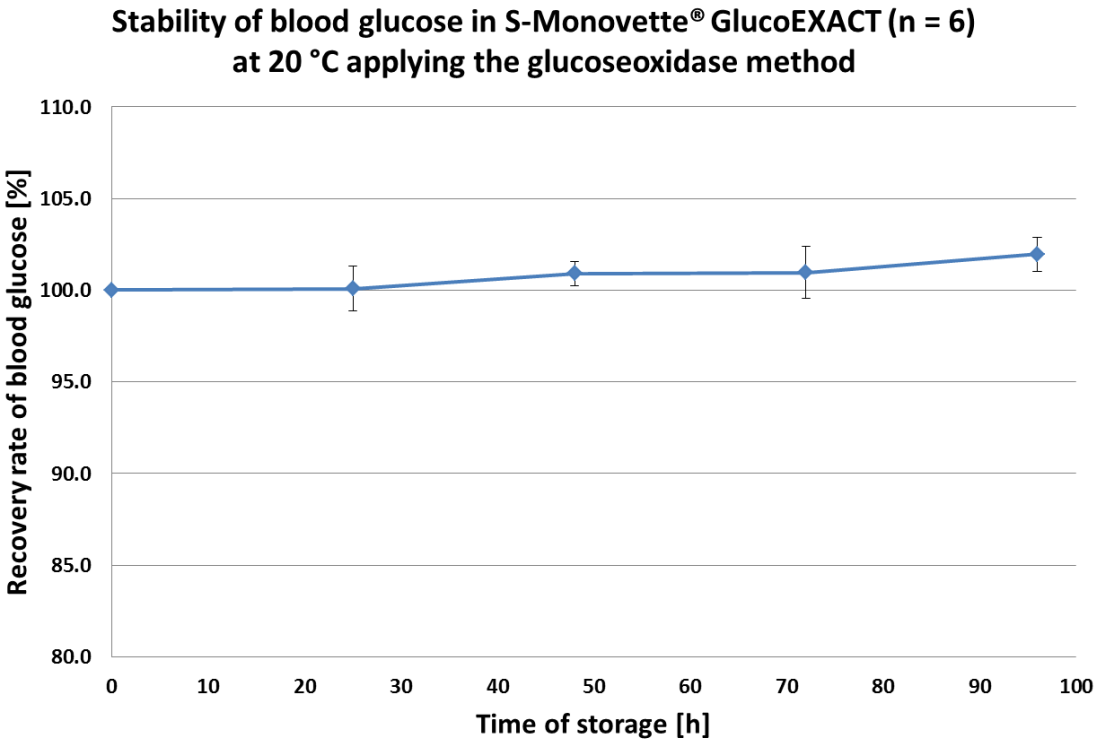

Figure 2: Depicted are the average recovery rates with standard deviations of blood glucose from 6 different donors up to 96 hours applying the glucoseoxidase method.

Discussion

In order to categorize people into different stages of hyperglycemia, a very accurate blood collection device is needed. EDTA/NaF alone is an incomplete glycolysis inhibitor as it needs around 2-4 hours until the full inhibitory effect in blood is achieved. During time a decrease of glucose of ~9 mg/dL per hour is observed. Uchida et al. [7] developed a mixture of EDTA/NaF/citric acid/citrate, which stops glycolysis immediately through additional acidification. S-Monovette® GlucoEXACT contains a preparation based on the above mentioned mixture, which combines the spontaneous glycolytic inhibitory effect of acidification (citric acid/citrate) and the long-term inhibitory effect of NaF.

Collecting blood into several appropriate blood collection devices directly from the vein, could give continuously rising or decreasing blood glucose values based on individual physiologic behavior of the donor [13]. Therefore we pooled an appropriate amount of neutral blood and aliquoted it into different S-Monovettes GlucoEXACT, one for each measuring point. The first aliquot at t = 0 h was cooled on ice directly in order to prevent any glycolysis. Blood glucose concentrations in succeeding aliquots were measured at storage intervals up to 96 hours at 20 °C. Applying the hexokinase method, glucose levels in blood were stabilized for at least 96 hours. Mean recovery rates showed maximum deviations from the reference value of 0.7 % after 96 hours. Applying the glucoseoxidase method, a continuous increase of glucose is observed with a mean of 2.0 % after 96 hours. Thus, a very little method dependent positive bias is detected during glucose measurements at 96 hours using the glucoseoxidase method. Standard deviation for each measurement was lower for the hexokinase method than for the glucoseoxidase method. Measured values from glucoseoxidase method are 4.7 % lower on average than with hexokinase method. This is a well-known phenomenon, described in the literature [12] and underlines the importance of method dependent reference values [14].

Conclusion

We developed a blood collection device, the S-Monovette® GlucoEXACT, which combines the inhibitory effects on glycolysis by citric acid as well as by fluoride. The glucose levels of the stabilized blood remains stable for at least 96 h. This minimizes the possibility of preanalytical errors and ensures a correct detection of glucose levels, even after prolonged transport of the sample.

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