Evaluation of **VACUETTE®** FC Mix blood collection tubes for diabetes testing

Background:

Greiner-Bio-One, Austria has been selling plastic evacuated tubes (**VACUETTE®**) for venous blood collection since 1986.

VACUETTE® FC Mix blood collection tubes contain an additive mix of Na_2EDTA, sodium fluoride, citric acid and sodium citrate. This mixture inhibits glycolysis and prevents coagulation. [1,2]

The **VACUETTE®** FC Mix blood collection tubes are used to stabilize the *in-vivo* glucose level in whole blood or plasma for up to 48 hours at room temperature and enable prolonged sample processing time including both storage and/or transportation. [3]

Diabetes is diagnosed by raised levels of blood glucose, a pathological OGTT (oral glucose tolerance test with 75g glucose) and elevated values for HbA1c (glycated hemoglobin A1C in EDTA blood).
Due to the incomplete inhibition of the glycolysis by sodium fluoride, new German guidelines recommend the usage of tubes containing the additive mixture of fluoride, citric acid and citrate. Furthermore, critical values in order to assess the glucose metabolism and diagnose diabetes mellitus are defined by those guidelines. [4/5]

Diabetes mellitus:	Casual plasma glucose	≥ 200 mg/dL	(≥ 11.1 mmol/L)
	Fasting plasma glucose	≥ 126 mg/dL	(≥ 7.0 mmol/L)
	OGTT-2h-value	≥ 200 mg/dL	(≥ 11.1 mmol/L)
	Hb1Ac	≥ 6.5%	(≥48 mmol/mol Hb)

Study Objective:

The study has been carried out to demonstrate that VACUETTE® FC Mix blood collection tubes are suitable for diabetes diagnosis by stabilization of glucose concentration for up to 48h after blood collection in comparison to Terumo VENOSAFE™ FC Mixture.

Study design and procedure:

Venous whole blood was collected from 50 non-diabetic donors and 50 diabetic subjects. Informed consent was given by all donors. The study has been approved by the Czech ethics commission. All specimens were drawn by using a **VACUETTE®** SAFETY Blood Collection Set (Item #450085) into the following tubes:

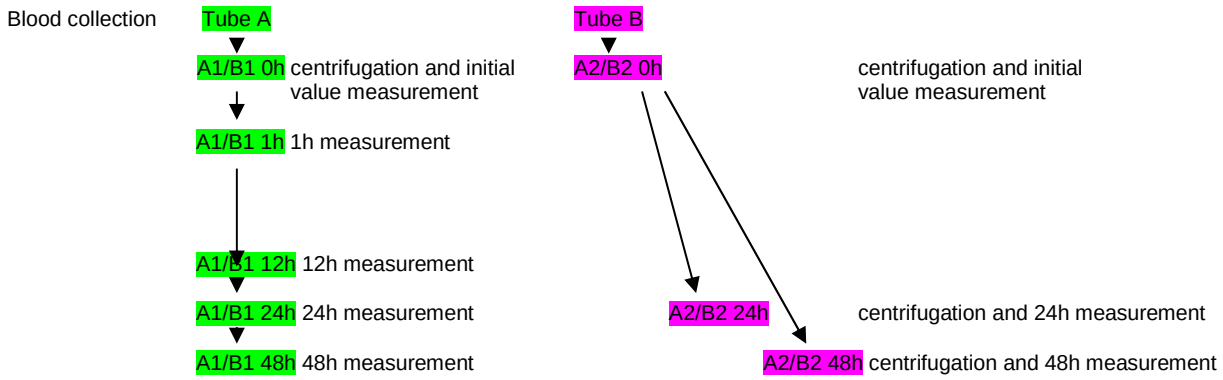
Sample A: VENOSAFE™ FC Mixture® (Prod. No. VF-052SCF)
Sample B: VACUETTE® FC Mix (Prod. No. 454511)

All samples were centrifuged at 1800g for 10 min at 20°C (centrifuge: Universal 30RF swing out bucket, Hettich). In order to test the robustness of whole blood, two tubes of each sample were taken. One of each sample was centrifuged immediately after blood collection. The second tube was split into three portions and centrifuged under two different time periods:

1) 24h after blood collection
2) 48h after blood collection

The sample tubes which were centrifuged initially after blood collection were analyzed for glucose at the initial time point, after 1h, 12h, 24h and 48h after blood collection on the COBAS 702 analyzer (Roche diagnostics, Mannheim). The sample tubes which were stored up to 48h after blood collection at room temperature were centrifuged and analyzed according to the time scheme given below. From each sample, the hemoglobin concentration was determined on the COBAS platform as well.

Time scheme:



Between measurements, all samples were stored in an upright position at room temperature. All samples were analyzed in duplicates.

Results:

1. Centrifugation directly after blood collection

The mean values of the glucose concentration for the samples which were centrifuged directly after blood collection are shown in Figure 1.

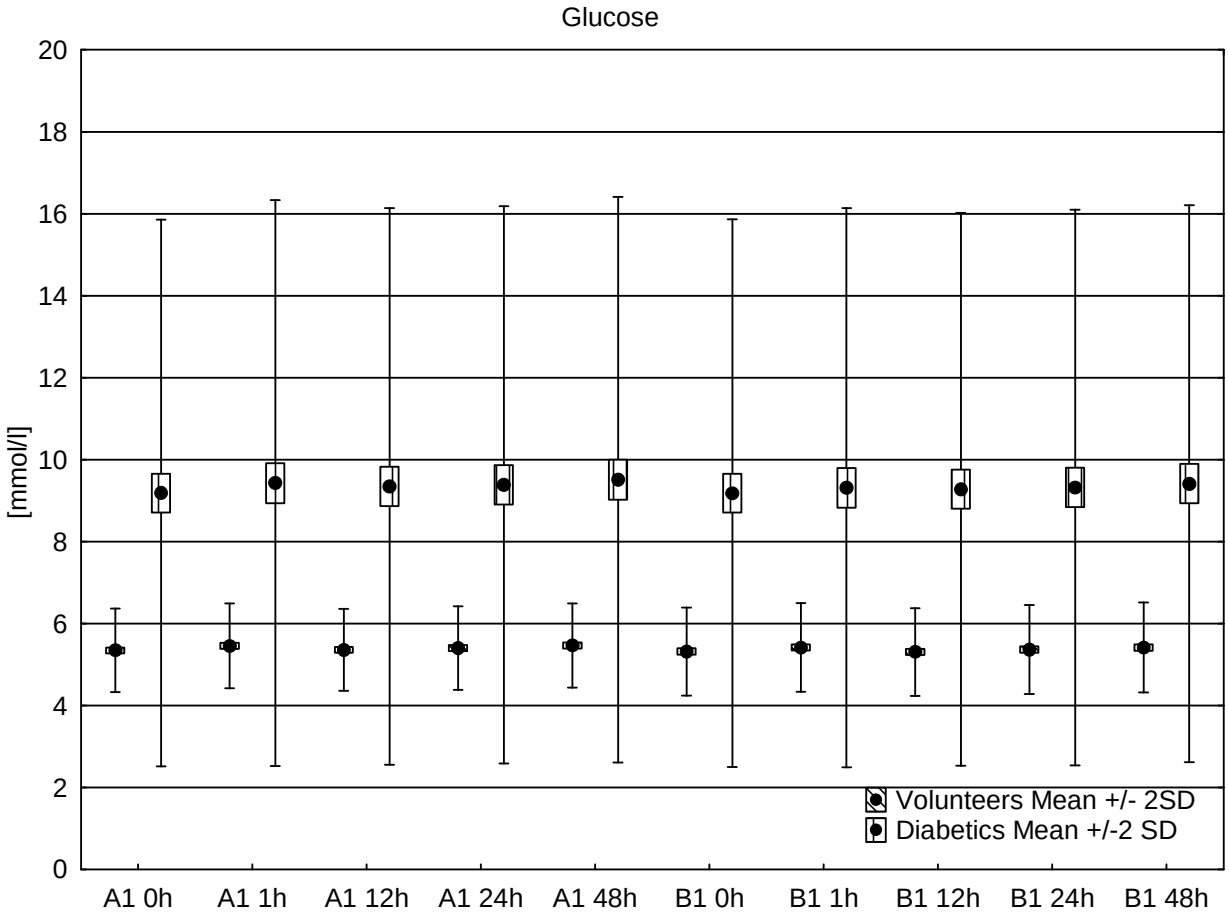


Figure 1 Mean results of glucose concentration [mmol/l] for samples centrifuged directly after blood collection (Initial values and replicates after 1h, 24h and 48h)

Comparison analysis was performed at all time points of determination. Statistics was performed with the t-test (α = 0.05) using StatSoft Software, Version 12.

Clinical evaluation was based on the allowed recommendation by the German Medical Association (RILIBÄK). [6].

Figure 1 illustrates the initial, 1h, 24h and 48h values for both samples centrifuged directly after blood collection. The stabilization of the glucose concentration has been demonstrated in both samples, no clinically or statistically significant deviations have been found comparing sample A1 to B1 at each point of time (highest single deviations: 4.2% initial, 8.0% after 1h, 5.2% after 12h, 6.1% after 24h, and 9.0% after 48h for diabetic subjects; 6.3% initial, 6.2% after 1h, 6.2% after 12h, 8.5% after 24h, and 6.2% after 48h for volunteer donors). When comparing the time points, the following deviations have been found for sample A1 after 48h (A1 0h to A1 48h: 6.9% and B1 0h to B1 48h: 7.7% for diabetic subjects and A1 0h to A1 48h: 7.5% and B1 0h to B1 48h: 6.2% for volunteer donors).

On the basis of the healthy or subjects diagnosed with diabetes tested, the equivalence in the performance of the samples that were centrifuged directly after blood collection has been shown

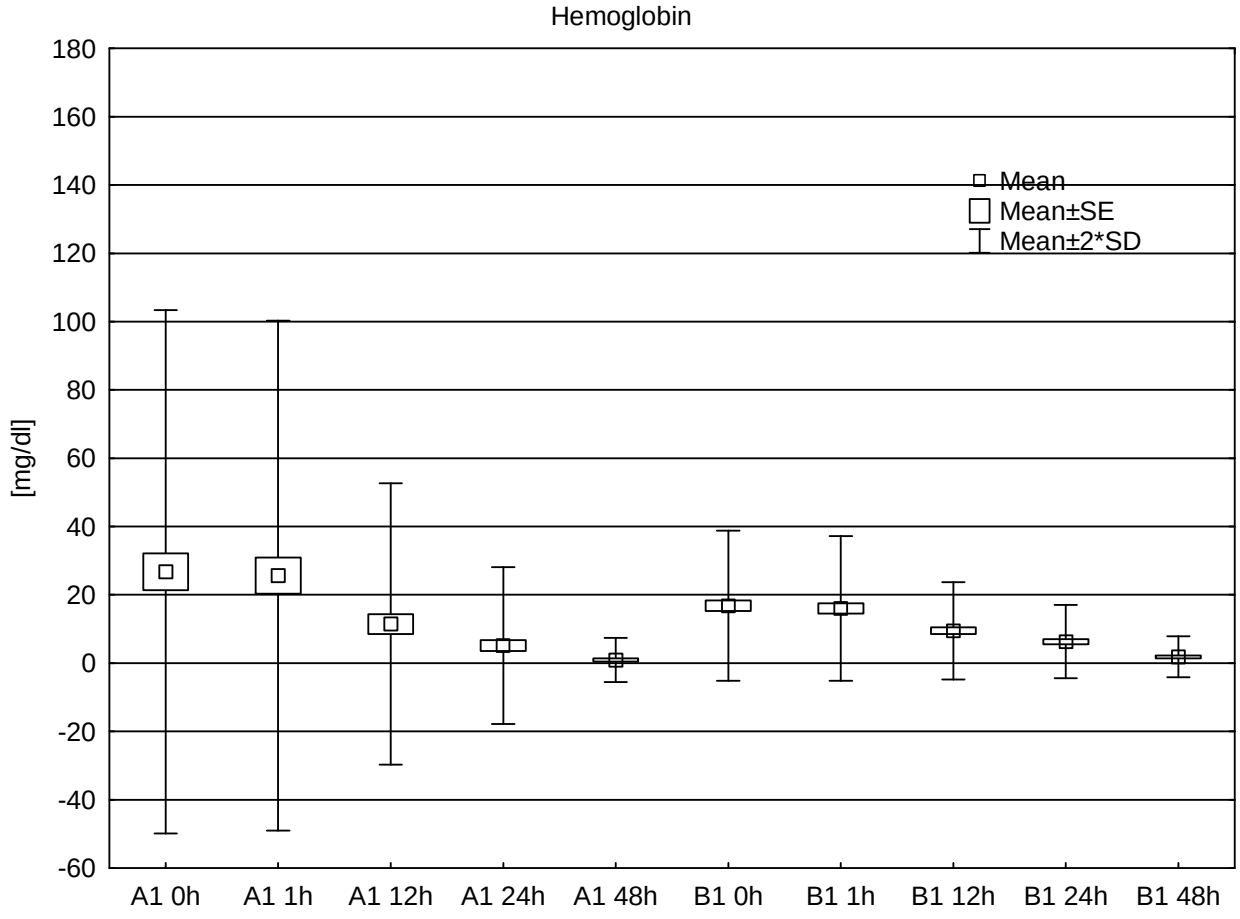


Figure 2: Hemoglobin concentration

The hemoglobin concentration in the tubes that were immediately centrifuged was very low. As indicated by Figure 2, the likelihood of hemolysis was found to be slightly higher in sample A, however without any impact on the determination of glucose concentration on the COBAS analyzer.

2. Storage of whole blood aliquots

Figure 3 summarizes the results of aliquotes stored as whole blood aliquots without immediate centrifugation.

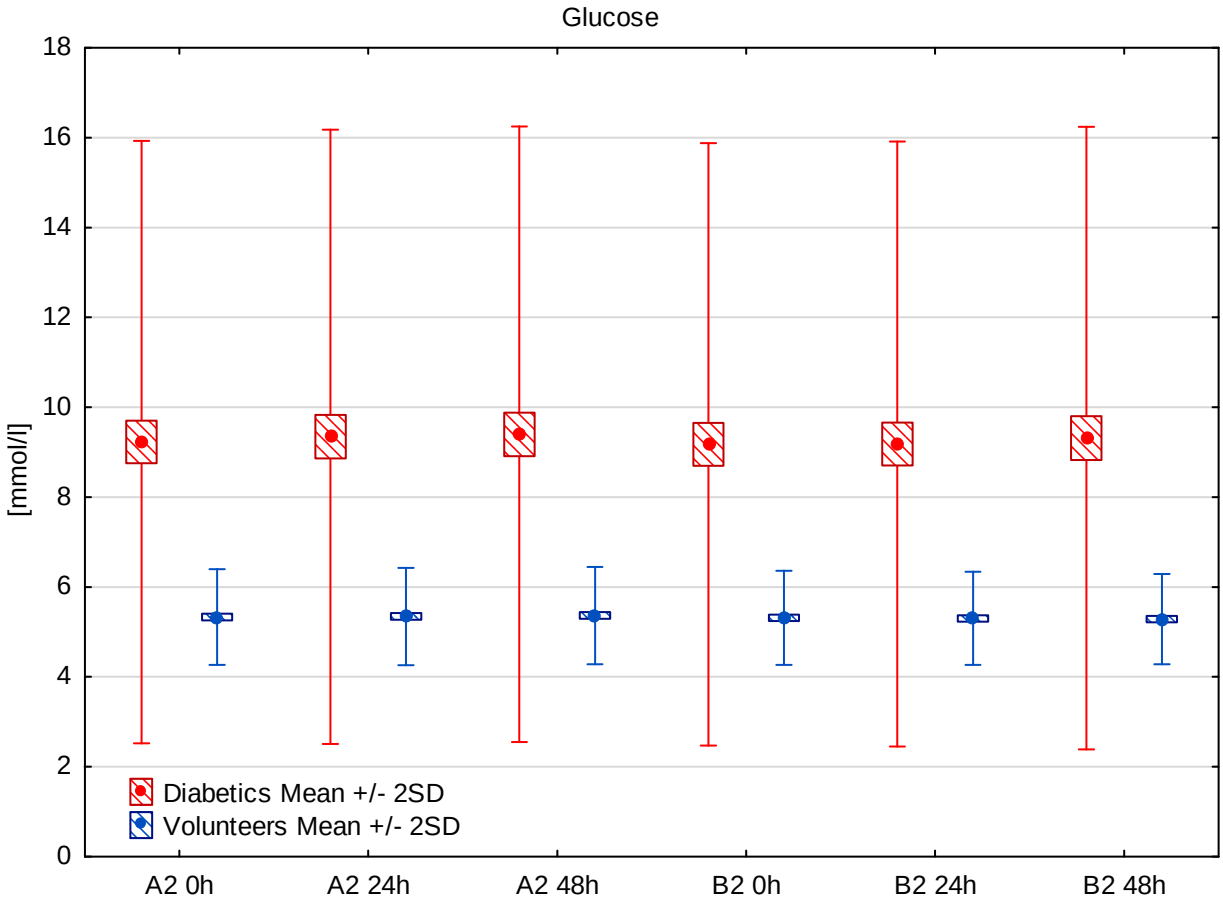


Figure 3: Storage as whole blood aliquots
Tube 2: Specimen aliquots centrifuged after 24h (A2 24h/B2 24h) or 48h (A2 48h/B2 48h) after blood collection

The stability of the glucose concentration in sample B2 (48h in whole blood) has been demonstrated as shown in Figure 3. The comparison between the initial values of sample B1 and the 48h values of sample B2 did not result in statistically or clinically significant differences due to sodium fluoride acting as an enolase inhibitor being responsible for the long term stabilization of the glucose concentration.

The stability of the glucose concentration in samples A2 24h, A2 48h as well as B2 24h, B2 48h (24h and 48h stored as whole blood aliquots) has been demonstrated by Figure 3. The highest deviations have been found for the comparison of A2 0h to A2 48h with 6.0% and B2 0h to B2 48 with 5.7% for diabetic subjects and of A2 0h to A2 48h with 6.0% and B2 0h to B2 48 with 3.6% for volunteer donors.

These results lead to the conclusion that storage for whole blood aliquots illustrates the equivalence in the performance of both blood collection tubes used for comparison testing.

Conclusion:

Using blood collection tubes containing the additive combination of citrate, EDTA and fluoride, acting as an enolase inhibitor being responsible for the long term stabilization of the glucose concentration, the stabilization of glucose concentration in the **VACUETTE**® FC Mix blood collection tubes in comparison to VENOSAFE™ FC Mixture (Terumo) was demonstrated for up to 48h at room temperature for donors who were healthy or diagnosed with diabetes. No clinically significant differences were observed between VENOSAFE™ FC Mixture and **VACUETTE**® FC Mix tube at any time point when stored in an upright position. Furthermore, stability of glucose concentration in whole blood aliquots was shown regardless of delayed centrifugation time up to 48h after the blood collection. These results indicate the suitability of **VACUETTE**® FC Mix blood collection tubes for reliable determination of glucose concentration for diabetes subjects if prolonged processing times including transport and/or storage times occur. On the basis of utilization of **VACUETTE**® FC Mix Blood collection tube, an impact of hemolytic index up to 250 mg/dl on the glucose concentration could not be proven.

References:

[1] E. Yagmur, J. van Helden, A. Koch, J. Jadem, F. Tacke, Ch. Trautwein: Effektive Glykolyse-Inhibierung im Citrat-gepufferten venösen Vollblut und Plasma, J Lab Med 2012; 36 (3): 169-177.

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[3] T. Koschinsky, P.B. Luppa, Neues und Kontroverses zur Blutglukosemessung bei der Diabetesdiagnostik: Konsequenzen der aktualisierten DDG- und AWMF-Leitlinien, J Lab ,Med; 2012; 36: 159

[4] W. Kerner, J. Brückel, Definition, Klassifikation und Diagnostik des Diabetes mellitus, Praxisempfehlungen der DDG, Diabetologie 2012: 7 (Suppl. 2): 84-87

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