Suitability of VACUETTE® FC Mix blood collection tubes for gestational 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 stabilize the *in-vivo* glucose level in whole blood and enable prolonged sample processing times including storage and/or transportation and the following analysis of glucose in the laboratory within 24h as required in the guideline [3].

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 and gestational diabetes are defined by those guidelines [4/5].

Gestational diabetes	fasting plasma glucose	> 92 mg/dL	(> 5.1 mmol/L)
	OGTT-1h-value	> 180 mg/dL	(> 10.0 mmol/L)
	OGTT-2h-value	> 153 mg/dL	(> 8.5 mmol/L)

Study Objective:

The study has been carried out to demonstrate that VACUETTE® FC Mix blood collection tubes are suitable for gestational diabetes diagnosis of pregnant women by stabilization of glucose concentration for up to 48h after blood collection in initially centrifuged tubes as well as for up to 24h in tubes stored as whole blood aliquots in comparison to Terumo VENOSAFE™ FC Mixture.

Study design and procedure:

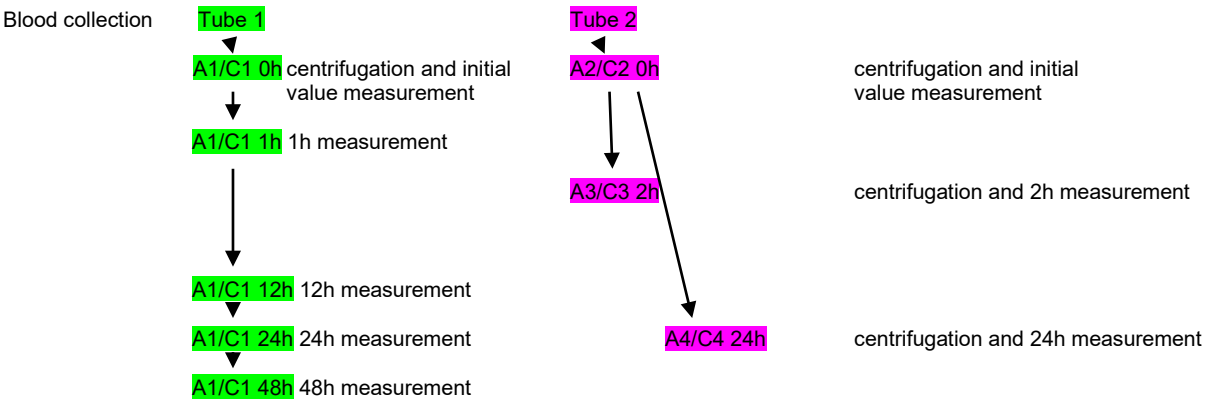
Venous whole blood was collected from 43 pregnant donors who were healthy (n=19) or diagnosed with gestational diabetes (n=24) by using a VACUETTE® SAFETY Blood Collection Set (Item #450085) into the following tubes:

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

All samples were centrifuged at 1800g for 10 min at 20°C (centrifuge: Eppendorf 5810R). 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) 2h after blood collection and
2) 24h after blood collection

Time scheme:



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 8000 analyzer (Roche diagnostics, Mannheim). The sample aliquots which were stored up to 48h after blood collection at room temperature were centrifuged and analyzed according to the time scheme given above. From each sample, the hemolytic index was determined on the COBAS 8000 as well.

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

One value is missing (A3, Donor 21) due to an analyzer error.

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 Table 1.

Table 1 Results of glucose concentration [mmol/l] for samples centrifuged directly after blood collection

Sample		Valid N	Mean	±SD
A1 0h:	Terumo VENOSAFE™ FC Mixture initial	43	6,81	1,76
A1 1h:	Terumo VENOSAFE™ FC Mixture	43	6,78	1,76
A1 12h:	Terumo VENOSAFE™ FC Mixture	43	6,77	1,81
A1 24h:	Terumo VENOSAFE™ FC Mixture	43	6,92	1,79
A1 48h:	Terumo VENOSAFE™ FC Mixture	43	6,93	1,77
C1 0h:	Greiner VACUETTE® FC Mix initial	43	6,76	1,74
C1 1h:	Greiner VACUETTE® FC Mix	43	6,73	1,72
C1 12h:	Greiner VACUETTE® FC Mix	43	6,77	1,73
C1 24h:	Greiner VACUETTE® FC Mix	43	6,82	1,74
C1 48h:	Greiner VACUETTE® FC Mix	43	6,83	1,74

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].

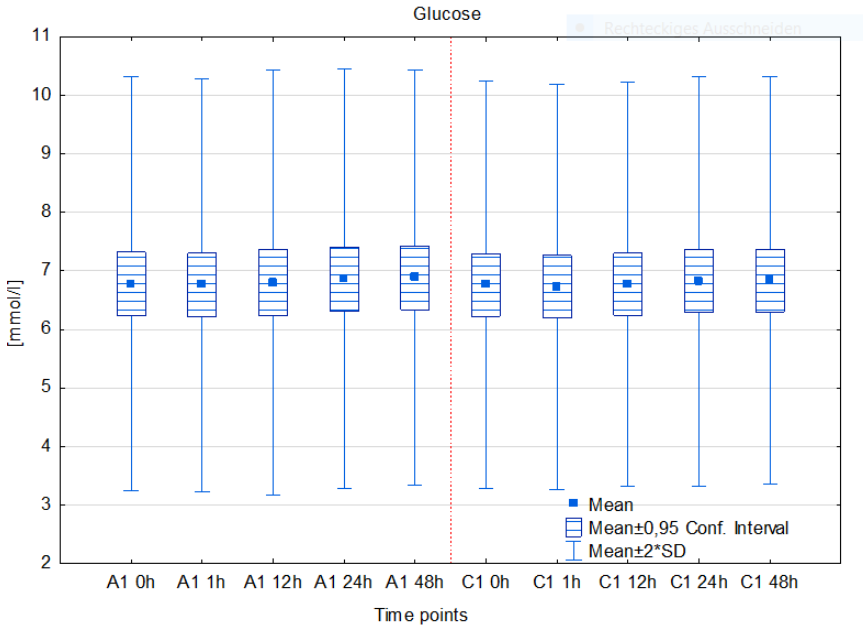


Figure 1: Initial, 1h, 12h, 24h and 48h values
Tube 1: specimen centrifuged immediately after blood collection, replicate measurements after 1h, 12h, 24h and 48h.

Figure 1 illustrates the initial, 1h, 12h, 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 C1 at each point of time (highest single deviations: 5.50% initial, 4.98% after 1h, 6.08% after 12h, 6.07% after 24h and 6.40 after 48h). The highest deviation when comparing the time points has been found for sample A1 after 48h (A1/0h to A1/48h: -5.37% and C1/0h to C1/48h: 6.56%).

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

The hemolytic index in the tubes, that were immediately centrifuged was very low (Hemolytic index <50 mg/dl). Storage of whole blood samples increases the likelihood of hemolysis as found for some samples centrifuged later (Hemolytic index <250 mg/dl), however without any impact on the determination of glucose concentration on the COBAS 8000 analyzer.

2. Storage of whole blood aliquots

Table 2 summarizes the results of aliquots stored as whole blood without immediate centrifugation.

Table 2 Results of glucose concentration [mmol/l] for storage of whole blood aliquots

Sample		Valid N	Mean	±SD
A2 0h:	Terumo VENOSAFE™ FC Mixture initial	43	6,78	1,76
A3 2h:	Terumo VENOSAFE™ FC Mixture	42	6,80	1,81
A4 24h:	Terumo VENOSAFE™ FC Mixture	43	6,77	1,82
C2 0h:	Greiner VACUETTE® FC Mix initial	43	6,76	1,74
C3 2h:	Greiner VACUETTE® FC Mix	43	6,74	1,78
C4 24h:	Greiner VACUETTE® FC Mix	43	6,69	1,79

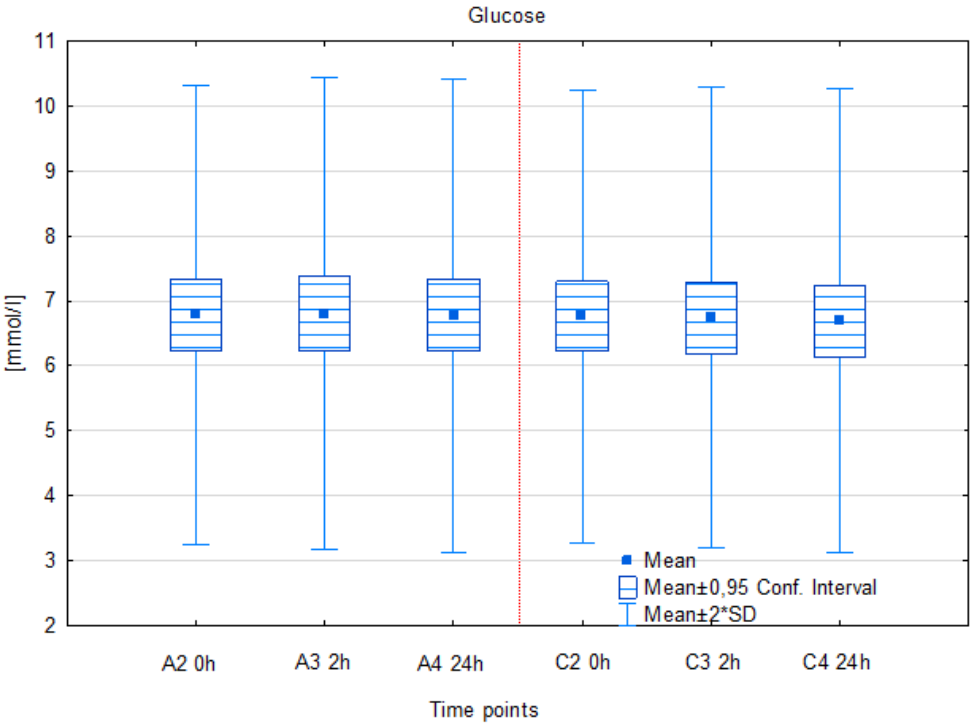


Figure 2: Storage as whole blood aliquots
Tube 2: specimen aliquots centrifuged after 2h (A3/C3) or 24h (A4/C4) after blood collection

The stability of the glucose concentration in sample C4 (24h in whole blood) has been demonstrated as shown in Figure 2. The comparison between the initial values of sample C2 and the 24h values of sample C4 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 aliquots A3, A4 as well as C3, C4 (2h, 24h stored as whole blood) has been demonstrated. The highest deviations have been found for the comparison of A2/0h to A4/24h with 10.32% and C2/0h to C4/24h with 8.84%.

These results lead to the conclusion that storage for whole blood aliquots also 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 initially centrifuged VACUETTE® FC Mix blood collection tubes in comparison to VENOSAFE™ FC Mixture (Terumo) was demonstrated for up to 48h at room temperature for pregnant donors who were healthy or diagnosed with gestational 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 was shown in whole blood aliquots regardless of delayed centrifugation time up to 24h after the blood collection. These results indicate the suitability of VACUETTE® FC Mix blood collection tubes for reliable determination of glucose concentration for pregnant women if prolonged processing times including transport and/or storage times occur. Further research with a larger group could be done to further sustain this conclusion. 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:

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