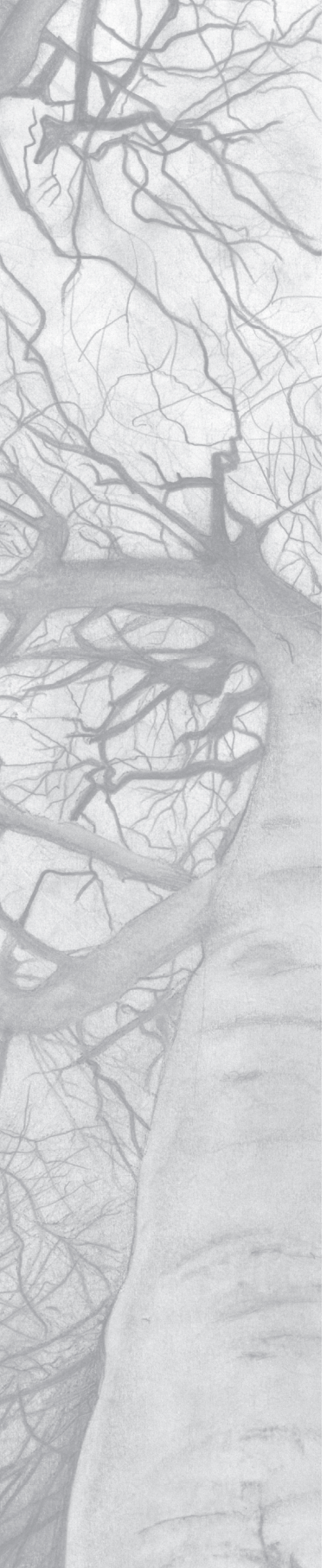

CHAPTER 8

The effect of time between venipuncture, processing and freezing on the measurement of coagulation factor levels

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INTRODUCTION

Coagulation factor levels are commonly measured in research studies evaluating etiology of diseases, in particular vascular diseases. Levels in the upper 10 percent (>P90) of the population distribution of procoagulant factors prothrombin, FVIII, FIX, FXI, and low levels of anticoagulation proteins are associated with an increased risk of venous thrombosis. [1-5] Many pre-analytical variables may affect the accuracy of these assays, including the duration of time from venipuncture to specimen processing and storage environment. [6,7] Clinic-based research settings are able to minimise the time from venipuncture to blood processing. However, immediate processing of blood samples is not feasible with some study designs, e.g., when samples are drawn in participant homes. In these settings, optimal sample handling should minimise pre-analytical factors that could impact coagulation factor level measurements.

Factor VII, factor VIII, and von Willebrand factors levels may be affected by a cold environment prior to centrifugation. [8-10] Pre-centrifugation storage temperature and time exposed to cold temperature prior to centrifugation did not appear to influence measured levels of factors prothrombin, V, VII, IX, XI and XII in the study of Favaloro. [8] Recommendations regarding the optimal temperature for storage prior to centrifugation of whole blood for performing coagulation factor assays are given in the fifth edition of the guidelines of the *Clinical and Laboratory Standards Institute*. [11] Cold storage prior to centrifugation of samples for factor VIII and von Willebrand factor is not advised in this guideline; however, no strict advice is given regarding the optimal sample handling conditions for other coagulation factors. This is due to the limited available evidence. The aim of this study was to assess the effect of differences in the time-interval between venipuncture and processing of blood on the levels of fibrinogen, prothrombin, factors VIII, IX, XI and antithrombin. Furthermore, the effect of storage of blood samples at different temperatures prior to centrifugation was assessed.

METHODS

A blood sample was obtained from 10 healthy volunteers; 4 males and 6 females with a mean age of 28 years (range 24–41 years). Venous blood was collected from the antecubital vein into three tubes of 3.8% sodium citrate (*Starstedt®*). Venous access was achieved at first attempt in all participants and a tourniquet was placed for a maximum of 25 seconds. All volunteers gave informed consent and the study was approved by the Medical Ethical Committee of the Leiden University Medical Centre in the Netherlands. The three citrate tubes were processed separately. Tube A, the reference sample, was processed directly after blood sampling. The sample was centrifuged at 18°C for 10 min

at 2500g and the plasma was immediately stored at -80°C. Tube B was stored for 2.5 hours at room temperature (~21˚C) while tube C was put on ice for 2.5 hours immediately after the venipuncture (~4˚C). After 2.5 hours both tube B and C were processed and stored similarly as tube A.

Analyses of the coagulation factor levels were all performed according to the instructions of the manufacturer of the STA-R analyser (Diagnostica Stago, Asnières, France). Fibrinogen (g/L) was determined according to methods of Clauss. [12] Levels of prothrombin, factors VIII, IX, XI, (*one-stage clotting assays*) and antithrombin (*amidolytic assay*) were measured as activity assays with mechanical clot detection methods. Mean coagulation factor levels were compared between the different methods of sample handling using a Student's paired t-test. Mean differences with 95% confidence intervals (CI) were calculated.

In addition, Bland-Altman plots were used to visualise the differences in coagulation factor levels between different storage conditions (on the *y*-axis) versus the mean coagulation factor level of the two storage conditions (*x*-axis). [13] The 95% limits of agreement of the three methods of storage were assessed to evaluate if the magnitude of the measurements affected the mean and the standard deviation of the difference.

RESULTS

The mean levels of the measured coagulation factor levels in tube A (immediately processed), tube B (processed after 2.5 hours storage at room temperature), and tube C (processed after 2.5 hours storage on ice) are shown in table 1. The table also shows the mean differences in coagulation factor levels among the three methods of sample handling. None of the coagulation factor levels (fibrinogen, prothrombin, factor VIII, factor IX, factor XI, and antithrombin) were affected by varying the time between venipuncture and centrifugation as shown by the mean differences between tubes A and B. Varying storage temperature, i.e., room temperature (tube B) versus cold storage on ice (tube C), only affected the measured levels of fibrinogen and coagulation factor VIII. For fibrinogen the mean difference was -0.014 g/L. By contrast, measured factor VIII levels were 16% *lower* after 2.5 hours of storage on ice compared with immediate centrifugation. Bland-Altman plots showed that the differences of the mean levels and their standard deviations were not influenced by the mean levels of fibrinogen, prothrombin, factor VIII, factor IX, factor XI and antithrombin, i.e., the difference between measured levels after different storage conditions was similar for individuals with high or low coagulation factor levels (Data not shown).

Table 1. Mean levels of coagulation factors in different storage conditions

N=10				Mean difference (95% confidence interval)		
Coagulation factor (range in adult population)	**Mean tube A**	**Mean tube B**	**Mean tube C**	**Tube A vs. tube B**	**Tube A vs. tube C**	**Tube B vs. tube C**
Fibrinogen (2–4 g L^{-1})	2.69	2.55	2.83	0.14 (−0.07 to 0.4)	−0.14 (−0.34 to 0.06)	−0.28 (−0.54 to −0.02)
Prothrombin (70–120%)	94.0	91.8	93.8	2.2 (−3.3 to 7.7)	0.1 (−4.2 to 4.5)	−2.1 (−7.0 to 2.9)
Factor VIII (60–150%)	86.6	91.7	70.6	−5.1 (−19.3 to 9.1)	16.0 (−1.6 to 33.7)	21.2 (13.1 to 29.2)
Factor IX (60–150%)	96.5	92.7	99.5	3.8 (−4.1 to 11.6)	−3.1 (−9.3 to 3.2)	−6.9 (−14.1 to 0.4)
Factor XI (60–150%)	100.3	99.4	98.0	0.9 (−3.1 to 4.9)	2.4 (−1.6 to 6.3)	1.49 (−2.4 to 5.4)
Antithrombin (80–120%)	106.8	105.3	107.2	1.5 (−0.08 to 3.1)	−0.4 (−2.3 to 1.5)	−1.9 (−4.9 to 1.1)

Tube A: direct processing. Tube B: room temperature (approximately 21°C), processing after 2.5 h. Tube C: cold environment (approximately 4°C), processing after 2.5 h. Mean differences with the 95% confidence intervals were calculated with Student's paired *t*-test.

DISCUSSION

Guidelines for blood sample storage time and temperature prior to centrifugation for coagulation factors are only available for von Willebrand factor and factor VIII, and are based on only a few studies. [8,9] We demonstrate here that time to centrifugation of blood samples up to 2.5 hours does not affect the levels of prothrombin, antithrombin and factors VIII, IX and XI. These findings are in accordance with the results of Favaloro et al. who reported no effect of time to centrifugation up to 3.5 hours, or differences in storage temperature (room temperature versus cold storage) for measurement of coagulation factors II, V, VII, IX, X, XI, XII. [8]

We also observed that sample temperature prior to centrifugation influenced fibrinogen and FVIII levels. The lower level of factor VIII in our study after exposure to a cold environment supports the idea that cold-activation of factor VIII may occur, so levels are less accurate after storage in a cold environment. [8,9] Differences for fibrinogen were too small to be of relevance.

In conclusion, we demonstrated that storage of blood samples on ice resulted in lower levels of coagulation factor VIII, and did not alter the levels of prothrombin, factor IX, factor XI and antithrombin. Moreover, somewhat higher fibrinogen levels were found after storage of the tubes on ice. Potentially, the effect of storage temperature may differ among different subgroups of the study population, e.g., a different coagulation factor activation in men and women, however the small study size did not allow us to perform subgroup analyses. Storage of the blood samples at room temperature for up to 2.5

hours prior to processing did not affect the measurement of the coagulation factor levels. This indicates that research studies where samples are drawn in participant homes or other non-laboratory settings, can obtain reliable coagulation factor level results when storing the samples at room temperature rather than on ice.

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