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Monitoring of Heparin and Low Molecular Weight Heparin with Capillary and Venous Whole Blood

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Key words

Heparin – Low molecular weight heparin – Coagulation assays – Chromogenic substrate assays

Summary

A method for determination of antifactor Xa-like activity in capillary whole blood obtained from the fingertip is described, which employs the Heptest coagulation assay. Values obtained with capillary whole blood are compared to values of corresponding plasma and venous whole blood samples. Normal values in plasma, venous whole blood, and capillary blood from the fingertip were 17.1 ± 2.1, 10.0 ± 1.3 and 10.4 ± 1.3 sec, respectively. The intraindividual coefficients of variations range from 0.4 to 1.8% in all assays. The day to day coefficient of variation of normal values ranged between 0.8 and 2.0% for all assays. The within assay coefficients of variation ranged from 3.0 to 7.7% for whole blood samples and from 1.5 to 2.2% for plasma samples after addition of no, 0.2 or 1.0 units of normal or LMW heparin to the samples. After administration of heparin or LMW heparin in healthy persons the coagulation values of the different coagulation assay systems displayed coefficients of correlation between r = 0.87 and r = 0.95. Correlation coefficients between the coagulation tests and the S 2222 chromogenic substrate method ranged from r = 0.77 to r = 0.92. In patients, who received LMW heparin for prophylaxis of thromboembolism the coagulation assay correlated between r = 0.78 and 0.92. The coagulation assays and the S 2222 method displayed coefficients of correlation between r = 0.74 and r = 0.83. The data indicate that Heptest sensitively measures antifactor Xa-like activity in capillary whole blood as well as venous whole blood samples containing low quantities of heparin or LMW heparin.

Introduction

The antithrombotic potential of low molecular weight (LMW) heparins is currently being investigated. In postoperative care (1–3) and in medical patients (4–6) various LMW heparins have been shown to prevent thromboembolism with a single dose daily, while treatment for thromboembolism appears to require repeated daily subcutaneous injections (7–8).

Impairment of renal function increases the half life of normal (9) as well as of LMW heparin (10). Elderly patients tend to accumulate heparin (11). These facts obviate the need for accurate monitoring of the anticoagulant effect of heparin therapy.

Anticoagulant therapy with heparin is routinely monitored with a variety of coagulation as well as chromogenic substrate assays, including activated partial thromboplastin time (12), thrombin clotting time tests (13), and chromogenic substrate methods measuring factor IIa or factor Xa inhibition (14). Another coagulation assay proved to be highly sensitive to low and high doses of normal heparin (15) as well as low molecular weight heparin (16). In patients treated with heparin or LMW heparin for a long period of time frequent withdrawal of venous blood becomes necessary. This may be difficult in some patients and quite strenuous for the patient as well as the staff.

The measurement of the effect of oral anticoagulants has been simplified by the introduction of a whole blood clotting assay using capillary blood obtained from the fingertip (17). An analogous test system using Heptest, for monitoring anticoagulant therapy with heparin has been developed. Results obtained with capillary whole blood from the fingertip are compared with results obtained with plasma and venous whole blood after administration of heparin and LMW heparin in healthy persons and in patients, who received LMW heparin for prophylaxis of thromboembolism.

Materials and Methods

Blood Sampling

Venous blood was collected by clean venipuncture using 3.8% sodium citrate as anticoagulant. One volume of anticoagulant was gently mixed with 9 volumes of blood in plastic tubes. Whole blood was tested in the coagulation assay immediately after withdrawal. Plasma was prepared by centrifugation at 1,800 g for 10 min. Plasma samples were analyzed within 30 min. Capillary blood was obtained from a small puncture in the fingertip using lancettes, and siliconized glass tubes (both from Merz & Dade, Munich, FRG) which were prefilled with 10 µl of 3.8 sodium citrate.

The clotting assays using venous and capillary whole blood were carried out as follows:

Heptest clotting assay in plasma was performed as described earlier. Coagulation assays were done with a Coa Screener analyzer (Laborgeräte, Ahrensburg, FRG) (16, 19, 20).

The chromogenic S 2222 substrate assay was performed in plasma as described earlier using the microtiter plate technique coupled to a computerized program (18).

Coagulation Assays

50 µl of whole blood were incubated for 2 min at 37° C. 50 µl factor Xa (Haemachem, St. Louis, USA) were added. After another 2 min of incubation at 37° C, coagulation was induced by addition of 50 µl Recalmix (Haemachem, St. Louis, USA). Clotting assays were performed with a Coa 1000 coagulation analyzer (Laborgeräte, Ahrensburg, FRG). Standard curves with whole blood were made using a pool of venous whole blood from 10 healthy subjects of the same blood group. The 4th international standard for normal heparin and the first international standard for low molecular weight heparin were used for calibration. Unfractionated and low molecular weight heparin were expressed as units/ml (U/ml) on the basis of these standards.

Coefficients of Variations

Capillary and venous blood and plasma samples were taken from 20 healthy male and female persons aged from 22 to 58 years on 5 days. The intraindividual variation and the day to day variation of the whole blood

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and plasma assays were determined. The mean values and standard deviation of high and low concentrations of heparin and LMW heparin were determined by adding 0.2 or 1.0 U/ml of heparin or LMW heparin to normal plasma and normal whole blood. 20 measurements of each sample were carried out and the within assay coefficient of variation was determined.

Administration of Heparin

7,500 U heparin (Braun, Melsungen, FRG) or LMW heparin (Kabi 2165, Kabi, Munich, FRG) were administered i.v. to 3 volunteers each and several subsequent capillary blood samples, whole blood samples, and plasma samples were taken. If coagulation time exceeded 120 sec, samples were diluted with whole blood or plasma prior to the assay. Hematocrit was measured from blood anticoagulated with EDTA using Coulter Counter (Coulter, Krefeld, FRG). For values obtained after injection of heparin median values are given. Linear regression analysis was performed between the different test systems.

Table 1 Mean values and standard deviation (sec) of Heptest coagulation assay in plasma, venous whole blood and capillary whole blood from 20 healthy subjects on 5 days (n = 20, mean and SD)

Assay	Day 1	2	3	4	5
Plasma	17.3 ± 2.0	17.5 ± 2.5	17.1 ± 2.1	16.7 ± 1.6	17.1 ± 2.4
Venous whole blood	10.1 ± 1.2	10.5 ± 1.7	9.9 ± 1.3	10.0 ± 1.1	9.7 ± 1.0
Capillary whole blood	10.6 ± 1.3	10.3 ± 1.0	10.3 ± 1.7	10.7 ± 1.4	10.4 ± 1.4

Table 2 Mean (x) and standard deviation (SD) of Heptest values determined on 5 subsequent days in 20 subjects

Subject	Capillary whole blood		Venous whole blood		Plasma	
	x	SD	x	SD	x	SD
1	10.9	1.12	10.3	0.48	18.9	0.67
2	10.6	1.01	10.7	1.57	17.8	0.75
3	8.8	0.92	8.5	0.39	16.0	1.42
4	11.3	1.17	10.3	0.93	17.5	0.88
5	11.3	0.78	9.6	0.62	16.5	1.36
6	11.4	1.28	11.3	0.65	19.3	1.74
7	10.7	1.30	9.6	0.30	15.6	0.89
8	12.8	1.80	12.0	1.20	19.5	1.08
9	9.5	0.60	9.1	0.60	13.4	0.45
10	9.1	0.50	8.8	0.80	16.3	1.10
11	10.2	0.82	10.5	1.11	18.0	1.15
12	8.9	1.01	8.7	0.50	15.2	0.70
13	11.3	0.60	11.1	0.50	21.3	1.50
14	10.5	0.80	9.9	0.80	15.6	1.10
15	11.7	0.90	12.1	1.70	19.9	1.20
16	9.4	0.60	9.3	0.30	16.8	1.05
17	10.2	0.90	10.2	0.50	16.1	1.00
18	9.6	0.70	9.5	0.50	15.4	0.20
19	10.1	0.40	9.9	0.50	18.2	1.40
20	10.2	0.40	9.0	0.75	15.5	0.75

Patients Receiving LMW Heparin

Thirty-three outpatients, who received one daily subcutaneous injection of LMW heparin (Kabi 2165) for prophylaxis of thromboembolism, were asked for additional blood sampling by puncture of the fingertip. Prophylaxis of thromboembolism was indicated in these patients due to side effects on conventional anticoagulants and recurrent thromboembolism or artificial heart valve replacement. The daily subcutaneous dose of LMW heparin ranged from 2,500 to 15,000 aXa units based on the body weight and bleeding risk (5). Coagulation assays of capillary, venous blood, and plasma samples as well as the chromogenic antifactor Xa S 2222 method were compared in the patients from samples obtained 2 to 4 hrs or in some cases about 15 hrs after the subcutaneous administration of the LMW heparin.

Results

Normal Values, Coefficients of Variation

Normal values of Heptest from capillary whole blood obtained from the fingertip ranged from 7.7 to 15.7 sec with a mean value of 10.4 ± 1.3 (SD) sec. Values for day to day variation and intraindividual variation in 20 healthy persons are given in Tables 1 and 2. Within-assay coefficients of variation were not determined, since only two tests can be performed from one sample of capillary blood, and repeated puncture of fingertips within a few minutes was refused by subjects.

Heptest values in normal venous whole blood ranged from 7.8 to 15.2 sec (10.0 sec ± 1.3). Heptest-values in normal plasma samples ranged from 13 to 20 sec (mean: 17.1 sec ± 2.1 sec). The day to day variation and the intraindividual variation in 20 healthy subjects are given in Tables 1 and 2. The within assay coefficient of variation of Heptest in plasma and whole blood samples containing no, 0.2, or 1.0 IU heparin or LMW heparin/ml sample is shown in Table 3. The variations are somewhat higher in whole blood samples compared to plasma. Coagulation times in whole blood samples are shorter than in plasma samples because the same amount of factor Xa and Recalmix are added to all test systems and whole blood samples contain only about half the amount of plasma compared to the plasma samples.

Coagulation of whole blood prior to performance of the assay did not occur, if siliconized glass capillaries with 3.8% sodium citrate were used (1/9 – citrate/blood). The fact that Heptest values for venous and capillary whole blood are nearly identical demonstrates that careful blood sampling from the fingertip does not influence normal coagulation times using Heptest reagents.

Comparison after Administration of Heparins

Coagulation times. The coagulation values of Heptest in capillary blood from the fingertip were compared with the results obtained with venous whole blood and plasma after i.v. administration of normal or LMW heparin in healthy subjects. High coefficients of correlation (linear regression analysis) were obtained between the coagulation times (sec) of venous blood –plasma, capillary blood–plasma, and capillary blood–venous blood. The equation of the regression lines are given in Table 4. Comparison of Heptest values of plasma samples with values of whole blood samples requires consideration of the cellular portion

Table 3 Mean and standard deviation (n = 20) of Heptest coagulation assay using whole blood and plasma without heparin and after addition of 0.2, or 1.0 units of normal or LMW heparin/ml

	No heparin (sec)	LMW 0.2 E/ml (sec)	LMW heparin 1.0 E/ml (sec)	Heparin 0.2 E/ml (sec)	Heparin 1.0 E/ml (sec)
Whole blood	10.4 ± 0.3	42.9 ± 2.9	87.5 ± 5.1	42.4 ± 1.4	125.4 ± 9.6
Plasma	17.4 ± 0.9	60.1 ± 1.3	115.0 ± 1.7	61.0 ± 1.3	120.3 ± 2.0

Table 4 Regression lines and coefficients of correlation of the Heptest coagulation values (sec) using capillary, or venous blood, or plasma after administration of heparin or LMW heparin to healthy subjects or LMW heparin to patients for prophylaxis of thromboembolism

sec – sec	Heparin		LMW heparin		Patients, LMW heparin	
Venous blood – plasma	y = 18.18 + 0.37x	r = 0.89	y = 6.94 + 0.52x	r = 0.90	y = 10.98 + 0.45x	r = 0.78
Capillary blood – plasma	y = 14.60 + 0.4x	r = 0.90	y = 8.93 + 0.46x	r = 0.87	y = 5.16 + 0.54x	r = 0.87
Capillary blood – venous blood	y = 1.76 + 0.95x	r = 0.92	y = 3.34 + 0.88x	r = 0.95	y = 1.04 + 0.95x	r = 0.92

of whole blood. The coagulation time of whole blood is generally shorter than the coagulation time of a corresponding plasma sample. However, correction of coagulation time values by use of the hematocrit as conversion factor is not possible.

Correlation coefficients between values for capillary and venous blood from the fingertip are r = 0.92 (normal heparin) and 0.95 (LMW heparin, Table 4). The slopes of the correlation curves are 0.95 and 0.88, respectively. These data demonstrate a high correlation between both assays and indicate that blood sampling from the fingertip does not influence the coagulation time values in heparinized blood.

Heparin activities. The correlation coefficients of the coagulation values (U/ml) of venous blood and plasma, capillary blood and plasma, capillary blood and whole blood samples are high and range between 0.82 and 0.94. The values for whole blood are lower due to the smaller amount of plasma in the test system. Therefore the slopes of the correlation curves are relatively small when comparing whole blood test-systems to assays performed with plasma samples. Comparison of the clotting values obtained with whole blood and capillary blood results in a rather steep correlation curve indicating that the activities of heparins are not essentially influenced by the cellular portion of blood.

A correction of the heparin activities in whole blood by hematocrit does not improve the correlation coefficients of the assays but – as expected – the correlation curve becomes steeper (data not shown).

Comparison of the coagulation tests with S 2222 method. The correlation of the coagulation test from venous blood, capillary blood, and plasma with the S 2222 chromogenic substrate assay ranged between r = 0.74 and 0.92 (Table 6). Values, which are corrected for hematocrit do not improve the coefficients of correlation although the slopes of the correlation curves are steeper (data not shown).

The time course of inhibition of factor Xa after i. v. injection of normal heparin, measured by S 2222 chromogenic substrate assay, and Heptest assay in plasma, venous and capillary whole blood are shown in Fig. 1. Values of whole blood samples were corrected for the hematocrit. The data indicate that there are no major differences in the time course of heparin activity using these assays. The time courses of antifactor Xa activity are also very similar after i. v. administration of LMW heparin. No major differences can be detected in the time course of heparin activity using the samples from capillary blood, venous blood, and plasma, and the S 2222 chromogenic substrate method (Fig. 2). The areas under the activity time curves of the different assays did not differ significantly with both heparins (Kruskal Wallis Test).

Treatment of Patients with LMW Heparin

The antifactor Xa like activities were correlated in capillary whole blood, venous whole blood, and plasma using the Heptest clotting assay, and in plasma using the S 2222 method from patients (n = 33), who received LMW heparin for prophylaxis of thromboembolism. The correlation of the clotting times of the different coagulation assays range from r = 0.78 to r = 0.92. The coagulation times of the whole blood assays are shorter than the coagulation times of plasma samples because 50 µl samples of plasma and whole blood are used in the assay. The correlation of the coagulation times of capillary and whole blood samples is shown in Fig. 3. The regression lines are given in Table 4.

When the coagulation values are transformed to U/ml the coefficients of correlation range from r = 0.80 to r = 0.94 (Table 5). Correction of the whole blood values by hematocrit does not improve the correlation coefficients of the assays (data not shown).

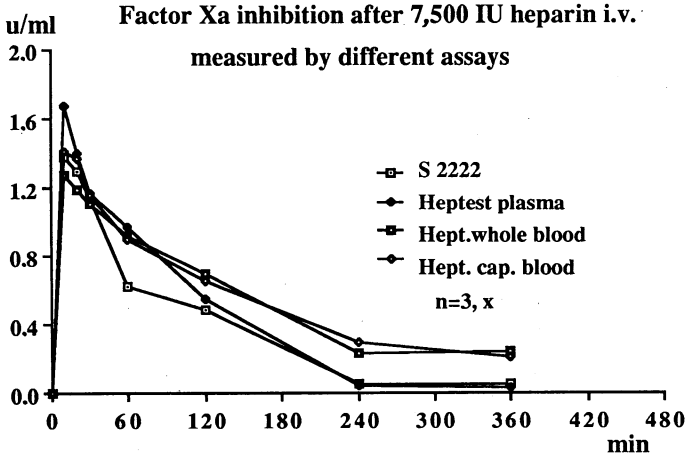

Fig. 1 The time course of the heparin-like activity (U/ml) is depicted after i. v. administration of 7,500 IU of normal heparin in healthy subjects using S 2222 chromogenic substrate assay, and the Heptest coagulation assay from plasma, venous, and capillary whole blood samples corrected by hematocrit (mean values)

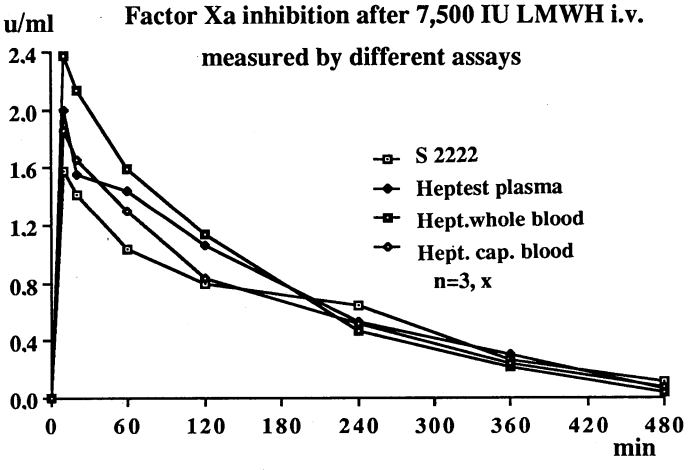

Fig. 2 The time course of the heparin-like activity (U/ml) is depicted after i. v. administration of 7,500 IU of LMW heparin Kabi 2165 in healthy subjects using S 2222 chromogenic substrate assay, and the Heptest coagulation assay from plasma, venous, and capillary whole blood samples corrected by hematocrit (mean values)

Table 5 Regression lines and coefficients of correlation of the Heptest coagulation assays (U/L) using capillary, or venous blood, or plasma after administration of heparin or LMW heparin to healthy subjects or LMW heparin to patients for prophylaxis of thromboembolism

U/L – U/L	Heparin		LMW heparin		Patients, LMW heparin	
Venous blood – plasma	y = 98.65 + 0.33x	r = 0.85	y = 12.17 + 0.7x	r = 0.94	y = 29.04 + 0.34x	r = 0.80
Capillary blood – plasma	y = 91.05 + 0.35x	r = 0.89	y = 52.12 + 0.53x	r = 0.82	y = 8.04 + 0.36x	r = 0.88
Capillary blood – venous blood	y = 13.23 + 0.97x	r = 0.94	y = 29.3 + 0.78x	r = 0.91	y = 0.92 + 0.98x	r = 0.94

Table 6 Regression lines and coefficients of correlation between the Heptest coagulation assays (U/L) from samples obtained from venous or capillary whole blood or plasma and the S 2222 chromogenic substrate assay (U/L)

U/L– U/L	Heparin		LMW heparin		Patients, LMW heparin	
Venous blood – S 2222	y = 105.81 + 0.16x	r = 0.77	y = 10.04 + 0.77x	r = 0.91	y = 34.49 + 0.36x	r = 0.74
Capillary blood – S 2222	y = 95.69 + 0.18x	r = 0.81	y = 22.23 + 0.60x	r = 0.83	y = 14.83 + 0.43x	r = 0.80
Plasma – S 2222	y = 17.30 + 0.50x	r = 0.91	y = 43.51 + 1.04x	r = 0.92	y = 46.6 + 0.96x	r = 0.83

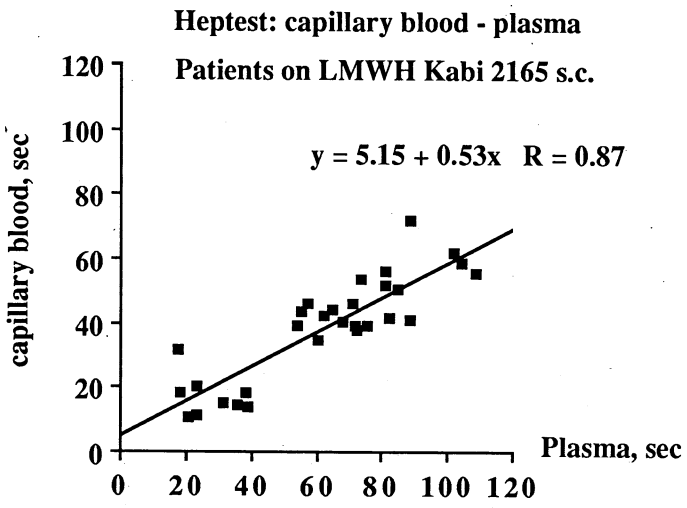

Fig. 3 The correlation between the coagulation times (sec) shown in Heptest coagulation assay using capillary blood and plasma samples obtained from patients receiving LMW heparin Kabi 2165 for prophylaxis of thromboembolism

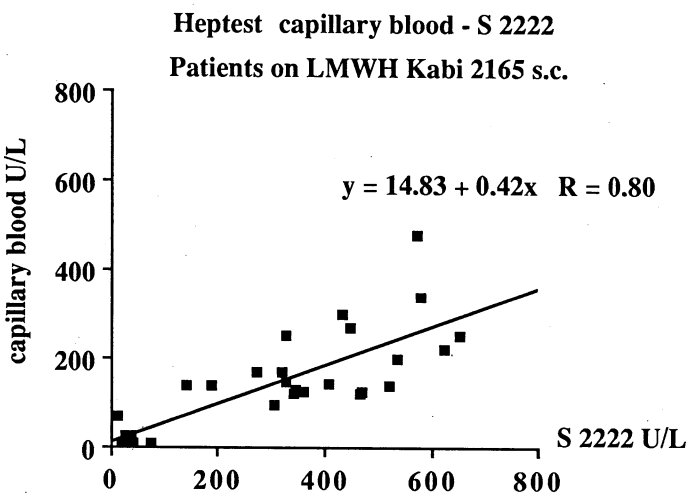

Fig. 4 The correlation between the Heptest coagulation values (U/L) from samples obtained from the fingertip and the S 2222 method from plasma is shown in patients receiving LMW heparin Kabi 2165 for prophylaxis of thromboembolism

The correlation coefficients of the clotting assays with the S 2222 chromogenic substrate method are shown in Table 6. When the results obtained with the whole blood methods are corrected by the hematocrit the coefficients of correlation do not improve (data not shown). The relation between values obtained from capillary whole blood and plasma S 2222 chromogenic substrate method is shown in Fig. 4 (r = 0.80).

Discussion

The development of thrombotest has considerably simplified the control of oral anticoagulation in outpatients (17). Use of capillary blood samples from the fingertip made monitoring of anticoagulant therapy by the patient himself possible.

Low molecular weight heparins are increasingly used in prophylaxis and treatment of thromboembolism (1–8). A heparin-sensitive coagulation test which could be performed by the patient himself would allow for long term heparin therapy in an outpatient setting. The present data support the assumption that centrifugation of blood samples is not necessary for measurement antifactor Xa-like activity in patients treated with heparin or LMW heparin using the Heptest coagulation assay. The results obtained with samples from healthy subjects demonstrate that measurement of antifactor Xa like activity using Heptest is also possible with very small samples taken from the fingertip.

The two methods to obtain whole blood – i.e. by venipuncture and from the fingertip – have been shown to be reproducible and valid with low intra- and interindividual variations for both whole blood test systems. Normal ranges for whole blood test systems are lower than for plasma system due to the relatively lower amount of plasma in the test tube. The high coefficients of correlation between the whole blood and plasma clotting methods demonstrate that the cellular portion of whole blood does not influence the antifactor Xa activity of heparins. At physiological quantities heparins bind tightly to antithrombin III and only to a negligible amount, if at all, to the cellular portion of whole blood. Therefore antifactor Xa like activities of heparin and LMW heparin is the same in whole blood and plasma samples. The results demonstrate that the method, by which the whole blood samples are obtained, do not affect the coagulation time in normal and heparinized samples. Thus, our data indicate that heparin can be sensitively determined in whole blood obtained from the fingertip.

The high coefficient of correlation of the whole blood test systems with the S 2222 method reflects a high sensitivity of Heptest reagents in the whole blood samples towards factor Xa. The S 2222 chromogenic substrate assay is based on an amidolytic reaction in a specific and sensitive biochemical system. It is largely uneffected by alterations of the individual patients coagulation pathway. Plasma coagulation assays are based upon fibrin formation in the patient's plasma sample and are influenced by non-heparin related alterations of the patients coagulation pathway. But the influence of cellular components of blood on fibrin formation is not included in these coagulation assays. Whole blood test systems as used in our study reflect effects of heparin and LMW heparin on plasmatic as well as cellular components of blood, thereby providing an estimate of the anticoagulant effect, which might be closer to the actual in vivo situation. However, in blood of healthy subjects, correction of heparin-like activity values of whole blood samples for hematocrit results in values which are almost identical to the values obtained with corresponding plasma samples in Heptest and S 2222 chromogenic substrate assay. This remains to be investigated for anticoagulants, and hyper- or hypocoagulabile states in patients.

In summary we conclude that Heptest coagulation time values of capillary whole blood samples from the fingertip and venous whole blood samples are highly reliable for detection of antifactor Xa like activities in patients receiving LMW heparin. The therapeutic ranges of the capillary and venous whole blood coagulation assays in patients treated with heparin or LMW heparin remain to be established.

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