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Manufactured By: HYPHEN BioMed

Hemoclot
Thrombin Time (T.T.)

Technical File
(Ref. ACK011K/L)

Clotting assay for the determination of thrombin time in human citrated plasma.

(offering an excellent sensitivity to low concentrations of heparin in plasma)

CE

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HEMOCLOT T.T. technical file (Ref. ACK011K/L)

Assay principle

- Hemoclot T.T. is an in vitro clotting assay developed for the determination of the clotting time induced by bovine thrombin (Thrombin Time, T.T.), in presence of calcium, on human citrated plasma, and exploration of the anti-thrombin activities.
- Excellent sensitivity to low concentrations of heparin in plasma (from 0.05 to 0.10 IU/ml Unfractionated Heparin (UFH), and from 0.20 IU/ml Low Molecular Weight Heparin (LMWH), in plasma).
- Manual, semi-automatic or automatic clotting method .

Intended use: IVD

CE

Measurement of the Thrombin Time (clotting time induced by bovine thrombin), in presence of calcium, on plasma, and exploration of the anti-thrombin activities, especially for low concentrations of heparin in plasma, and hirudin (qualitative detection).

Kit presentations:

CK011K: 6 x 20 tests (using KC10 or manual method).

CK011L: 6 x 80 tests (using KC10 or manual method).

Reagent (R1): Bovine thrombin, lyophilised in the presence of calcium, and stabilized.

Procedure:

- Specimen: citrated human plasma, undiluted

Assay Characteristics:

- **Assay optimized to offer an excellent sensitivity to low concentrations of Unfractionated Heparin (UFH) (from 0.05 to 0.10 IU/ml in plasma), of Low Molecular Weight Heparin (LMWH) (from 0.20 IU/ml in plasma), and of hirudin or other direct thrombin inhibitors.**
- Total assay time : **1 minute** or below
- Intra assay reproducibility: **CV ≤ 2-6 %** (for a normal plasma clotting time, using KC10 instrument)
- Inter assay reproducibility: **CV ≤ 3-8 %** (for a normal plasma clotting time, using KC10 instrument).
- Can be used with: manual, semi-automated or automated methods.

Intra-lot homogeneity and and inter-lots reproducibility

Tested using KC10 instrument, by measuring the clotting time (CT or thrombin time, in seconds) obtained for a lyophilised normal plasma pool. N≥ 10 independent vials are tested for each lot of Hemoclot Thrombin Time (T.T.):

Lot	060522A	060531B	060531C	060601C	060601D
N vials	10	10	10	10	10
Mean CT (sec)	23.9	22.9	23.6	22.5	22.6
SD (sec.)	0.30	0.45	0.23	0.26	0.30
CV (%)	1.25	1.96	0.97	1.14	1.31

For the 5 manufacturing lots, the obtained CV is <3%, in compliance with the specifications.
Excellent homogeneity within a same manufacturing lot is ensured, as well as between the successive manufacturing lots.

Stability of reconstituted reagents

Lot 060601D CT (sec):	Plasma 1	P1asma 1+UFH 0.05 IU/ml	Plasma 2	P1asma 2+UFH 0.05 IU/ml
Fresh	22.4	38.6	22.3	36.0
7days / 2-8°C	22.5	na	21.7	37.5
48h / RT	22.0	39.0	na	na

Excellent preservation of performances of reconstituted reagents, stored at 2-8°C for 7 days or at RT for 48 hours, compared with those of freshly reconstituted vials.

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Overheating study

	Lot 060531B		Lot 060601D	
CT (sec):	Plasma	P1asma +UFH 0.05 IU/ml	Plasma	P1asma +UFH 0.05 IU/ml
Fresh	23.0	37.2	21.7	34.9
30°C	22.4	38.3	21.6	37.0

Excellent preservation of performances following storage of lyophilised products for 3 weeks at 30°C comparatively to those stored at 2-8°C. Kits can be shipped at RT for a short period without damage.

Sensitivity to heparin; intra- and inter-assay reproducibility.

Comparison of the Unfractionated heparin (UFH) and Low Molecular Weight Heparin (LMWH) influence on the measured Thrombin Times, using the KC10 instrument, between different lots of Hemoclot Thrombin Time (T.T.) and the Stago reagent (Thrombin (2)).

Tested sample : normal plasma (45303), with addition of UFH (0-0.05-0.10 IU/ml) or LMWH (0.1-0.2-0.3 IU/ml).

	Reagent	060522A	060531B	060531C	060601C	060601D	Mean HBM	Stago
CT (sec)								
Normal Plasma		22.2	24	22.5	19.6	19.2	**21.5**	**14.3**
+ UFH (IU/ml)	**0.05**	27.1	*29.5*	*41.9**	26.1	25.8	**30.1 (*excl: 27.1)**	**15.5**
	0.10	NC	NC	NC	93.2	NC	**NC**	**18.7**
+ LMWH (IU/ml)	**0.10**	22.4	24.8	24.4	21.9	21.8	**23.1**	**15.8**
	0.20	33.6	31.8	35	30.8	29.8	**32.2**	**18.8**
	0.30	59.2	57.3	59.6	NC	56.3	**58.1**	**23.5**

(NC: no coagulation).

Conclusion : Excellent sensitivity to low concentrations of UFH (from 0.05 to 0.10 IU/ml), and to LMWH from 0.20 IU/ml, as compared with the Stago reagent.

INTER-ASSAY REPRODUCIBILITY of clotting times obtained with or without addition of UFH 0.05 IU/ml, using the KC10 instrument:

Each value corresponds to the clotting time of a normal plasma (NP44103), or a of the same plasma supplemented with UFH at 0.05IU/ml, tested with different vials of Hemoclot TT in independent series.

Lot	060531B (2ml)		060601D (8ml)		Stago	
Vial	NP	NP +0.05 UFH	NP	NP +0.05 UFH	NP	NP + 0.05 UFH
n	5	5	5	5	4	4
Mean	**20.4**	**32.8**	**19.7**	**28.7**	**15.6**	**16.3**
median	**20.5**	**30.5**	**19.8**	**27.9**	**15.3**	**16.3**
SD (sec)	0.3	4.5	0.2	2.2	1.0	0.4
CV (%)	**1.3**	**13.8**	**1.3**	**7.6**	**6.4**	**2.4**
Min	20.0	27.2	19.2	25.8	14.7	15.8
Max	20.7	39.9	19.9	32.1	17.3	16.7

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Each value corresponds to the clotting time of a normal plasma (NP45303), or a of the same plasma supplemented with UFH at 0.05IU/ml, tested with different vials of Hemoclot TT in independent series.

	Inter assay CV			
	Lot 060531C		Lot 060601D	
	NP	NP+ 0,05 UFH	NP	NP+ 0,05 UFH
N	9	9	8	8
Mean	**24.2**	**51.9**	**22.0**	**41.6**
Median	24.5	53.5	21.8	41.15
SD (sec)	1.28	2.81	0.88	3.03
CV (%)	**5.3**	**5.4**	**4.0**	**7.3**

Conclusion: inter assay CV for normal plasmas are in compliance with the specifications (≤10%). Obtained CV are slightly higher for plasmas supplemented with UFH at 0.05 IU/ml , as the measured clotting times for those specimen can vary "more widely" ("weak coagulability of plasma resulting from the presence of trace amounts of heparin). An inter assay CV of 2-6% can be claimed for normal plasma, using the KC10 instrument.

INTRA-ASSAY REPRODUCIBILITY of clotting times obtained with or without addition of UFH 0.05 IU/ml, using the KC10 instrument:

Each individual value corresponds to the clotting time of a normal plasma (NP45302), or a of the same plasma supplemented with UFH at 0.05 IU/ml or LMWH at 0.20 IU/ml, tested with a same vial of Hemoclot TT in a same series.

Intra assay reproducibility								
Lot	060531C				060601D			
Plasma	NP	NP +UFH 0.05	NP +UFH 0.05	NP+LMWH 0.2	NP	NP +UFH 0.05	NP +UFH 0.05	NP+LMWH 0.2
N	**10**	10	10	10	**10**	10	10	10
Mean CT(sec)	**20.8**	40.3	43.2	38.4	**21.6**	35.4	31.4	36.2
SD (sec)	**1.15**	15.49	4.80	7.66	**0.39**	6.89	4.93	10.20
CV (%)	**5.5**	/	/	20.0	**1.8**	/	/	/
Median	**20.3**	31.4	44.4	40.3	**21.7**	34.4	29.3	31.7
Min	**19.4**	30.3	29.6	29.0	**20.7**	27.9	27.8	28.0
Max	**22.8**	81.2	48.3	50.1	**22.1**	43.8	41.8	59.0

Conclusion: intra assay CV for normal plasmas are in compliance with the specifications (3-8%). Obtained CV are "similar" or higher for plasmas supplemented with UFH at 0.05 IU/ml, as the measured clotting times for those specimen can vary "more widely" ("weak coagulability" due to presence of trace amounts of heparin). Intra assay CV of 2-6% can be claimed for normal plasma, using the KC10 instrument.

Discrimination is excellent for plasmas containing low concentrations of heparin (from 0.05 to 0.10 IU/ml UFH, and from 0.20 IU/ml LMWH).

Sensitivity to Hirudin

Goal: To evaluate the effect of hirudin concentrations in plasma up to a given concentration, on the Thrombin Time measured with the Hemoclot T.T. assay.

Materials and reagents: Hemoclot T.T: lots 060531C and 060601D; test run using the KC10 instrument.

Preparation of tested plasma samples: 3 normal plasmas (I, II, III) are supplemented with increasing concentrations of hirudin, and then tested for clotting time using the Hemoclot T.T. assay.

Results:

Plasma	(I)		(II)		(III)		Mean N=3		Global mean CT (sec)
Lot	060531C	060601D	060531C	060601D	060531C	060601D	060531C	060601D	
HIR (ATU/ml)	CT (sec)						Mean CT (sec)		
0	22.5	21.9	22.7	23	21.8	21.7	22.3	22.2	22.3
0.125	26.4	23.3	25.3	26.5	24.6	24.5	25.4	24.8	25.1
0.25	30	25.8	28.2	29.9	28.4	27.6	28.9	27.8	28.3
0.50	50.5	37.2	45.2	43.9	41.1	42.3	45.6	41.1	43.4
1	NC*	NC	NC	NC	NC	NC	NC	NC	NC

Conclusion: A good Thrombin Time sensitivity is observed for low concentrations of hirudin (clotting time prolonged from 0.25 ATU/ml or 17 ng/ml, using the KC10 instrument). (NC*: No coagulation)

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Interference of fibrinogen degradation products, bilirubin, & haemoglobin

Goal: To evaluate the interference of fibrinogen degradation products, bilirubin, and haemoglobin concentrations in plasma up to a given concentration, on clotting time measurement using the HEMOCLOT T.T. assay.

Materials and reagents:
-Hemoclot T.T: lots 060531C and 060601D.
-Fibrinogen degradation products (FDP) (HBM), Bilirubin (Bil) and Haemoglobin (Hb) (Sigma).
-Normal plasma samples (P).

Preparation of tested plasma samples: normal plasmas (P) are supplemented with increasing concentrations of FDP, bilirubin or haemoglobin, and then tested for Thrombin Time using Hemoclot T.T. assay.

Results (tested using KC10 instrument):

Plasma	P1		P2		P3		Mean N=3		Global mean CT (sec)
Lot	060531C	060601D	060531C	060601D	060531C	060601D	060531C	060601D	
	CT (sec)						Mean CT (sec)		
0	22.4	21.8	21.1	20.2	20.8	19.5	21.4	20.5	**21.0**
50	22.9	22.4	17.9	20.9	21.1	22.7	20.6	22.0	**21.3**
200	23.3	22.8	19.6	19.9	21.6	22.4	21.5	21.7	**21.6**
500	23.3	24.2	20.7	19.5	22.4	24	22.1	22.6	**22.4**
1000	22.9	24.9	20.2	19.9	23	23.1	22.0	22.6	**22.3**

	(4)		(5)		(6)		Mean N=3		Global mean CT (sec)
Lot	060531C	060601D	060531C	060601D	060531C	060601D	060531C	060601D	
Bili. (mg/ml)	CT (sec)						Mean CT (sec)		
0	20.7	21.5	20.6	21.6	20.5	20.0	20.6	21.0	20.8
0.01 mal»	20.2	21.6	19.3	21.4	19.2	20.0	19.6	21.0	20.3
0.10	19.8	22.3	18.7	21.2	18.7	19.2	19.1	20.9	20.0
0.25	19.4	21.6	19.5	19.9	19.9	19.2	19.6	20.2	19.9
0.50	18.8	20.7	18.9	19.7	18.1	21.5	18.6	20.6	19.6

	(1)		(2)		(3)		Mean N=3		Global mean CT (sec)
Lot	**060531C**	**060601D**	**060531C**	**060601D**	**060531C**	**060601D**	**060531C**	**060601D**	
Hb. (mg/ml)	**CT (sec)**						**Mean CT (sec)**		
0	20.7	21.5	20.6	21.6	20.5	20.0	20.6	21.0	**20.8**
0.1 mal»	20.6	21.0	19.7	20.6	18.4	19.5	19.6	20.4	**20.0**
1.0	20.7	22.2	19.4	na	18,8	19.9	19.6	21.1	**20.3**
2.5	19.9	21.9	21.2	20.1	18.9	19.8	20.0	20.6	**20.3**
5.0	19.6	21.5	18.6	18.6	18.1	20.0	18.8	20.0	**19.4**

Conclusions: No significant interference on clotting times obtained for FDP until 1mg/ml in plasma, for bilirubin until 0.25 mg/ml in plasma (25x normal concentration), and for haemoglobin until 2.5 mg/ml in plasma (25 x normal concentration), using the KC10 instrument. The incidence of FDP directly generated in the tested plasma (by Streptokinase) needs to be evaluated.
However, Thrombin Time may be affected by many usual drugs, and further studies should be run to determine the source of unexpected abnormal results.
In order to get the full assay performances, the working instructions must be carefully observed.

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HEMOCLOT T.T. technical file (Ref. ACK011K/L)

Adaptations to intruments.

Goal: Comparison of the device performances for clotting time measurement on different instruments.

Material: Hemoclot T.T. lot 060531C.

Samples: 31 normal frozen citrated plasma samples, 9 plasmas from patients under dicoumarol therapy, and normal plasmas with addition of various concentrations of UFH or LMWH.

Protocols on the different instruments: according to the specific adaptation for each instrument.

Results :

Normal frozen plasmas	KC10
N	56
Mean CT (sec)	20.8
median	20.8
SD	1.72
min	16.9
max	24.5
mean+2SD	24.2

Instrument	KC 10	STA	Amax Destiny (mecha.)	Amax Destiny (optic.)	ACL (optic.)
CT (sec)					
NORMAL PLASMA SAMPLES					
N	31	31	31	31	31
Mean	20.9	21.6	23.2	18.0	17.3
SD	2.00	1.86	1.72	1.60	1.25
Min	16.9	17.0	20.9	15.8	15.4
Max	**24.5**	**25.8**	**26.9**	**21.6**	**20.3**
Median	20.5	21.3	22.8	17.5	17.4
Mean+2SD	**24.9**	**25.4**	**26.7**	**21.2**	**19.8**
PLASMAS OF PATIENTS UNDER DICOUMAROL THERAPY					
N	9	9	9	9	9
Mean	**18.1**	**18.0**	**19.6**	**10.3**	**<15.4**
SD	1.31	2.46	0.99	0.70	na
Min	16.5	14.8	17.4	9.5	na
Max	**20.3**	**23.8**	**20.9**	**11.9**	**na**
PLASMAS WITH ADDITION OF HEPARIN					
0	19.3	21.0-21.4-22.2	23.2	18.2	17.2
0.1 LMWH	24.3	27.8	30.2	21.4	21.4
0.2 LMWH	35.0	51.0-53.8-55.1	NC-NC	29.5-34.8	29.1
0.3 LMWH	84	NC	NC	82	41.7
0.05 UFH	30.6	41.5-42.2-41.3	36.7	25.2	25.6
0.1UFH	NC	NC	NC	NC	120.1

Conclusion:

•Good discrimination of plasmas containing low concentrations of heparin will all instruments.

•Similarities and differences are noticed between the methods:

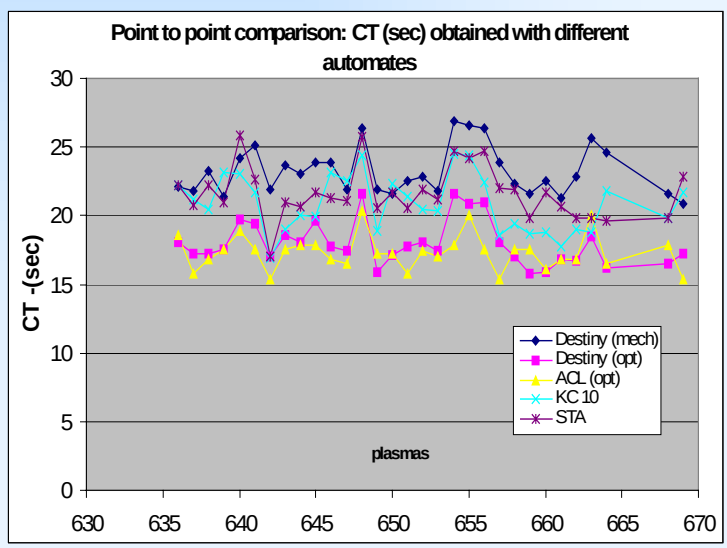
-*According to the detection mode*: clotting times obtained with « optical » methods are shorter than those obtained with « mechanical » methods.

- Obtained clotting times using ACL or Destiny (optical) are similar.

- Obtained clotting times using the STA and KC10 (mechanical methods) are similar.

-Obtained clotting times using the AMAX Destiny are higher than those obtained with other « mechanical detection », but « similar aspect » of the curve.

•Note: each laboratory should confirm the range of clotting times values obtained for normal plasmas, that can vary with the reagent lot and the instrument used. Further studies should be systematically run to investigate the source of unexpected abnormal results.



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Inter–lots performances comparison, and comparison with Diagnostica Stago device (Thrombin (2)), on normal frozen or lyophilised plasmas, and on plasmas of patients under Dicoumarol therapy, with the KC10 instrument.

Goal: Inter-lots comparison of the Hemoclot T.T. device performances (5 lots) , and comparison with a commercial device, for clotting time measurements on plasma samples, using KC10.

Material:

Reagent lots	**060522A**	**060531B**	**060531C**	**060601C**	**060601D**	Stago lot 051442
Expiration dates	2008-11	2008-11	2008-11	2008-12	2008-12	2007-05
Vol. Reconst.	8ml	2ml	8ml	2ml	8ml	2ml

Samples: 49 samples (37 normal frozen plasmas, 4 lyophilised normal plasmas, and 8 plasmas of patients under dicoumarol therapy (AVK)).

Protocol on KC10 instrument: according to the device insert.

Results:

	Clotting times (in seconds) for normal frozen/thawed citrated plasmas					
	Lot 060522A	**Lot 060531B**	**Lot 060531C**	**Lot 060601C**	**Lot 060601D**	**Stago**
N	**37**	**37**	**37**	**37**	**37**	**37**
Mean	**22.3**	**20.0**	**20.8**	**19.8**	**19.1**	**16.0**
Median	**21.8**	**19.9**	**20.8**	**20.5**	**19.0**	**15.1**
SD (sec)	**1.83**	**1.88**	**1.78**	**1.95**	**1.89**	**2.26**
Mean-2SD	18.6	16.3	17.2	15.9	15.3	11.5
Mean+2SD	25.9	23.8	24.4	23.7	22.9	20.6
Min	19.7	17.1	17.1	15.2	15.0	13.1
Max	**27.1**	**25.5**	**24.4**	**24.8**	**24.1**	**20.6**

	Clotting times (in seconds) for normal lyophilised citrated plasmas					
	Lot 060522A	**Lot 060531B**	**Lot 060531C**	**Lot 060601C**	**Lot 060601D**	**Stago**
N	4	4	4	4	4	4
Mean (sec)	21.0	21.7	20.7	20.2	18.8	18.5
SD	0.50	1.42	1.31	0.49	0.75	0.35

	Clotting times (in seconds) for plasmas of patients under dicoumarol therapy					
	Lot 060522A	**Lot 060531B**	**Lot 060531C**	**Lot 060601C**	**Lot 060601D**	**Stago**
N	8	8	8	8	8	8
Mean	20.0	19.0	18.8	17.4	18.4	15.7
SD	1.93	1.81	1.66	1.52	1.34	1.91
Min	16.7	15.9	15.1	15.4	16.6	11.0
Max	23.9	21.3	20.4	20.2	20.8	17.5

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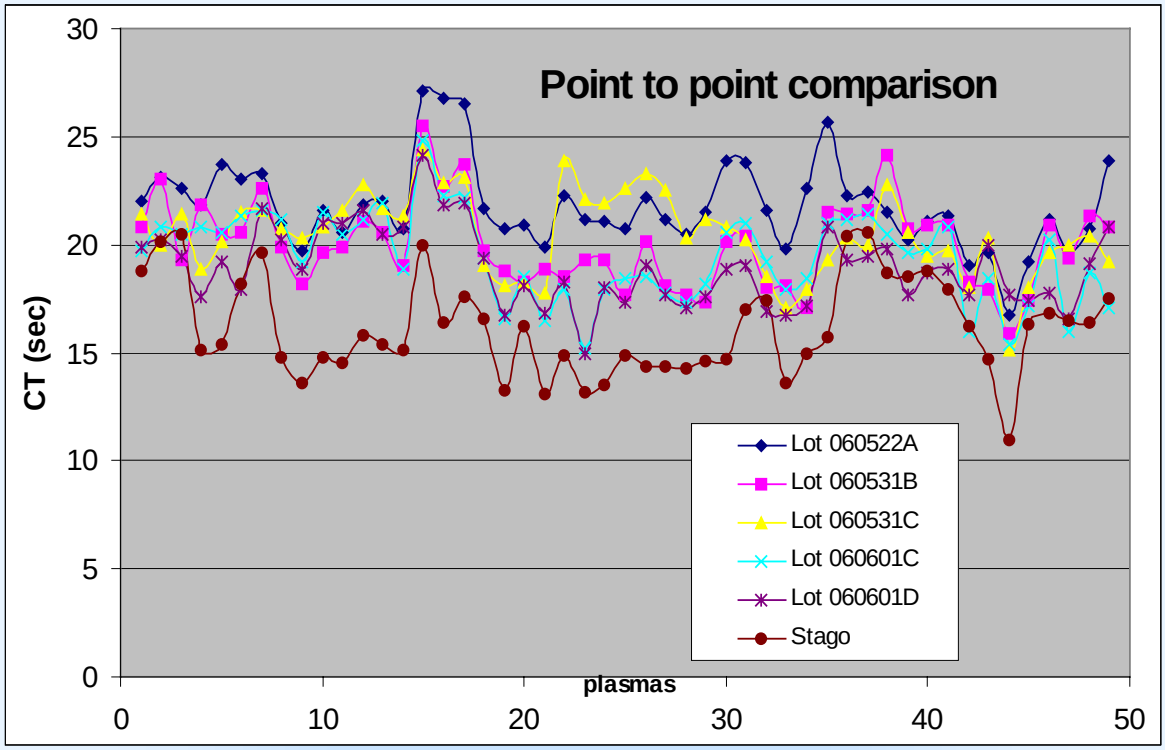

CONCLUSIONS :

Obtained clotting times, for the 5 Hemoclot T.T. lots, using KC10 instrument, on normal citrated frozen and thawed human plasmas, are in the range 15 to 25 seconds (min-max or mean ±2SD for N=37).

Obtained CT are slightly higher with the first produced lot (060522A).

Obtained clotting times with Stago reagent are slightly shorter (at least for the lot used).

The obtained SD is <2seconds for Hemoclot T.T. lots, and slightly higher for Stago reagent.

Clotting times for lyophilised plasmas or plasmas from patients under dicoumarol therapy remain significantly unchanged (CT ≈18-20 sec).

Clinical applications

Determination of the thrombin time on human citrated plasma.

Excellent sensitivity to low concentrations of heparins (from 0.05 to 0.10 IU/ml UFH, and from 0.20 IU/ml LMWH in plasma), and for all direct Thrombin Inhibitors.

References:

Samama MM., Elalamy I., Conard J., Achkar A., Horellou MH., « Hémorragies et thromboses : du diagnostic au traitement », Paris : Masson, 15-16, 60, 2004.

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