



BIOPHEN PROTHROMBIN - « Performances results extract » :

Performance comparison of BIOPHEN Prothrombin (chromogenic assay) with a clotting based assay for prothrombin measurement; normal range, specificity and reproducibility.

Goal: This study aims at comparing BIOPHEN Prothrombin assay performances with those of a “standard” clotting assay for prothrombin measurement on the following samples: .

- Control of the expected normal range by assaying normal plasmas (men and women)
- Quality control: Calibrator, normal and abnormal control plasmas.
- «Pathological samples»: plasmas from patients under AVK therapy from our sample stock, and FII deficient plasma.

The calibration curve is established with the 2nd international standard (SSC/ISTH lot 2) for prothrombin (89% FII)

Material:

Chromogenic assay: Biophen II lot 60401.

Clotting assay : FII deficient plasma lot 040720B (Exp : 2007-01) (Rec with 1ml dist. water)
Stago Neoplastin (rec with 5ml of R2 from Stago)

Samples:

- Plasma NIBSC 2nd : Lot 2 (89% FII)
- Biophen Plasma Calibrator, Normal Control and Abnormal Control.
- FII Deficient plasma: 040720B Exp : 2007-01
- Precision Biologics « normal plasmas » (men and women)
- “AVK treated” plasmas samples

Protocol for the chromogenic assay (Biophen Prothrombin, manual method):

- Calibration curve prepared from SSC/ISTH lot 2 standard (89% FII) diluted 1:25 ; 1:50 ; 1:100 ; 1:200 ; and 0 in prediluted 1:10 R3 buffer.
- Working dilution: each sample is tested at the standard 1:50 dilution in prediluted 1:10 R3 buffer. (note: plasma calibrator, normal an abnormal control are tested at the 1:50 and 1:100 dilution).

Protocol for the prothrombin clotting assay (manual method):

- Calibration curve prepared from SSC/ISTH lot 2 standard (89% FII) diluted 1:10 ; 1:20 ; 1:40 ; 1:80 ; 1:160 in Owren Koller buffer.
- Working dilution: each sample is tested at the 1:20 dilution (note: the standard dilution for the tests is 1:10; plasma calibrator, normal an abnormal control are tested at the 1:10 and 1:20 dilution).

Protocol: 100µl FII deficient plasma
100µl calibration curve or diluted sample
1 min at 37°C
200µl Neoplastin preincubated at 37°C
Measure clotting time (CT, in sec)

Results:

	Clotting assay			Calibration curve (SSC/ISTH standard)	Biophen Prothrombin		
	%FII	DILUTION	CT (sec)		FII %	DILUTION	A405
Calibration curve (SSC/ISTH standard)	89%	1/10	22,6 sec	Calibration curve (SSC/ISTH standard)	178%	1/25	1,805
	45%	1/20	30,5 sec		89%	1/50	0,952
	22%	1/40	43 sec		44,50%	1/100	0,508
	11,13%	1/80	60,7 sec		22,25%	1/200	0,259
	5,56%	1/160	91,9 sec		0%	0	0,012

Normal samples	Clotting assay	BIOPHEN Prothrombin
F3266	102%	107%
F3267	76%	70%
F3268	90%	94%
F3269	88%	90%
F3270	80%	80%
F3271	94%	91%
F3272	88%	91%
F3273	94%	91%
F3274	100%	99%
F3275	95%	100%
F3277	101%	107%
F3278	99%	104%
F3279	95%	98%
F3280	100%	106%
F3281	101%	109%
F3282	92%	96%
F3283	100%	108%
F3284	102%	116%
F3285	96%	113%
F3286	116%	120%
F3287 (fresh)	90%	89%
F3288	103%	111%
F3289	90%	94%
F3287 (freezed-thawed)	92%	85%
M3288	96%	94%
M3289	84%	82%
M3291	88%	82%
M3292	98%	104%
M3293	108%	105%
M3294	84%	83%

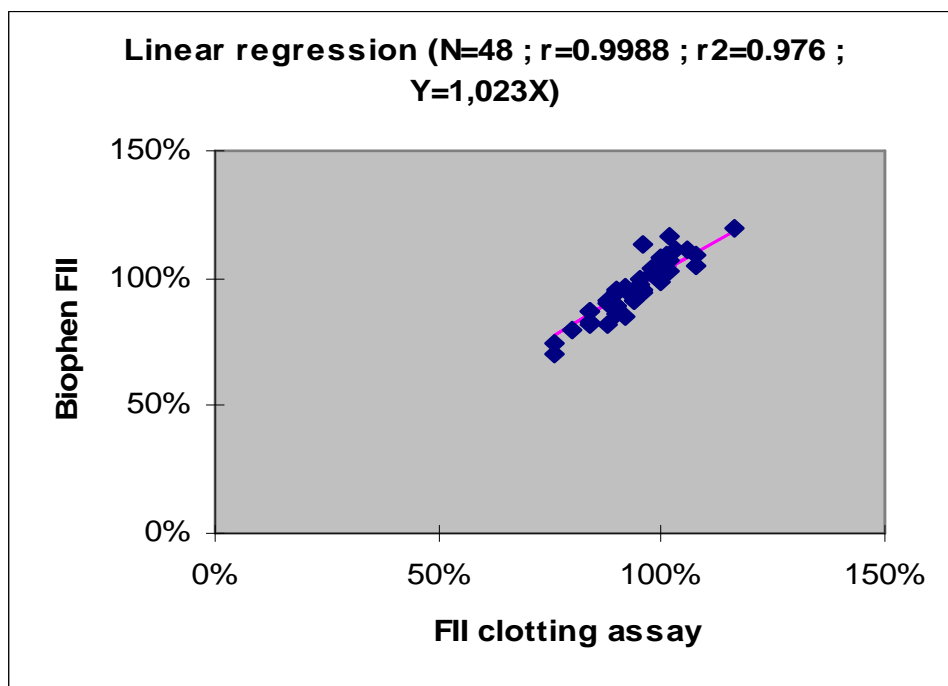
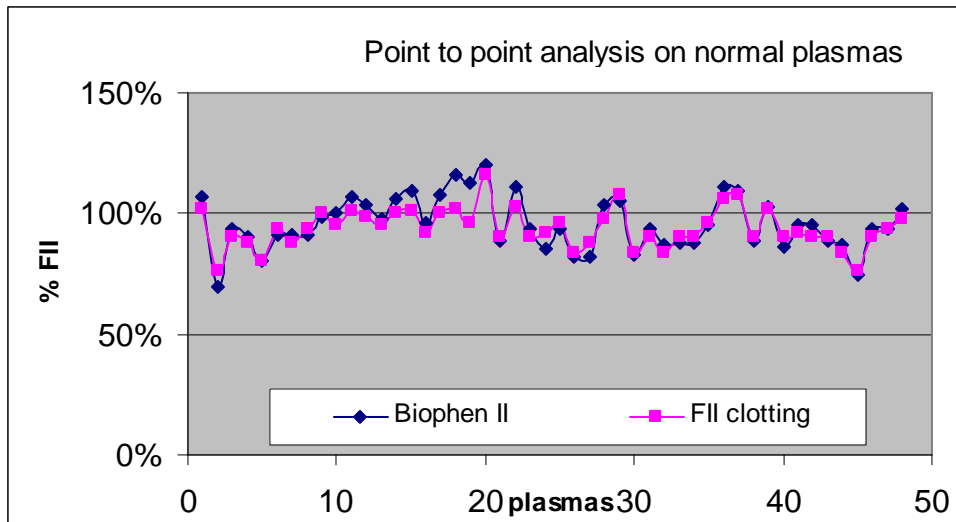
M3295	90%	94%
M3296	84%	87%
M3297	90%	88%
M3298	90%	88%
M3299	96%	95%
M32102	106%	111%
M32103	108%	109%
M32104	90%	89%
M32105	102%	103%
M32106	90%	86%
M32107	92%	95%
M32108	90%	95%
M32109	90%	89%
M32110	84%	87%
M32111	76%	75%
M32112	90%	94%
M32113	94%	94%
M32114	98%	102%

Women (F)	Clotting assay	Biophen Prothrombin
N	24	24
Mean	95%	99%
Median	95%	99%
SD	0,08	0,12
Min	76%	70%
Max	116%	120%

Men (M)	Clotting assay	Biophen Prothrombin
N	24	24
Mean	92%	93%
Median	90%	94%
SD	0,08	0,11
Min	76%	75%
Max	108%	111%

Total « Normal samples »	Clotting assay	Biophen Prothrombin
N	48	48
Mean	94%	96%
Median	93%	94%
SD	0,08	0,11
Min	76%	70%
Max	116%	120%

The expected normal range of about 70-130% is obtained.



Conclusion: there is a good correlation between the 2 devices (on N=48 normal samples).

	Clotting assay	Biophen Prothrombin
Calibrator	89%	92%
	88%	94%
	89%	94%
	92%	98%
	92%	93%
Mean	90%	94%
Indicative Assigned value	92%	
SD	0,02	0,02
CV	2,08	2,43

	Clotting assay	Biophen Prothrombin
Normal control	92%	87%
		88%
		92%
		89%
		94%
		92%
		93%
		96%
Mean	92%	91%
Indicative Assigned value	92%	
SD	/	0,03
CV	/	3,42

	Clotting assay	Biophen Prothrombin
Abnormal control	47%	51%
		51%
		54%
		51%
		54%
		54%
		48%
		52%
Mean	47%	52%
Indicative Assigned value	50%	
SD	/	0,02
CV	/	4,04

Pathological samples	Clotting assay	Biophen Prothrombin
AVK 7068	15%	36%
AVK 77402	22%	48%
AVK 7274	33%	79%
AVK 7360	22%	46%
AVK 6877	14%	39%
FII DEFICIENT PLASMA (040720B)	0%	0%

Conclusion : There is a good correlation between the 2 methods; the expected normal range of about 70-130% is confirmed.

For “AVK treated” patients samples, measured prothrombin values with BIOPHEN Prothrombin are slightly higher than those obtained with the clotting assay.

There is an excellent specificity (FII deficient plasma measured at <0.1% FII).

Reproducibility values obtained for plasma calibrator, normal and abnormal control plasma are satisfying.