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Please note that the uses described in the following page(s) have not been approved or cleared by FDA, with respect to the described assay or test.

In the US, the product is intended **For Research Use Only. Not for Use in Diagnostic Procedures.**

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Manufactured By: HYPHEN BioMed

BIOPHEN Factor X Technical File

Ref. A221705

Chromogenic assay for the quantitative determination of Factor X activity in human citrated plasma.

Assay range: 0 - 200% Factor X

Apr 2006

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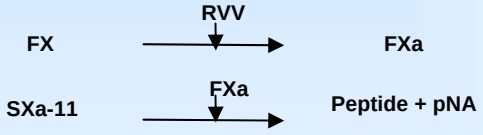
BIOPHEN Factor X technical file (Ref. A221705)

Assay principle

- BIOPHEN Factor X kit is an in vitro assay for the quantitative determination of Factor X in human citrated plasma, with a chromogenic assay, using manual or automated protocols.
- Factor X can be activated by both the intrinsic and extrinsic pathways of the blood coagulation cascade. Prothrombin is then converted to thrombin by the action of factor Xa, complexed with factor V in the presence of phospholipids and calcium.
- Factor X is measured following a specific activation with RVV, an enzyme extracted from snake venom (Russell's Viper Venom). Activated Factor X (FXa) then specifically cleaves the specific substrate SXa-11, releasing para-nitro-aniline (pNA), which color is measured at 405nm. There is a direct relationship between color development and Factor X activity in the tested plasma.



Intended use: For in vitro research use only

Diagnosis of congenital or acquired Factor X deficiency as a risk factor for bleeding disorders.

Measurement of Factor X activity in clinical samples where it can be reduced (dicumarol therapy, hepatic diseases, vit K def., etc…)

R1: Reagent 1: SXa-11 chromogenic substrate, lyophilised (4 vials).

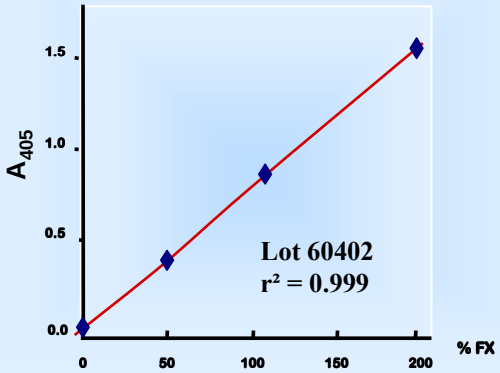
R2: Reagent 2: RVV : Highly purified enzyme, lyophilized and stabilized; RVV can specifically activate FX into FXa (4 vials).

R3: Reagent 3: Tris NaCl buffer "10xconc.": Contains sodium azide. Dilute ten fold with distilled water before use (4 vials).

Procedure

- Specimen: citrated human plasma.
- Plasma Dilution: 1:10.
- Calibration: Factor X calibrator, normal plasma pool or international standard.
- End-point method or kinetics protocols.

Calibration curve (0 to 200%)

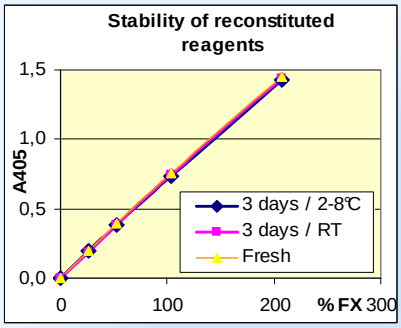


The assay has a dynamic range from 0 to 200% of factor X.

Assay Characteristics

- Total assay time : **10 minutes** or below
- Assay range : **0 to 200 %** of Factor X in plasma (corresponds to about 0 to 1µg/ml of Factor X in the assayed dilution).
- Reproducibility: **≤ 2 %** (N≥10 R1 or R2, tested with manual method on the 100% FX conc.)
- Detection limit (blank+3SD, N≥10): < **0.5 %** (specification ≤5%)
- Specificity: FX deficient plasma **< 0.5 %** (specification ≤5%)
- Can be used with: manual, automated, and microplate methods.

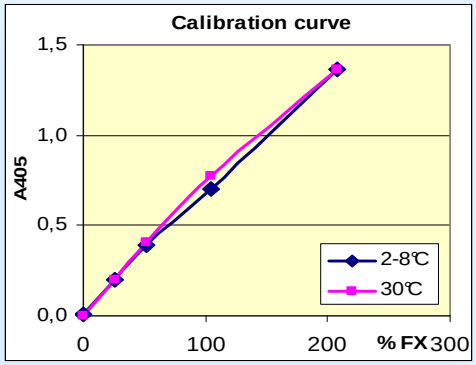
Stability of reconstituted reagents



Excellent preservation of performances of reconstituted Reagents, when stored at 2-8°C or at RT for 3 days, compared with freshly reconstituted vials.

Measured %FX	Fresh	3d/2-8℃	3d/RT
Normal control	109%	110%	108%
Abnormal control	62%	58%	59%

Overheating study



Excellent preservation of reagents 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.

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Recovery and accuracy

Goal: To validate the accurate recovery of FX measurement using the BIOPHEN Factor X assay; recovery is evaluated by spiking various concentrations of a normal plasma pool or of purified human FX into a FX deficient plasma.

Material:
- Biophen Factor X (# 221705): lot 60402 Exp 2008-07
- Human FX: lot 050505B Exp : 2006-11
- FX deficient Plasma: lot 050630B Exp : 2006-12
- Human Plasma Pool (Precision Biologics): Lot A1012 (assigned value: 100% FX), used to establish the calibration curve.

Preparation: preparation of FX deficient plasma, supplemented with variable amounts of FX by addition of various volumes of a normal plasma pool, or of purified human FX (note: the Factor X concentration in a normal plasma pool, titrating by definition 100 %, is of about 10 µg/ml), in order to obtain expected concentrations ranging from 0 to 100% FX. Each point is then diluted 1:10 in buffer (R3) for the assay.

Protocol: according to the device insert (manual method)

Results:

Calibration curve		
% FX	**Dil.**	**A405**
200	1:5	1.50
100	1:10	0.82
50	1:20	0.42
25	1:40	0.23
0	Buffer	0.00

	Expected FX (%)	Measured FX (%)
Addition of purified (h) FX	10µg (100%)	103%
	5µg (50%)	50%
	1µg (10%)	12%
	0.5 µg (5%)	5%
	0.1 µg (1%)	0.05%
Addition of plasma pool	100%	99%
	90%	87%
	70%	66%
	50%	53%
	20%	19%
	10%	8%
	0%	0.1%

Conclusion: Using BIOPHEN Factor X assay, there is an excellent recovery of purified FX or of normal pooled plasma Factor X spiked into FX deficient plasma.

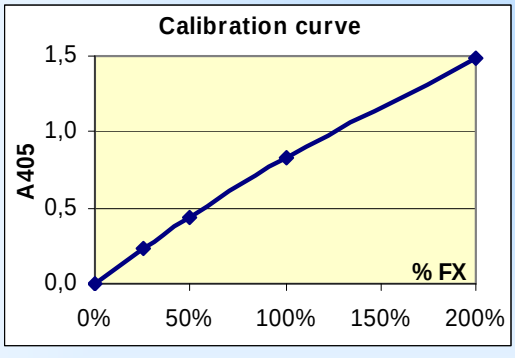
Heparin interference

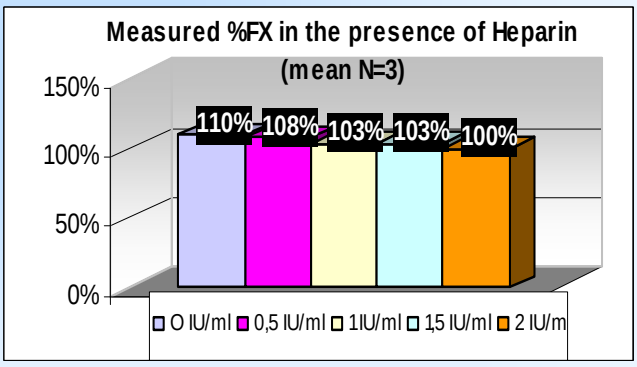
Goal: To check that there is no significant interference of heparin concentrations in plasma, up to 2IU/ml , on the FX measurement using the BIOPHEN Factor X assay.

Material:
- Biophen Factor X: lot 60402
- Human Plasma Pool (Precision Biologics): Lot A1012 (assigned value: 100% FX), used to establish the calibration curve.

Sample Preparation: 3 different normal plasmas supplemented with 0 to 2 UI/ml Heparin (LMWH, Lovenox).

Results:





Conclusion: No significant interference of heparin up to 1 IU/ml in plasma.

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Comparison of the BIOPHEN Factor X assay (chromogenic assay) with a clotting based assay for the FX measurement; (normal range, specificity and reproducibility).

Goal: Comparison between the measurement of Factor X using the BIOPHEN FX assay comparatively to a conventional clotting Factor X assay (using a Factor X deficient plasma and calcium thromboplastin reagent).

Material:

- Chromogenic assay: Biophen Factor X, lot 60402.
- Clotting assay : FX deficient plasma (Hyphen BioMed) lot 050630B; Stago Neoplastin (calcium thromboplastin).
-Calibration curve: established with the Secondary NIBSC Coagulation Standard (SSC/ISTH lot 2) (assigned to 94% FX).

Samples:

- Quality controls: Biophen Plasma Calibrator, Biophen Normal Control and Biophen Abnormal Control.
- Normal plasmas: Precision Biologics (men and women).
- Pathological samples: plasmas from patients with dicumarol therapy; FX Deficient plasma (HBM lot 050630B).

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

- Calibration curve: SSC/ISTH lot 2 standard (94% FX) diluted 1:5 (188%); 1:10 (94%) ; 1:20 (47%); 1:40 (23.5%) ; and 0 (0%) in the ten fold diluted R3 buffer.
-Working dilution: each sample is tested at the standard 1:10 dilution in the ten fold diluted R3 buffer.

Protocol for the FX clotting assay (KC10):

- Calibration curve: SSC/ISTH lot 2 standard (94% FX) diluted 1:10 (94%); 1:20 (47%); 1:40 (23.5%); 1:80 (11.75%) in Owren Koller buffer.
- Working dilution: each sample is tested at the 1:20 dilution, and the measured FX concentration is multiplied by 2.
- Protocol: 50µl FX deficient plasma
50µl standard or diluted sample
1 min at 37°C
100µl Neoplastin preincubated at 37°C
Measure clotting time (CT, in sec)

Results : * Calibration curves:

Clotting assay (mean of 4 series)		
% FX	Dil.	CT (sec)
94	1:10	25.4
47	1:20	31.1
23.5	1:40	39.1
11.75	1:80	51.9

Biophen FX (mean of 4 series)		
% FX	Dil.	A405
188	1:5	1.50
94	1:10	0.83
47	1:20	0.42
23.5	1:40	0.22
0	0	0

* FX measurement in normal samples: the expected normal range of 60-130% is confirmed :

«Normal samples»	Clotting assay	Biophen FX
N	45	45
Mean	118.5 %	104.3 %
Median	118 %	109 %
SD	21.5	19.3
Min	66 %	64 %
Max	154 %	136 %

Notes : some values are discrepant and could be related to the freezing-thawing of some plasma samples; repetitive freeze thawing can activate FVII, which can result in a shortened clotting time and an overestimation of clotting FX; Factor X concentrations above 100 % are measured with better accuracy with the chromogenic assay, Biophen FX.

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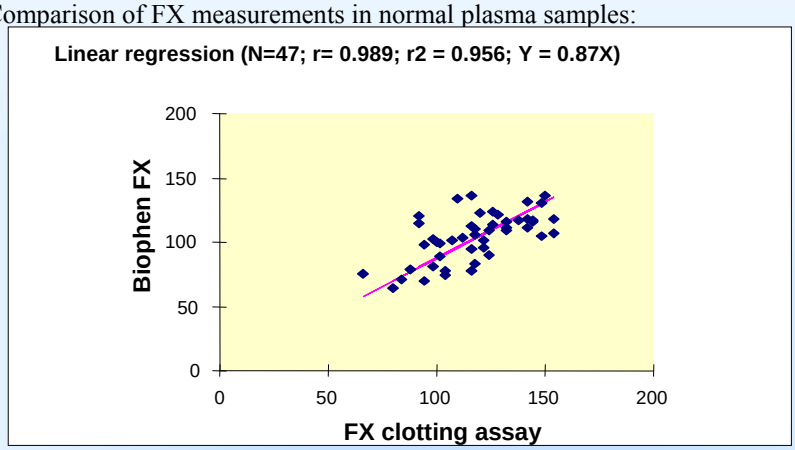
* Comparison of FX measurements in normal plasma samples:



* FX measurement on plasmas from dicumarol treated patients, and FX deficient plasma:

Pathological samples	Clotting assay	Biophen FX
Dicu. 7156	6%	28%
Dicu. 7399	34%	68%
Dicu. 6991	15%	34%
Dicu. 7014	12%	44%
Dicu. 7382	5%	47%
FX DeficientPlasma	0.5%	<0.1%

* FX measurement in normal and abnormal controls, and calibrator: reproducibility data:

% FX	Clotting assay	Biophen FX	
		Mean	CV (%)
Sample 1 (Cal)	107%	101%	5.4 (N=8)
Sample 2 (NC)	100%	100%	7.4 (N=8)
Sample 3 (AC)	53%	58%	5.4 (N=8)

Conclusions : **There is a good correlation between the 2 methods; the expected normal range for FX, of about 60-130%, is confirmed. For "dicumarol treated" patient samples, measured FX values with the BIOPHEN Factor X assay are slightly higher than those obtained with the clotting assay. The Biophen Factor X assay measures partly PIVKA Factor X. There is an excellent specificity (FX deficient plasma is measured at a concentration <0.1% FX). In the recommendeded conditions, the assay is specific for Factor X measurement (use of Russell's Viper Venom with a specific action on factor X activation, absence of phospholipids in the test, presence of specific inhibitors for thrombin (hirudin) and heparin (polybren)). Reproducibilities obtained for plasma calibrator and control plasmas are excellent and in compliance with the assay applications.**

Clinical applications

Various Factor X variants have been identified (1,2,4). Congenital or acquired Factor X severe deficiency is characterized by bleeding manifestations and an hemorrhagic state, similar to haemophilia. Pregnancy in women with congenital factor X deficiencies is often associated with adverse fetal outcomes (3). Factor X deficiency or decrease can result from vitamin K deficiency, severe liver disease, and therapeutic use of anticoagulant drugs (dicumarol or warfarin). Factor X chromogenic assay has been reported being useful to monitor patients under oral anticoagulant therapy with Anti Vitamin K drugs (5), inducing a decrease in Factor X concentration (15 to 80%) and a prolonged clotting time. However Factor X concentrations measured in patients treated with Vitamin K Antagonists are slightly higher than those obtained with a clotting based assay.

References:

1) Molecular analysis of the genotype-phenotype relationship in factor X deficiency", Millar DS, Elliston L, Deex P, Krawczak M, Wacey AI, et Al., Hum Genet, 106:249-257, 2000.
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3)"Congenital coagulopathies and pregnancy: report of four pregnancies in a factor X-deficient woman", Kumar M, Mehta P, Am J Hemat, 46:241-244, 1994.
4)"A family with heterozygous factor X Friuli defect outside Friuli", Girolami A., Lazzarin M, Procidano M, Luzzatto G, Blut, 46: 149-154, 1983.
5)"Dosage du facteur Stuart à l'aide d'un substrat synthétique au cours des traitements anticoagulants oraux. Résultats préliminaires", Conard J, Gradelet A, Samama M, Ann. Biol. Clin., 39:81-83, 1981.
6)"Determination of vitamin K sensitive coagulation factors in plasma. Studies on three methods using synthetic chromogenic substrates", Bergström K and Egberg N, Thromb. Res., 12:531-547, 1978.
7)"Activation of decarboxy factor X by a protein from Russell's viper venom. Purification and partial characterization of activated decarboxy factor X", Lindhout MJ, Kop-Klaassen BHM, Hemker MC, Biochim. Biophys. Acta, 533:327-341, 1978.
8)http://www.ncbi.nlm.nih.gov ; OMIM; "Coagulation factor X" (+227600).

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