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Manufactured By: HYPHEN BioMed

NEW STANDARDIZED CHROMOGENIC ASSAYS FOR AUTOMATED MEASUREMENTS OF FIX OR FIXa IN PLASMA AND THERAPEUTIC CONCENTRATES

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INTRODUCTION

- Automated measurements of Factor IX (FIX) and activated Factor IX (FIXa) are required for testing Prothrombin Complex Concentrates (PCC), or therapeutic recombinant Factor IX (eg. BeneFIX®), but also for quantitating plasma Factor IX (Haemophilia B), or for performing recovery studies in treated patients.
- Two fully homogeneous chromogenic assays, highly sensitive, and offering an extended working range, were developed for these applications.

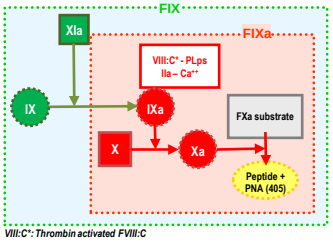
TESTED SAMPLES

- Normal citrated plasma (NI), and plasma from dicoumarol treated patients (VKA), and Haemophilia B patients (Haem. B).
- FIX deficient plasma (FIX DP), immunodepleted (FIX <0.1%).
- FIX pharmaceutical concentrates (BeneFIX®; recombinant FIX), are pre-diluted in FIX DP or in the assay diluent (this predilution is made at 1 IU/ml for the high range, or 0.2 IU/ml for the low range), then assayed at the standard assay dilution for the high (1:80 and further) or low (1:15) ranges , in the assay diluent and tested with the automated instrument STA-R (Diagnostica Stago).

MATERIAL AND METHODS

- FIX and FIXa (Act) chromogenic assays (based on FXa generation), designed with the use of highly purified human proteins, and well characterized synthetic phospholipids, and including an optimized diluent for full expression of FIX or FIXa activity :
  - Tested FIX is incubated with FXIa, FX, FVIII:C, FIIa, phospholipids and calcium.
  - FIX is then activated to FIXa, which activates FX to FXa in a dose-dependent manner.
  - FXa activity is measured with a chromogenic substrate (405 nm).
- FIXa (Activated FIX) is measured using the same principle, but without the FIX activation step by FXIa.
- Conventional aPTT based FIX clotting assay (FIX DP and Cephen aPTT reagent).
- Assay calibrations are standardized with the NIBSC standards for plasma FIX (SSC/ISTH secondary plasma standard lot 3) or activated FIX (97/562).

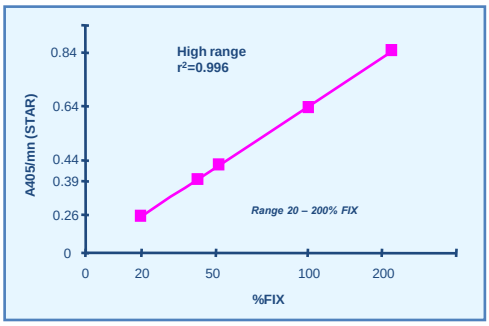
Assay principles

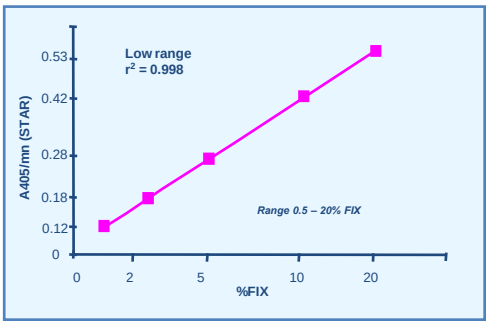


VIII:C*: Thrombin activated FVIII:C

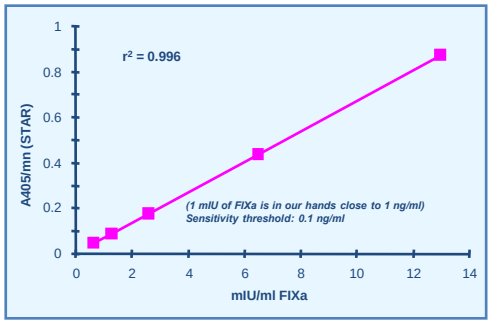
RESULTS

FIX chromogenic assay dose response curves for the High and the low range (STA-R)

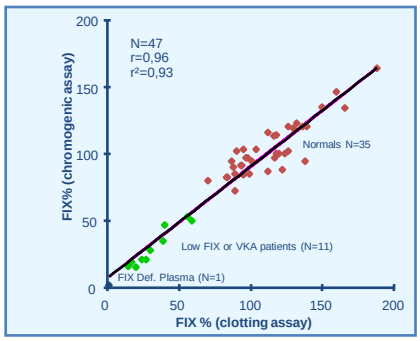




FIXa chromogenic assay dose response curve (STA-R)



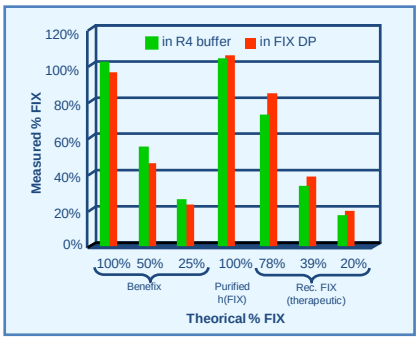
Correlation study for FIX measurement , using the FIX chromogenic assay or a conventional clotting assay, APTT based.



Results obtained for each group of patients with both FIX methods

Patients		% FIX (chromogenic, high range)	%FIX (clotting)
NI (N=30)	Mean (Min-Max)	108% [83-157)	103% [83-139]
VKA (N=10)	Mean (Min-Max)	34 % [16-74]	34% [15-70]
Haem. B (39%)		33%	38%
Haem. B (0.5%) (low range)		0.5%	0.5%

Compared reactivity and recovery of purified (h)FIX or two therapeutic preparations



Diluted into FIX deficient plasma or optimized assay diluent

DISCUSSIONS

FIX chromogenic assay (STA-R):

- **High range**: 0.025 IU/ml (125 ng/ml) to 0.0006 IU/ml (3 ng/ml) in diluted sample (1:80).
- **Low range**: detection threshold of 0.005 IU/ml in plasma.
- Excellent correlation with conventional FIX clotting method (r^2=0.93).
- No interference of other factors or heparin.
- Using the specific assay diluent, purified human FIX or two FIX pharmaceutical concentrates yield a similar reactivity than when diluted into FIX DP (80-105% recovery).

Factor IXa chromogenic assay:

- Dynamic range: 0.025 to 0.0005 IU/ml FIXa (0.001 IU~1 ng) (tested at 1:2 dilution).
- Sensitive and efficient for quantitating trace amounts of FIXa in FIX concentrates preparations (data not shown).

Further studies are in progress on Haemophilia B patients and various pharmaceutical preparations.

CONCLUSIONS

- Standardized assays (NIBSC), designed with optimized and secured raw materials, highly stable (72h at 2-8°C, or frozen) and fully automatable.
- These two chromogenic assays are easily performed on automated coagulation instruments and allow measuring FIX or FIXa activity with high sensitivity.
- No FIX deficient plasma is required for testing FIX or FIXa, and concentrates can be assayed directly diluted in the assay diluent.
- These assays represent a very helpful alternative to clotting methods for testing FIX in plasma, or the activation grade of PCC and other FIX therapeutic concentrates. These methods improve the laboratory practice for monitoring FIX preparations, but also for testing FIX in haemophilias or monitoring recovery of therapeutic concentrates in patients receiving substitutive therapy.

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