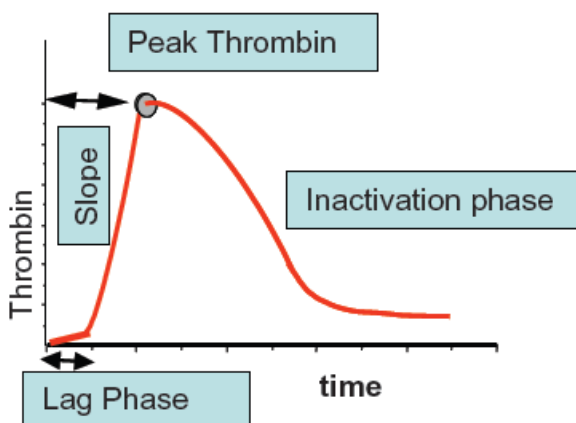
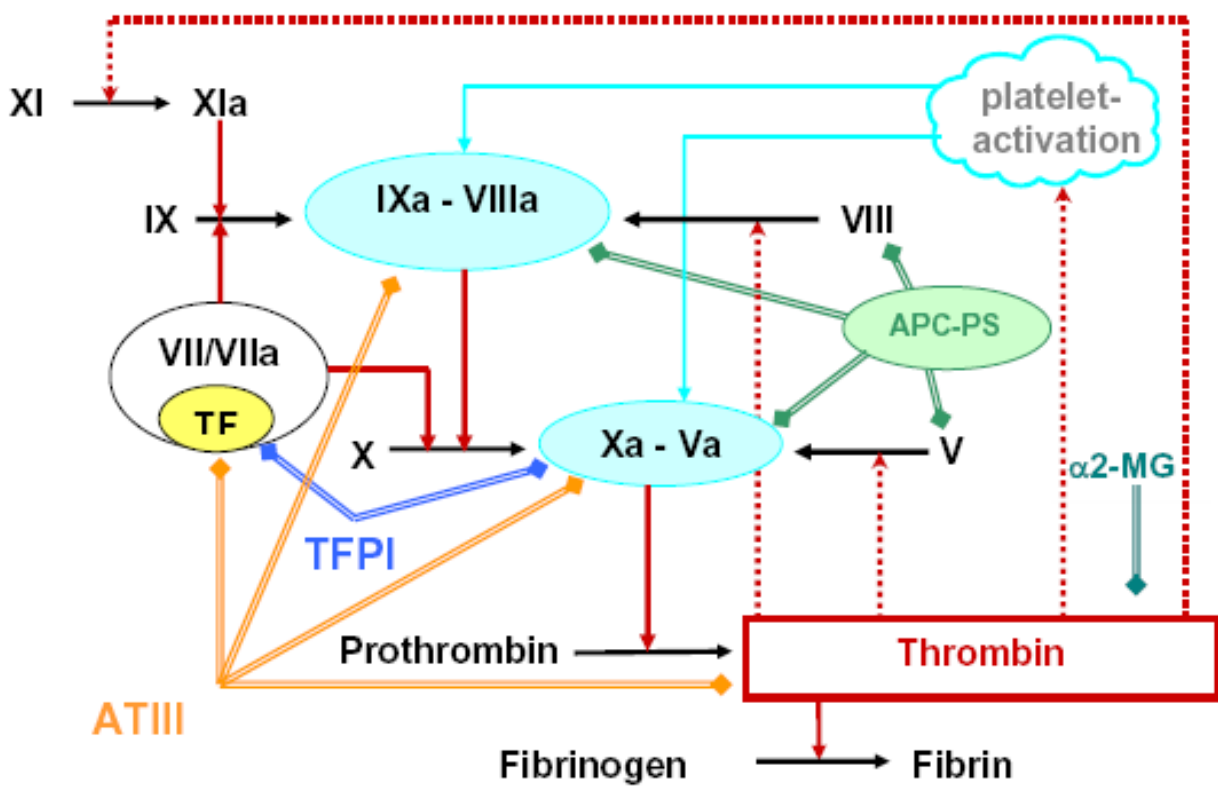


# TECHNOTHROMBIN<sup>®</sup> TGA

TECHNOTHROMBIN<sup>®</sup> TGA is a **thrombin generation assay (TGA)** based on monitoring the formation of thrombin by means of a fluorogenic substrate upon activation of the coagulation cascade by tissue factor. This assay can be used to monitor hemophiliacs during inhibitor bypassing therapy, to monitor anticoagulation therapy and to determine states of thrombophilia.



The use of TECHNOTHROMBIN<sup>®</sup> TGA for such diverse applications is made possible because TECHNOTHROMBIN<sup>®</sup> TGA allows to measure the whole kinetics of thrombin generation not only during the initiation phase of thrombin formation with the end point fibrin formation, but also during the phase of down regulation of thrombin formation and inactivation of the formed thrombin. TECHNOTHROMBIN<sup>®</sup> TGA is therefore a universal tool for analysis and monitoring of the haemostatic system on an individual basis.

## TEST PRINCIPLE

TECHNOTHROMBIN<sup>®</sup> TGA is based on monitoring the fluorescence generated by thrombin cleavage of a fluorogenic substrate over time upon activation of the coagulation cascade by different concentrations of tissue factor (RA, RB, RC Low, RC High and RD\*) and negatively charged phospholipids in plasma. From the changes in fluorescence over time, the concentration of thrombin (nM) in the sample can be calculated using the respective thrombin calibration curve (only one calibration has to be done for each lot). The rate of increase in thrombin concentration over time then allows to calculate generation of thrombin in the sample per minute and to plot this value over time for the whole coagulation process. This then results in the visualization of the different phases of clot formation.

The TECHNOTHROMBIN<sup>®</sup> TGA Kit 3x16 T. REF 5006010 contains :

mL	reagent	description
3 x 1.5	TGA substrate	Fluorogenic substrate 1 mM Z-G-G-R-AMC, 15 mM CaCl <sub>2</sub>
1 x 3	TGA buffer	Hepes-NaCl-buffer containing 1% bovine serum albumin
1 x 0.5	TGA thrombin calibrator	~1.000 nM thrombin in buffer with BSA
1 x 0.5	TGA reagent C Low (RC Low)	RC Low conc. of phospholipid micelles and pM rhTF
1 x 0.5	TGA reagent C High (RC High)	RC High conc. phospholipid micelles and pM rhTF
1 x 0.5	TGA reagent D (RD)	RD conc. of phospholipid micelles and pM rhTF
1 x 1	TGA control High (CH)	Human plasma with increased thrombin generation, Iyo.
1 x 1	TGA control Low (CL)	Human plasma with decreased thrombin generation, Iyo.

\* TGA Reagents RA, RB, RC Low, RC High and RD are particularly suitable for:

TGA RA = measuring circulating micro particles  
TGA RB, RC Low = measurement of thrombophilia and monitoring of F VIII inhibitor therapy (FEIBA or rFVIIa)

TGA RC High = monitoring anti-coagulant therapy  
TGA RD = monitoring heparin, direct thrombin and Xa inhibitor therapy

## QUANTITATIVE MEASUREMENT OF THROMBIN GENERATION

### Fluorescence Reader (manual method)

For assaying thrombin generation using TECHNOTHROMBIN<sup>®</sup> TGA you need a fluorescence reader equipped with filters ~360 nm and ~460 nm (excitation/emission). A free excel software for the evaluation of TECHNOTHROMBIN<sup>®</sup> TGA results is available for each reader ([www.technoclone.com](http://www.technoclone.com)).

Applications are available for:

BioTek<sup>®</sup> FLx 800<sup>™</sup> TC  
BMG Labtech FLUOstar OPTIMA  
Molecular Devices Gemini / SpectraMax<sup>®</sup>  
Perkin Elmer<sup>®</sup> Victor Wallac  
TECAN Genios<sup>™</sup>  
Thermo Fluoroskan<sup>®</sup>

### CEVERON<sup>®</sup> alpha w. TGA (fully automated method)

The new automated analyser CEVERON<sup>®</sup> alpha w. TGA (REF 9820010) is equipped with a fluorescence measurement module and thus suitable for assays such as TECHNOTHROMBIN<sup>®</sup> TGA. With CEVERON<sup>®</sup> alpha, standard clotting tests and thrombin generation assays can be performed.



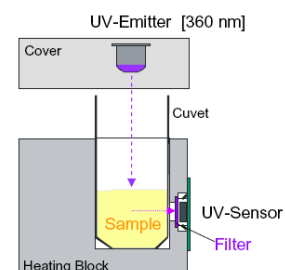
ceveron<sup>®</sup>  
alpha

Thrombin Generation is measured with a special adapted TGA fluorimetric module which is placed over the cuvette rotor (Fig.1). With an UV emitter (360 nm) placed in the module, thrombin generation can then be measured (Fig. 2).

**For more details on Thrombin Generation f. Ceveron<sup>®</sup> see separate folder.**



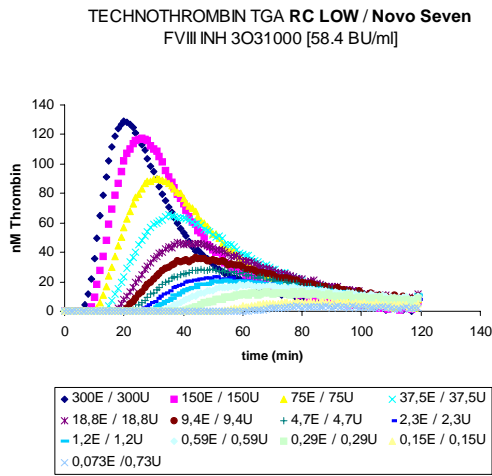
1. Fluorimetric module



2. Measuring principal of the TGA module

## FACTOR VIII INHIBITOR THERAPY

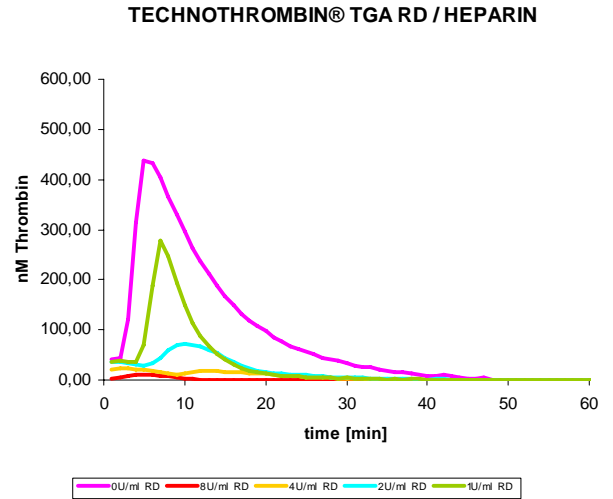
Dose dependent reconstitution of thrombin generation potential in Factor VIII inhibitor plasma after addition of FEIBA (Factor Eight Inhibitor Bypassing Activity) or rFVIIa (NovoSeven®).



## MONITORING OF HEPARIN OR DIRECT XA INHIBITORS

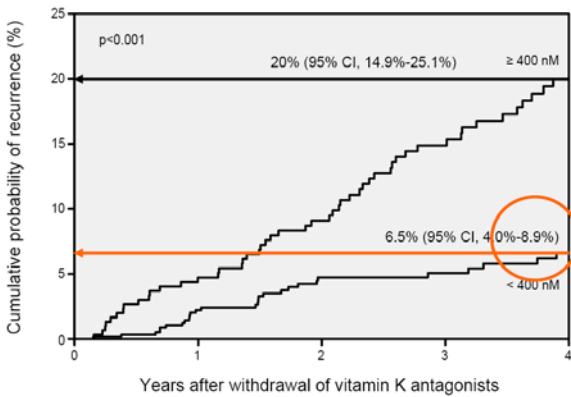
Dose dependent decrease of thrombin generation potential in plasma after addition of different heparin or direct Xa inhibitor concentrations.

(Example of addition of unfractionated heparin below)

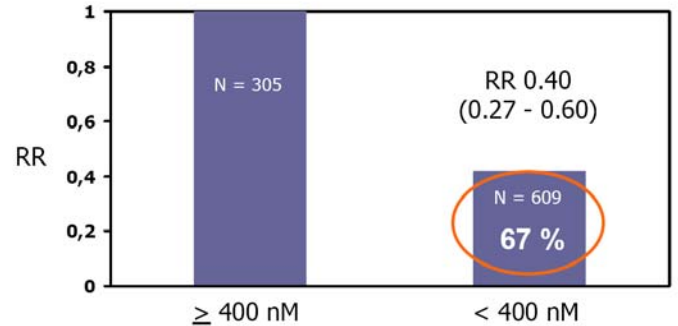


## ASSAYING THROMBOPHILIC TENDENCY

### Peak thrombin - Probability of Recurrent VTE



### Peak thrombin - Relative Risk of Recurrence



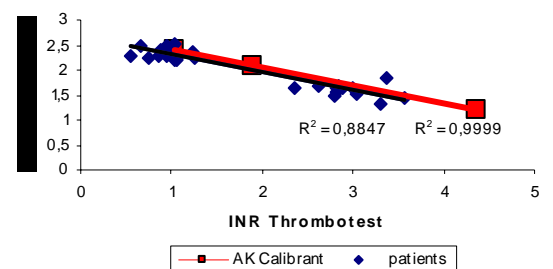
after adjustment for age, sex, BMI, location of first thrombosis, duration of oral anticoagulation, F V Leiden, and F II G20210A

Hron G. et al., JAMA, July 26, 2006 – Vol 296, No. 4, 397-402

## MONITORING OF ANTI-COAGULATION THERAPY

All forms of anti-coagulant therapies such as heparin therapy, direct thrombin inhibitors or oral anti-coagulants can be monitored using TECHNOTHROMBIN® TGA.

### Peak TECHNOTHROMBIN TGA (RC) / INR Thrombotest

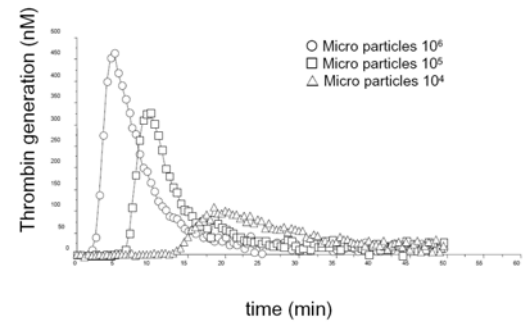


## THROMBIN GENERATION BY MICRO PARTICLES

Thrombin generation induced by micro particles can be measured by the TECHNOTHROMBIN® TGA assay.

The amount of thrombin generated is dependent on the number of micro particles present in the sample.

Micro particle induced thrombin generation / TGA RA



## SUMMARY

Reagent	Purpose
TGA RA	- to <b>monitor the activity of circulating micro particles</b> contained in platelet poor plasma
TGA RB, RC Low	- measurement of <b>thrombophilia tendency</b> (preferentially in standard PPP plasma) - to monitor <b>inhibitor bypass therapy with FEIBA or rFVIIa</b> in hemophiliacs with Factor VIII inhibitors
TGA RC High	- monitoring <b>anticoagulation therapy</b>
TGA RD	- monitoring <b>heparin, direct thrombin and Xa inhibitor therapy</b>

## ORDERING INFORMATION

Complete reagent kit:

REF 5006010 TECHNOTHROMBIN® TGA Kit



Modular reagents:

REF 5006345	TECHNOTHROMBIN® TGA CAL Set	1 Set
REF 5006205	TECHNOTHROMBIN® TGA RA	5 x 0.5 mL
REF 5006206	TECHNOTHROMBIN® TGA RA	50 x 0.5 mL
REF 5006209	TECHNOTHROMBIN® TGA RB	5 x 0.5 mL
REF 5006210	TECHNOTHROMBIN® TGA RB	50 x 0.5 mL
REF 5006212	TECHNOTHROMBIN® TGA RC Low	5 x 0.5 mL
REF 5006213	TECHNOTHROMBIN® TGA RC Low	50 x 0.5 mL
REF 5006214	TECHNOTHROMBIN® TGA RC High	5 x 0.5 mL
REF 5006216	TECHNOTHROMBIN® TGA RC High	50 x 0.5 mL
REF 5006220	TECHNOTHROMBIN® TGA RD	5 x 1.5 mL
REF 5006222	TECHNOTHROMBIN® TGA RD	50 x 1.5 mL
REF 5006235	TECHNOTHROMBIN® TGA SUB	5 x 1.5 mL
REF 5006230	TECHNOTHROMBIN® TGA SUB	50 x 1.5 mL

Additional Control:

REF 5006320	TECHNOTHROMBIN® TGA Control High	5 x 1 mL
REF 5006330	TECHNOTHROMBIN® TGA Control Low	5 x 1 mL
REF 5021100	TECHNOFROZEN Control N	50 x 1 mL

For TGA reagents for Ceveron® alpha see separate folder i

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