

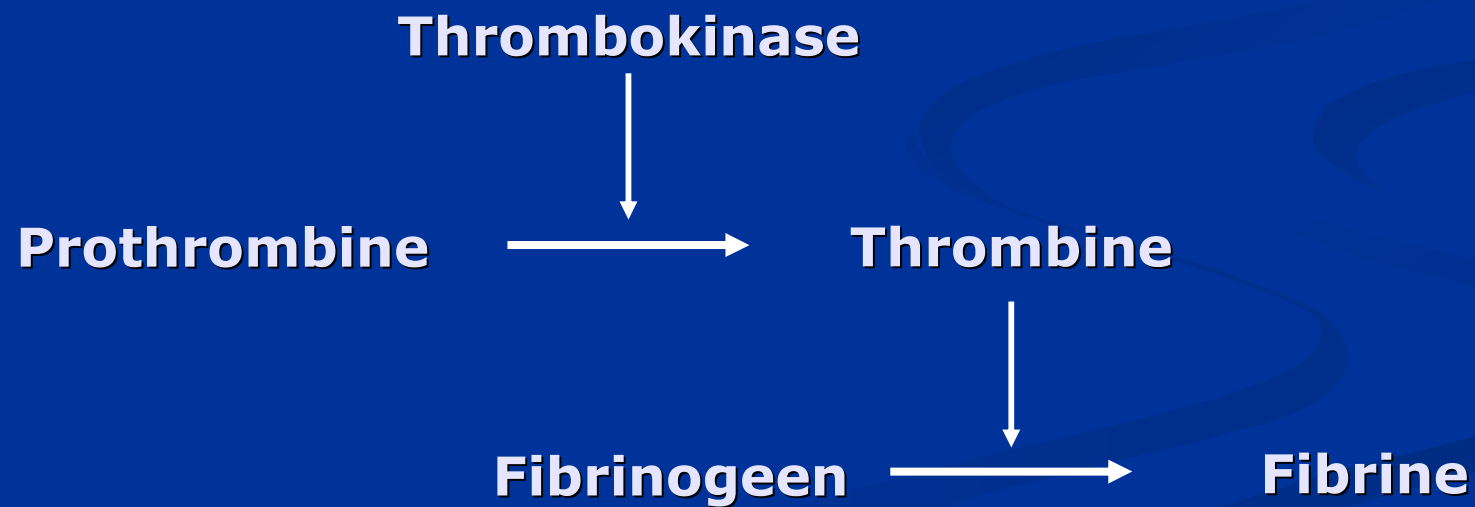
De Thrombine Generatie Test: Theorie en Praktijk

J. Rosing

**Cardiovascular Research Institute Maastricht
The Netherlands**

Vroeger was de stolling erg eenvoudig

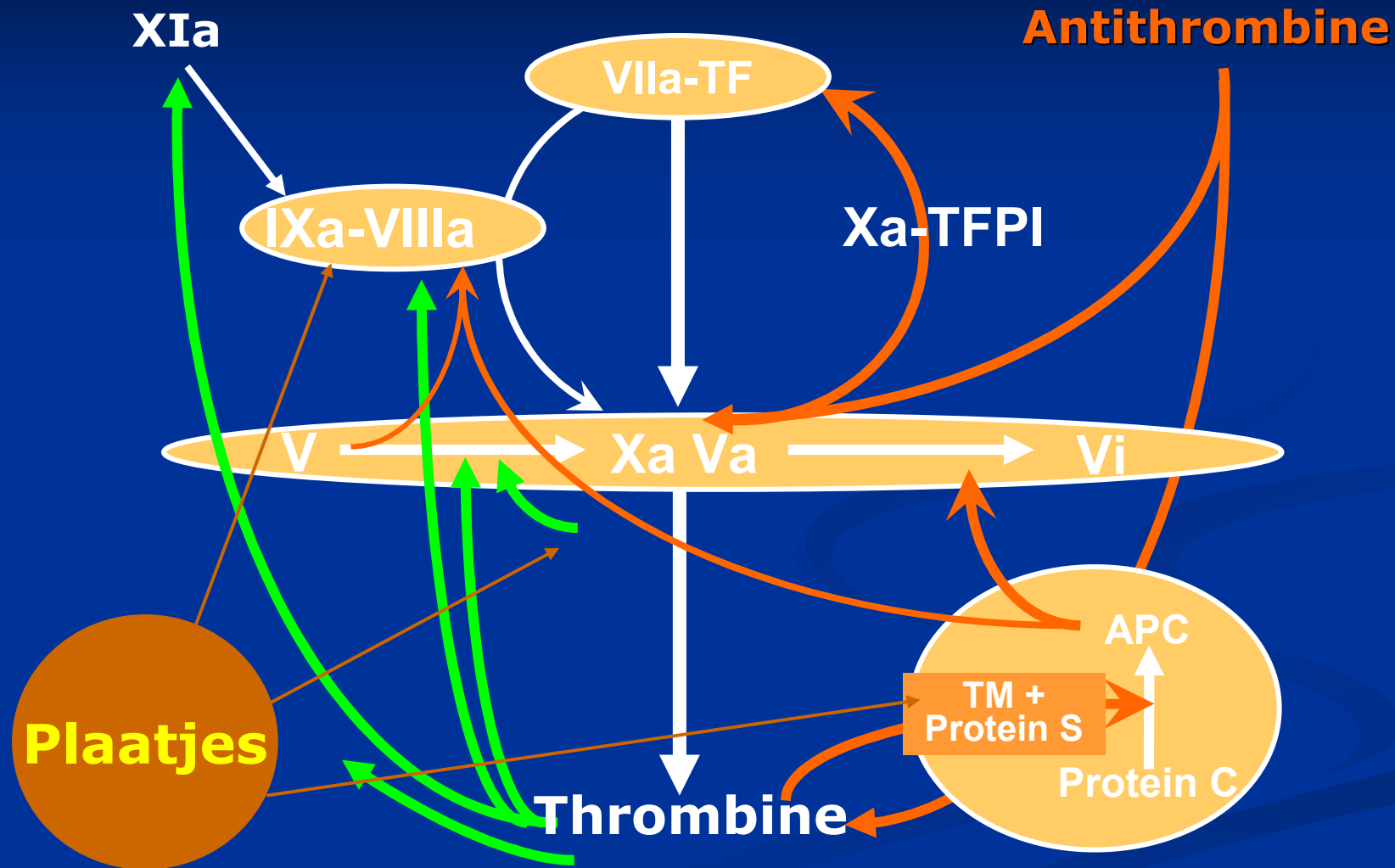
Morawitz 1905:



Maar toen kwam de biochemie

Positieve Feedback

Negatieve Feedback



en talloze ingewikkelde stollingstesten

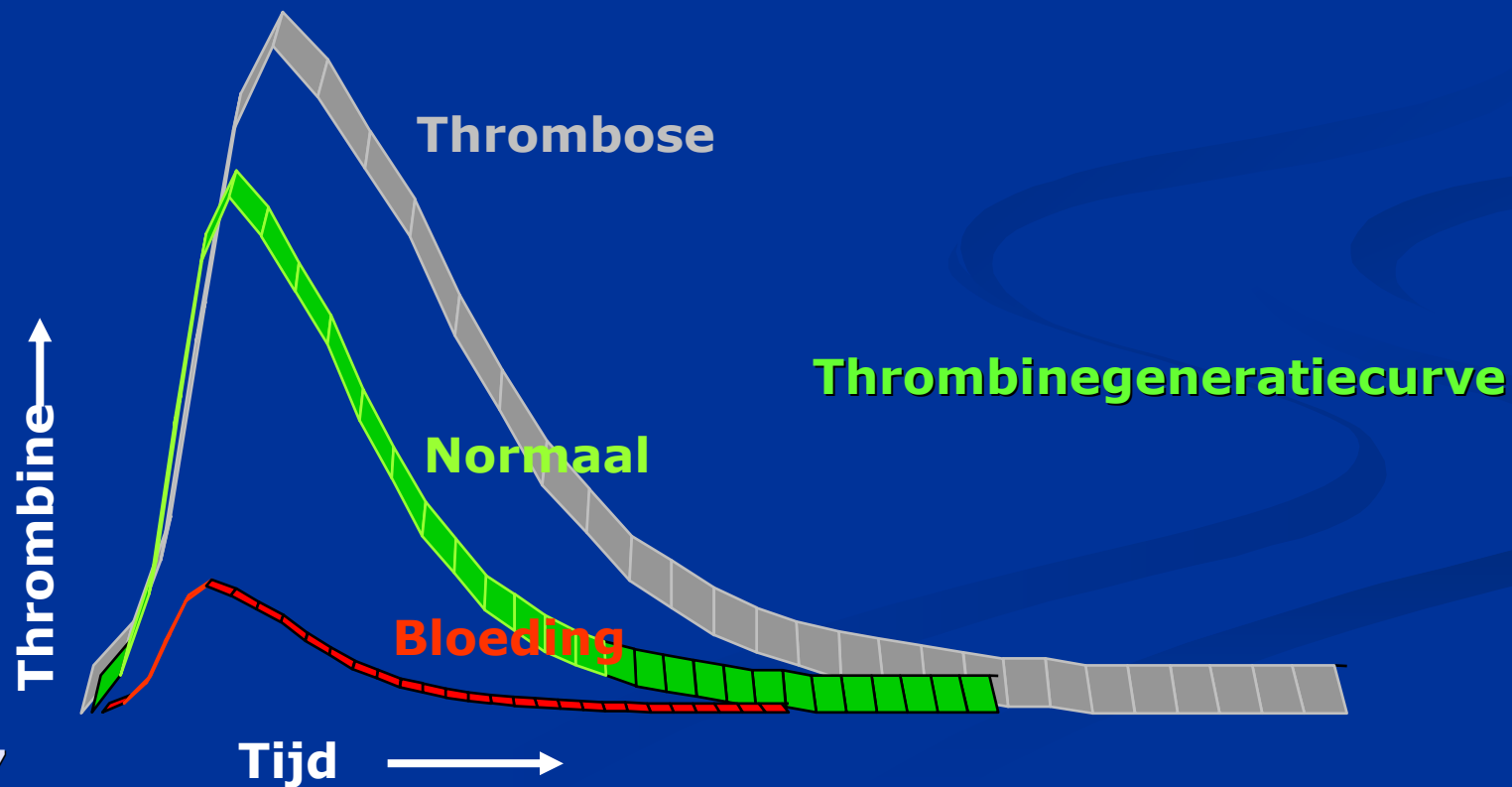
- **Stollingstesten (aPTT, PT, TT, etc.)**
- **Stollingsfactoren (activiteit/antigeen)**
- **Anticoagulante eiwitten**
- **Activatie markers (TAT, F1.2, D-dimer)**
- **Stolsel retractie**
- **Plaatjes aggregatie testen**
- **VWF testen**
- **enz.**
- **enz.**
- **enz.**

Waar worden al deze testen nu eigenlijk voor gebruikt?

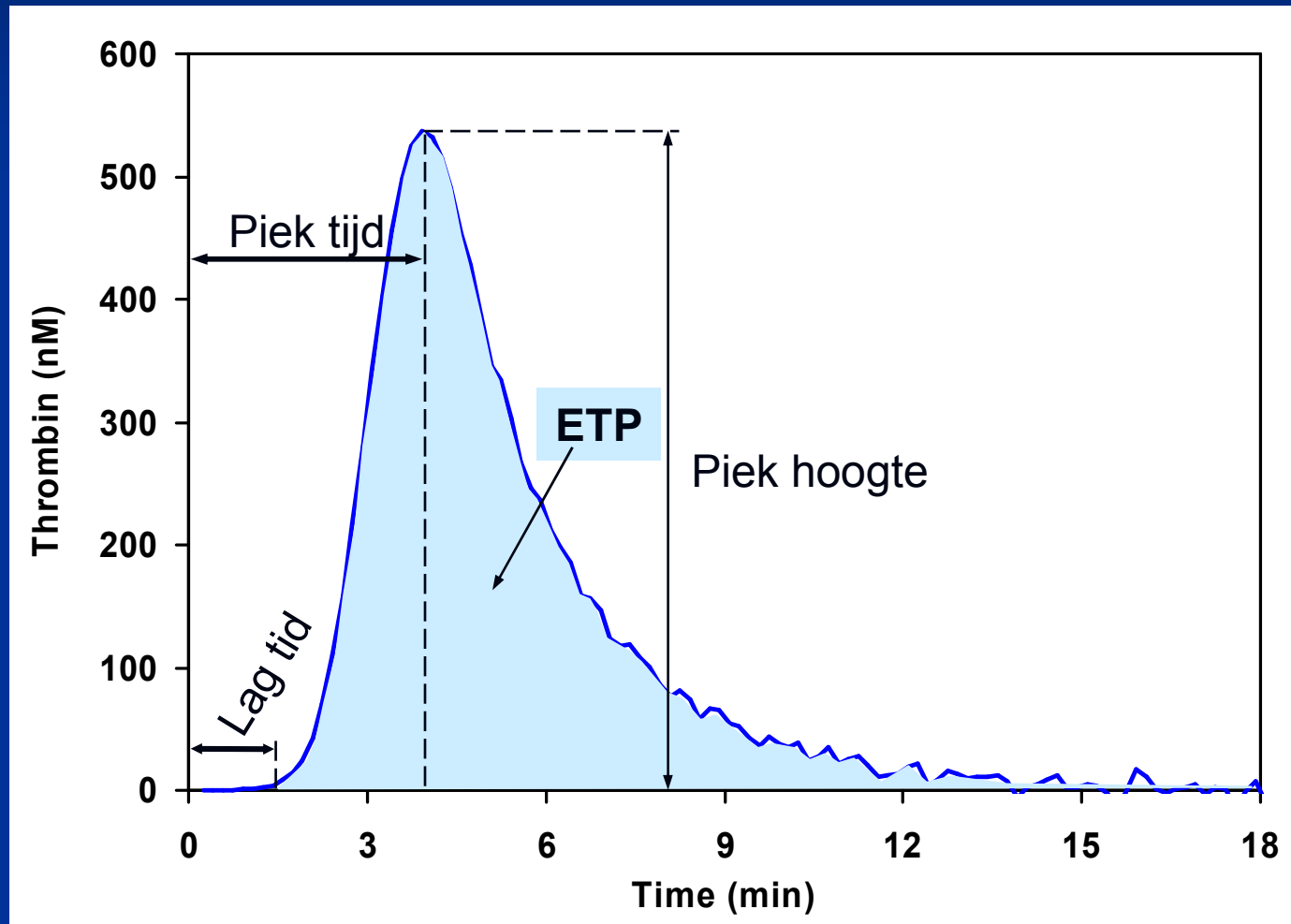
- **Diagnose van thrombose en bloedingsziekten**
- **Bepalen van (individueel) risico op thrombose of bloedingen**
- **Het bepalen van de effectiviteit van antistollings-therapie en van behandeling van hemofilie**
- **Ontwikkeling van nieuwe anticoagulantia en behandelmethoden van hemofilie**

Een functionele test gebaseerd op de eerste wet van de hemostase en thrombose

Veel thrombine minder bloeding maar meer thrombose
Weinig thrombine meer bloeding maar minder thrombose



Thrombine generatie parameters



1986: Kwantitering van thrombine met een chromogeen substraat in ~ 30 monsters die in de tijd uit plasma genomen werden



Hoe kan je thrombine generatie in de praktijk meten?

1986: Subsamenen op een chromogeen thrombine substraat

1993: Continu met een laag affiniteit chromogeen substraat voor thrombine in gedefibrineerd plasma

2000: Continu met een laag affiniteit fluorogeen substraat voor thrombine in vol plasma of bloed

Principe van de techniek

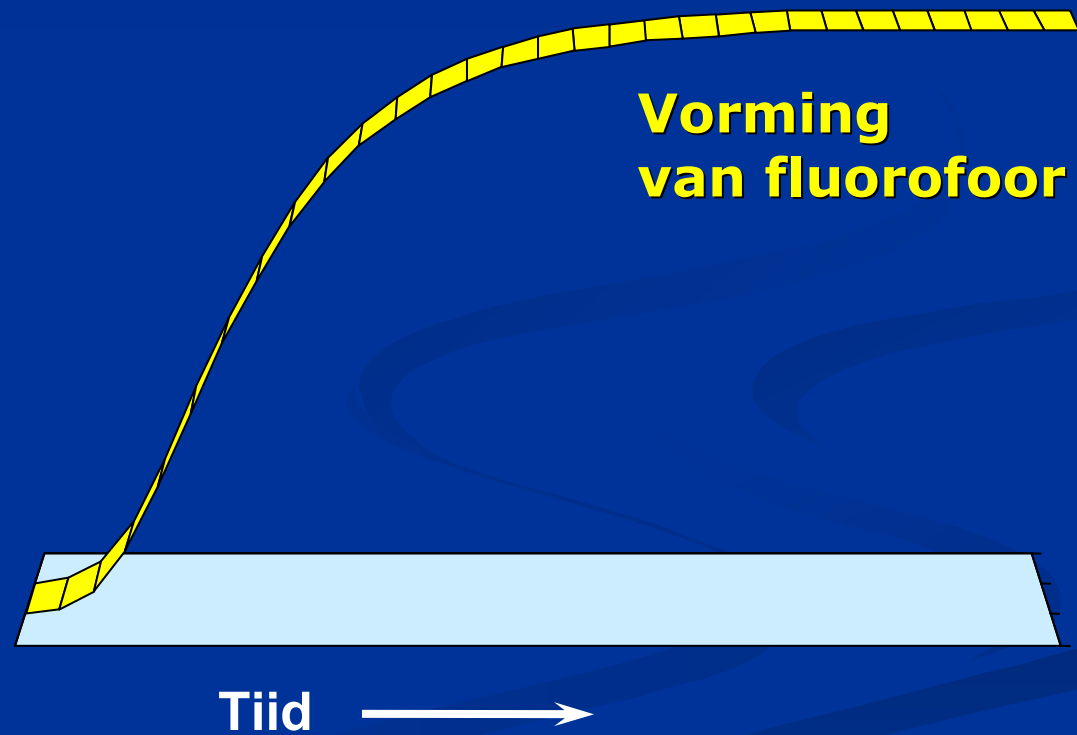
Prothrombine

ZGGR-AMC
plasma

Thrombine

AMC

Geremd Thrombine



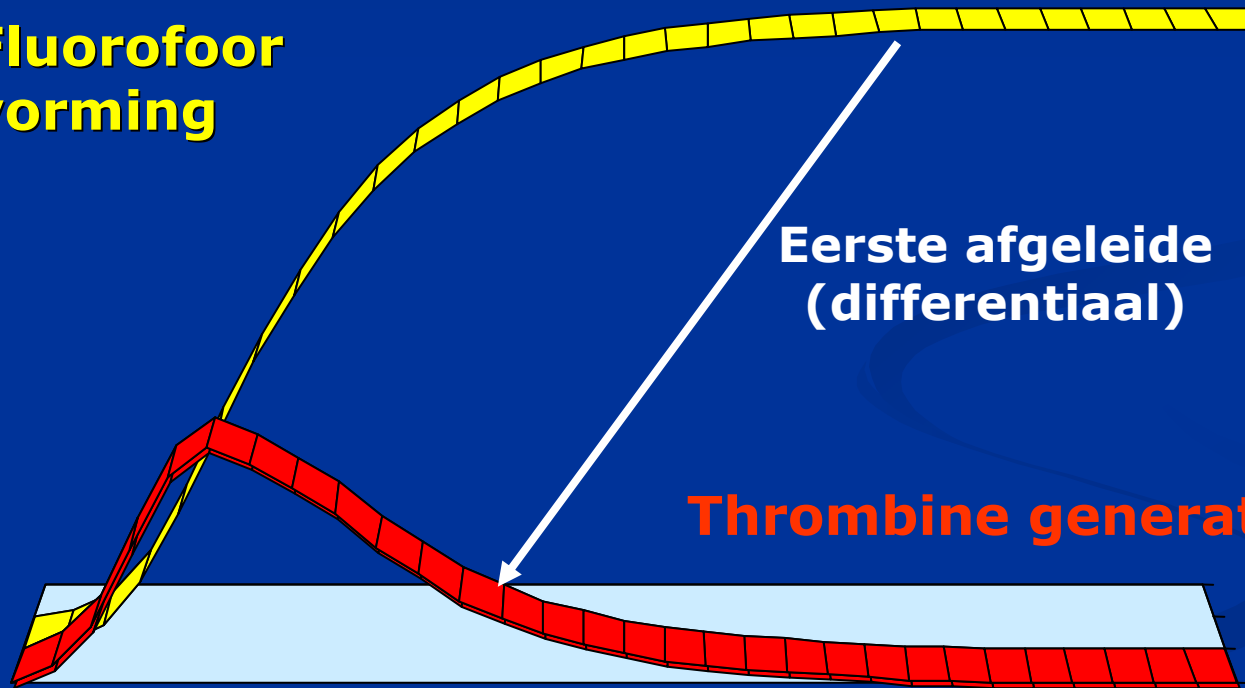
Berekening van thrombine generatie uit fluorescentie verandering

Fluorofoor
vorming

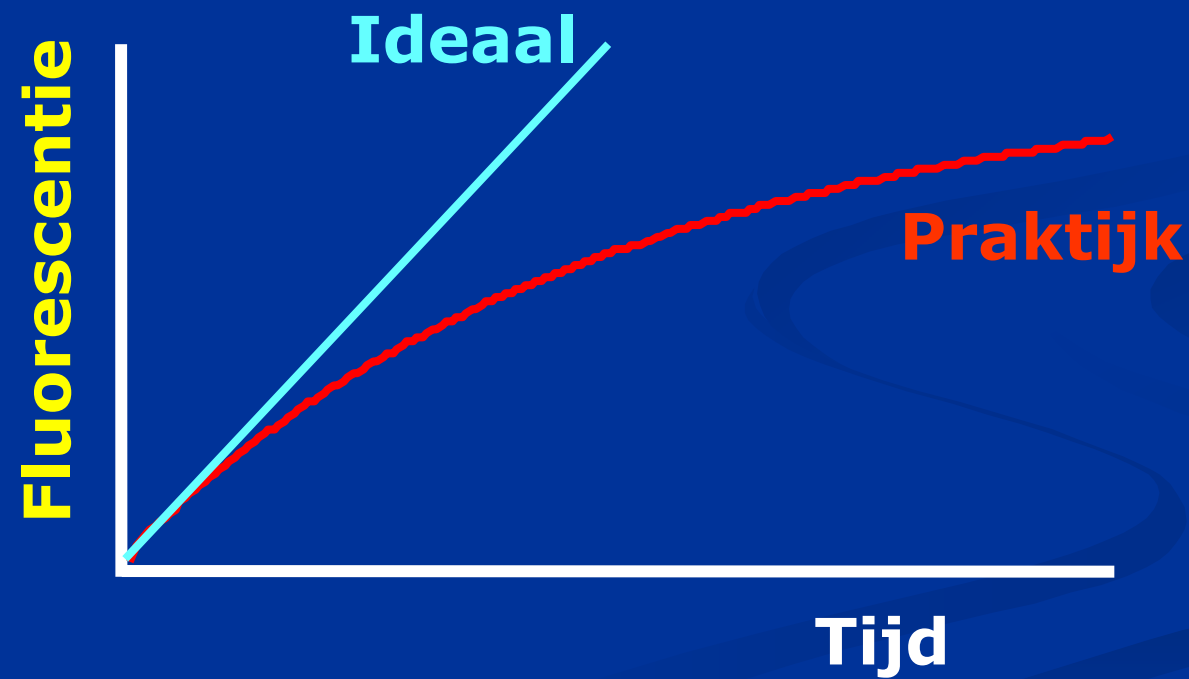
Eerste afgeleide
(differentiaal)

Thrombine generatie curve

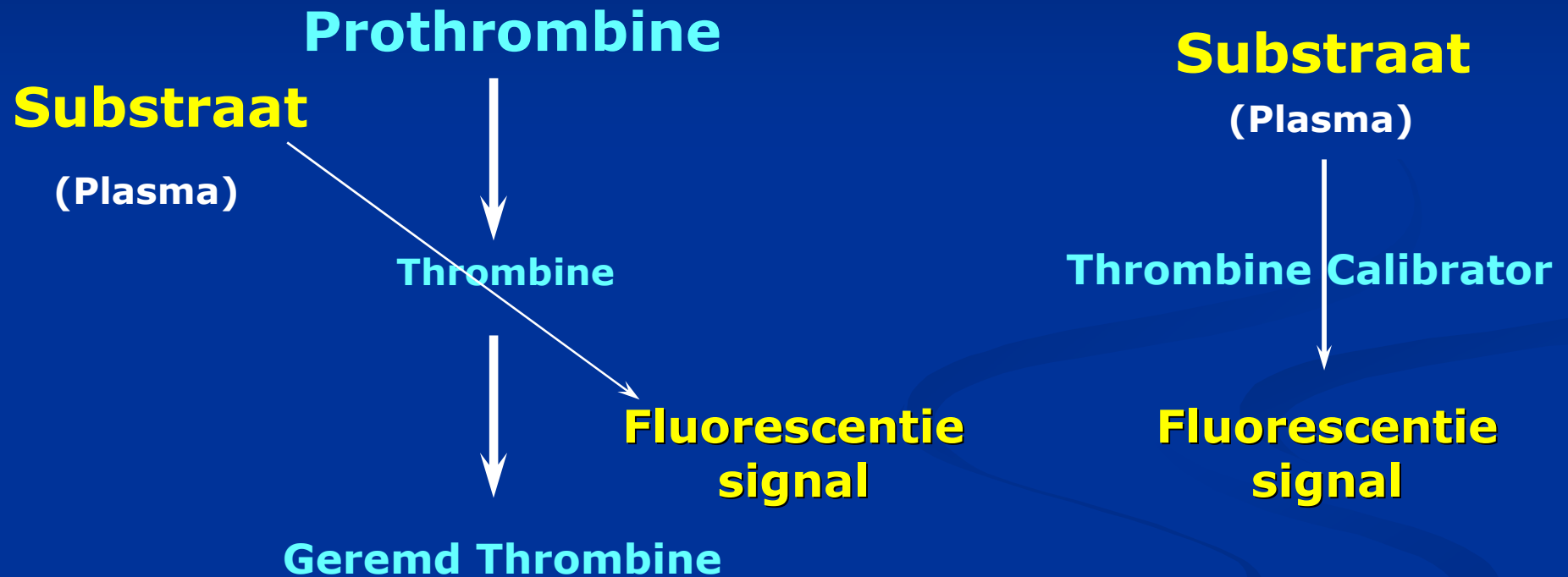
Tijd →



Een vaste hoeveelheid thrombine geeft geen lineaire fluorescentie verandering



Automatische Methode



**Vergelijking van de twee signalen →
de thrombine concentratie in plasma**

Continue real time meting van de thrombine vorming in een fluorimeter

Plate reader (fluorescentie), Substraat, Calibrator en Software

Well 1:

80 μ l Plasma

20 μ l Tissue factor

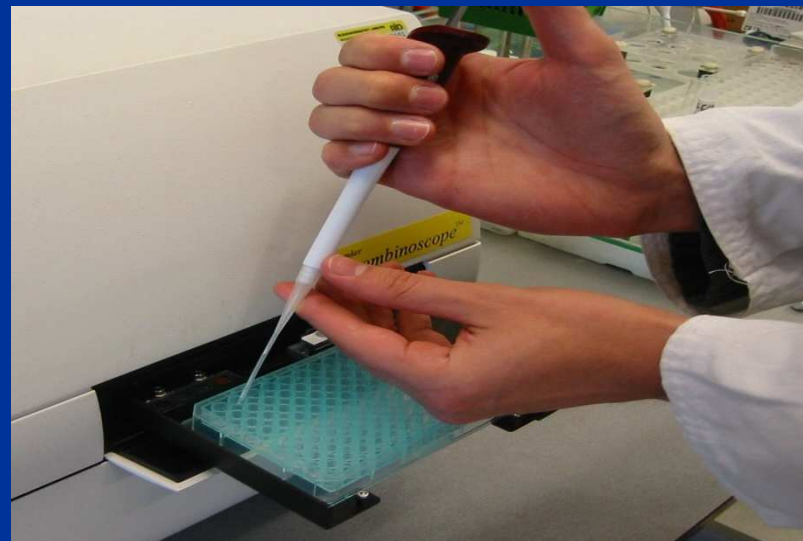
Well 2: Calibratie

80 μ l Plasma

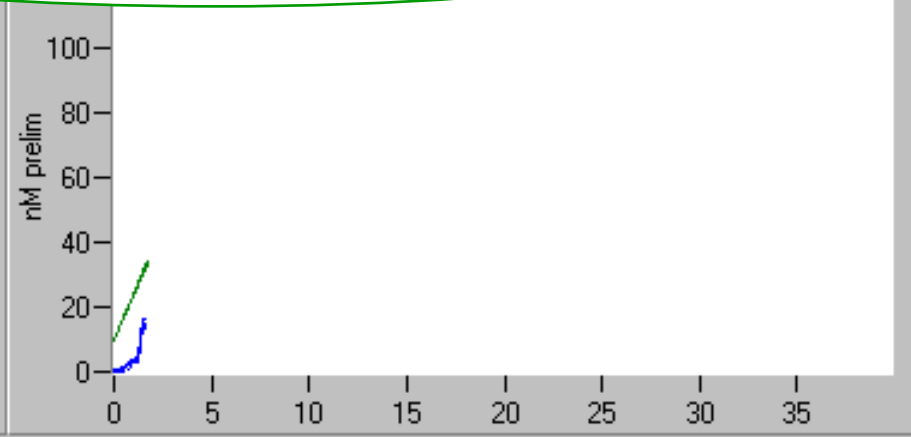
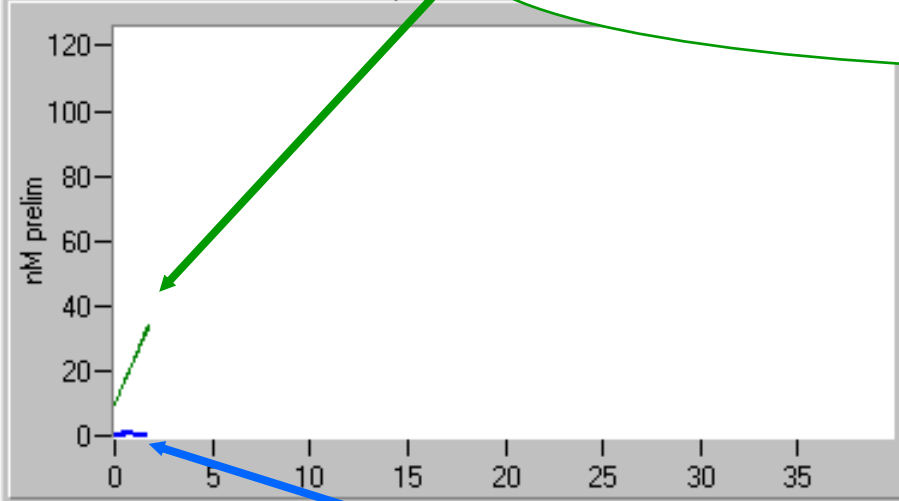
20 μ l Thrombine

Start met een dispenser:

20 μ l fluorogeen thrombine substraat + CaCl_2



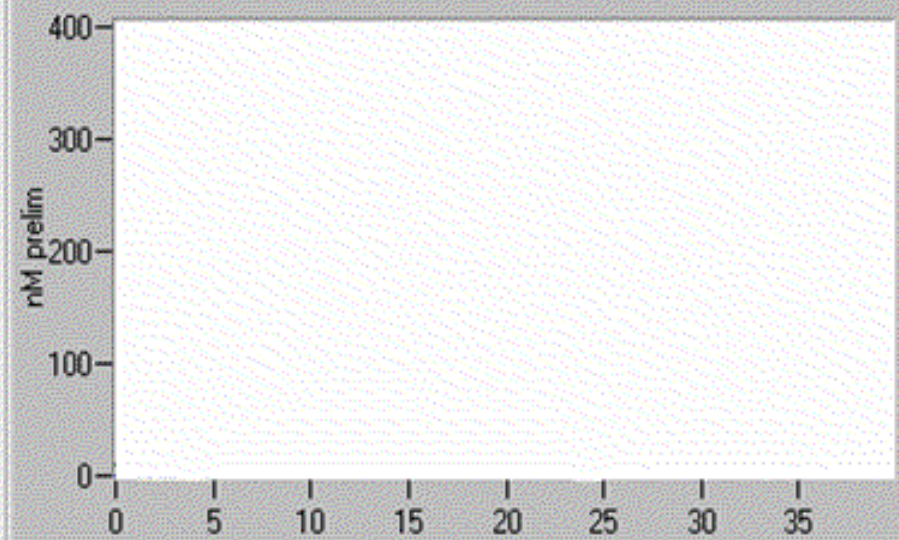
0.5 pM TF C



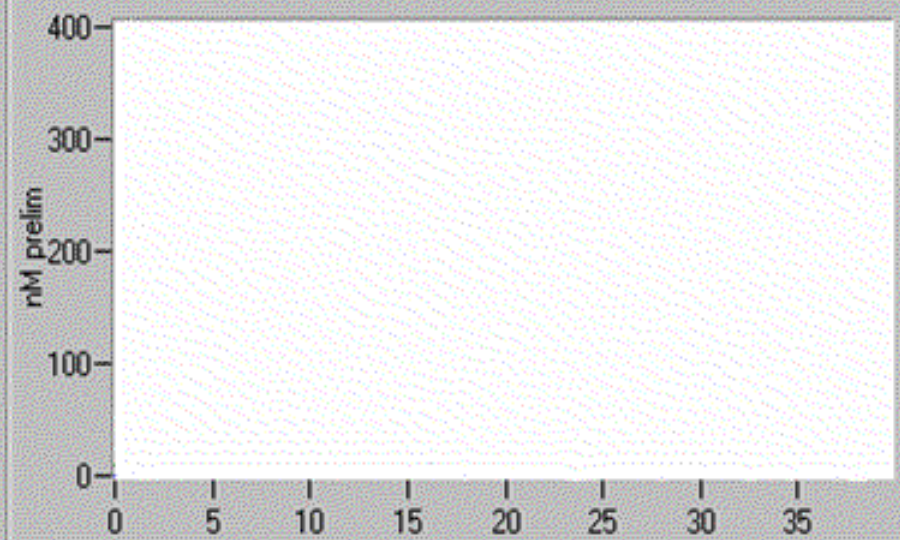
Green Signal = Calibrator Wells

Blue Signal = Plasma

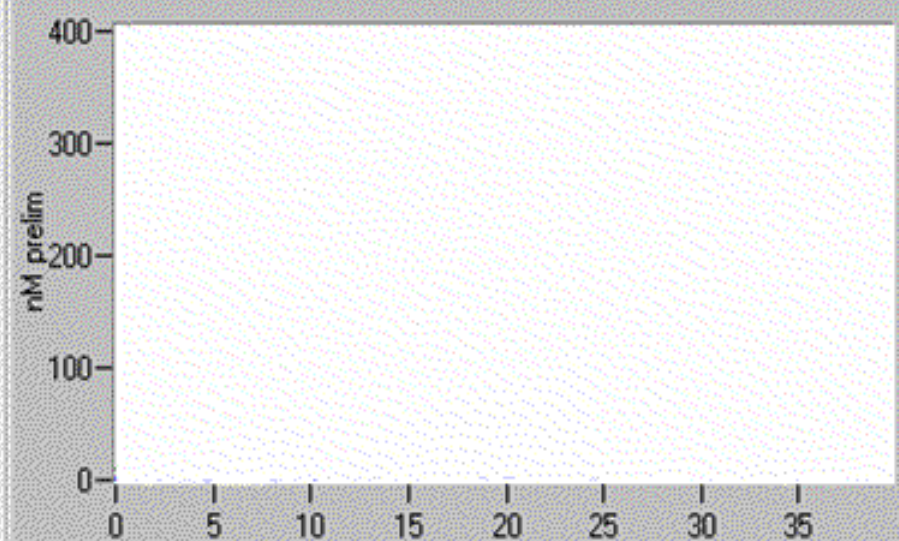
0.5 pM TF Control 1



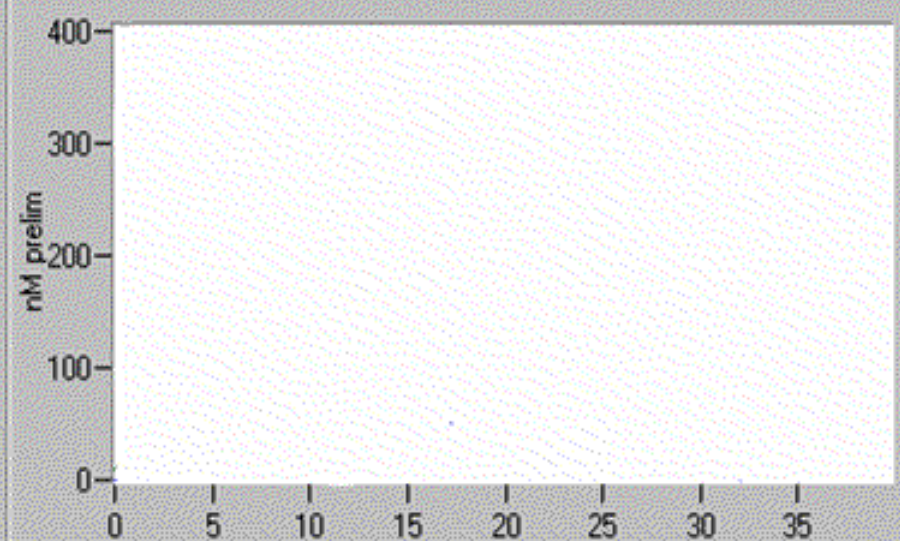
5 pM TF Control 2



0.5 pM TF Hep 3



5 pM TF Hep 4

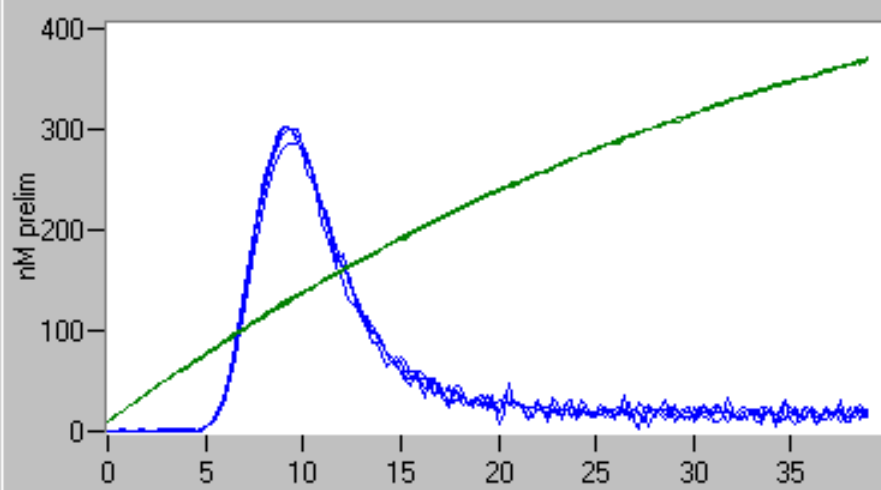


Graphs

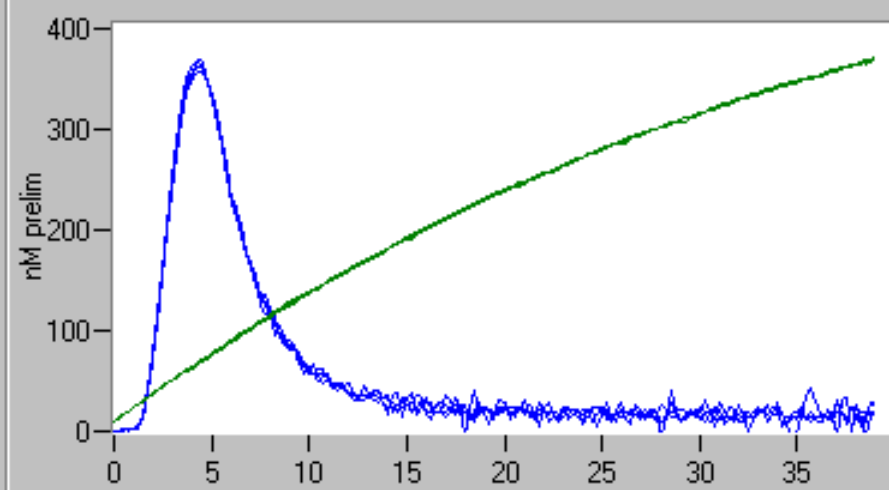


Plate in Plate out

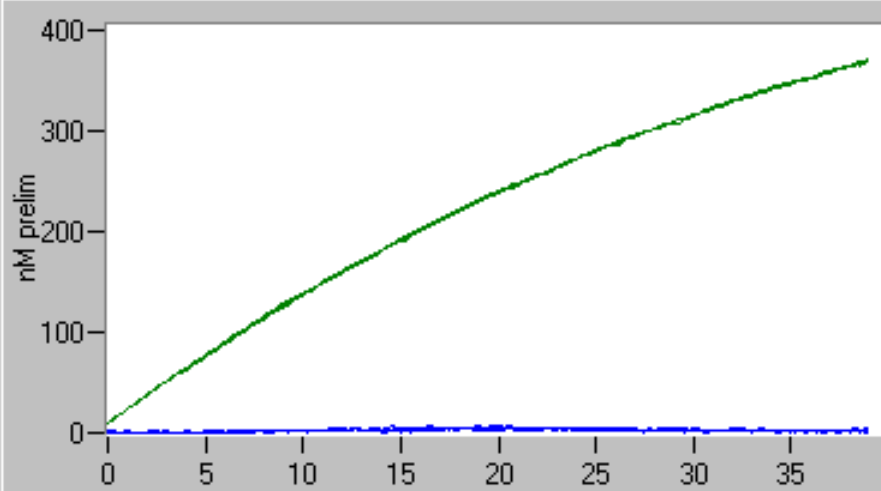
0.5 pM TF Control 1



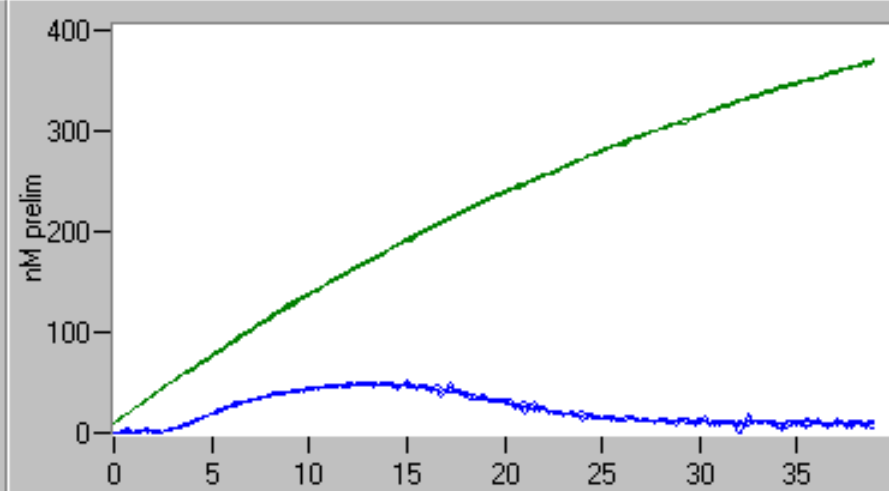
5 pM TF Control 2



0.5 pM TF Hep 3



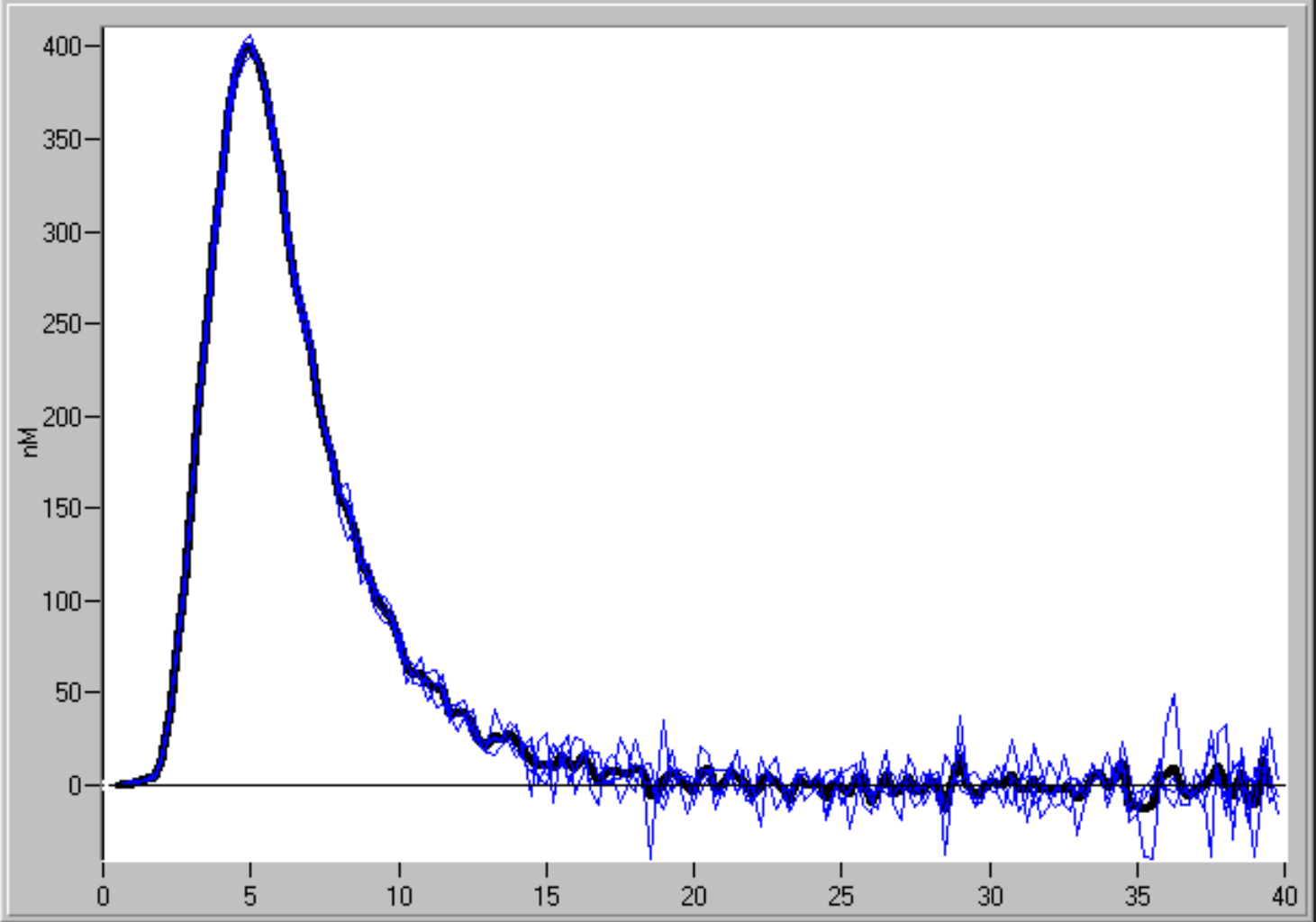
5 pM TF Hep 4



Graphs

5 pM TF Control 2 properties

nMIIa | dY/dt(Raw) | Raw | Standards



Calculated	
LagTime	StdDev
<input type="text" value="2.75"/>	<input type="text" value="0.00"/>
ETP	
<input type="text" value="2004.7"/>	<input type="text" value="22.30"/>
Peak	
<input type="text" value="400.37"/>	<input type="text" value="4.40"/>
ttPeak	
<input type="text" value="5.00"/>	<input type="text" value="0.00"/>
startTail	
<input type="text" value="22.75"/>	<input type="text" value="0.43"/>
Error	<input type="text"/>
WellWithError	<input type="text"/>

ZoomXY | Pan | DragCursor | | Xpos: | Ypos:

GeCalibreerde geAutomatiseerde Thrombine generatie meting in een fluorimeter (CAT)

Throughput:

96-wells microtiterplaat

48 wells voor de thrombine generatie meting in plasma monsters

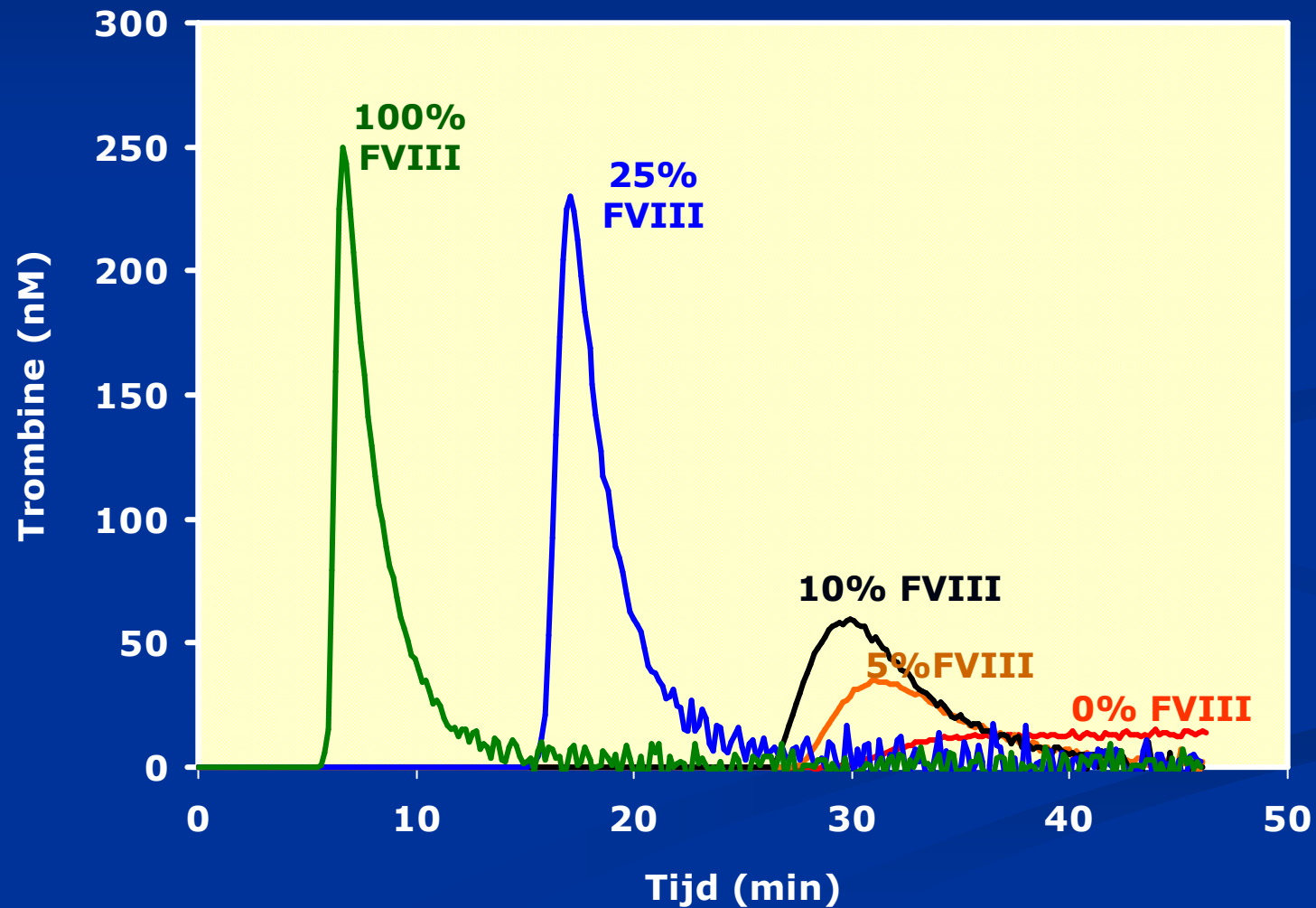
48 wells voor calibratie in elk plasma



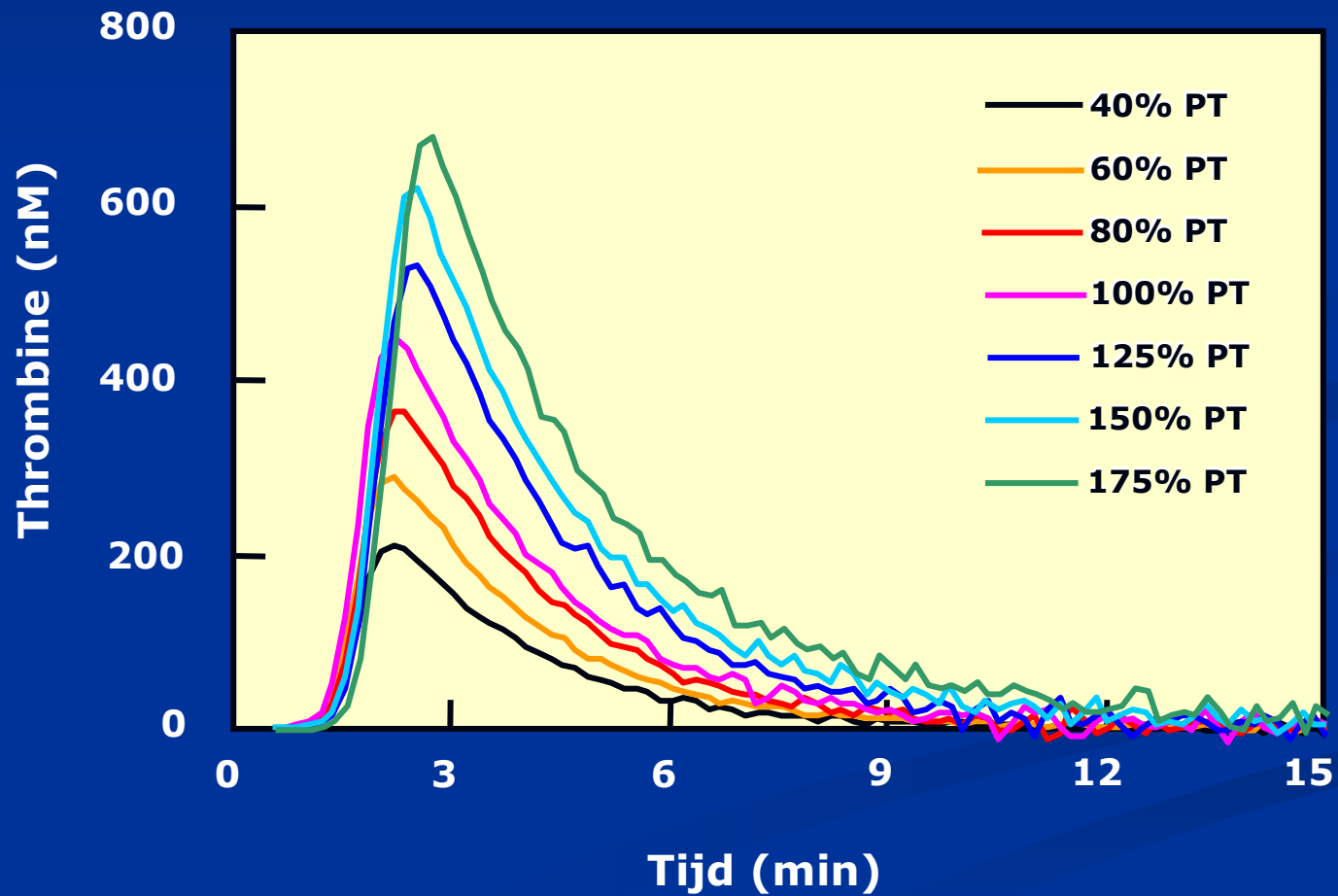
→ In 48 verschillende plasma monsters kan de thrombine generatie gemeten worden in ~ 40 min (hiervoor is 160 μ l plasma nodig)

Haemophilia

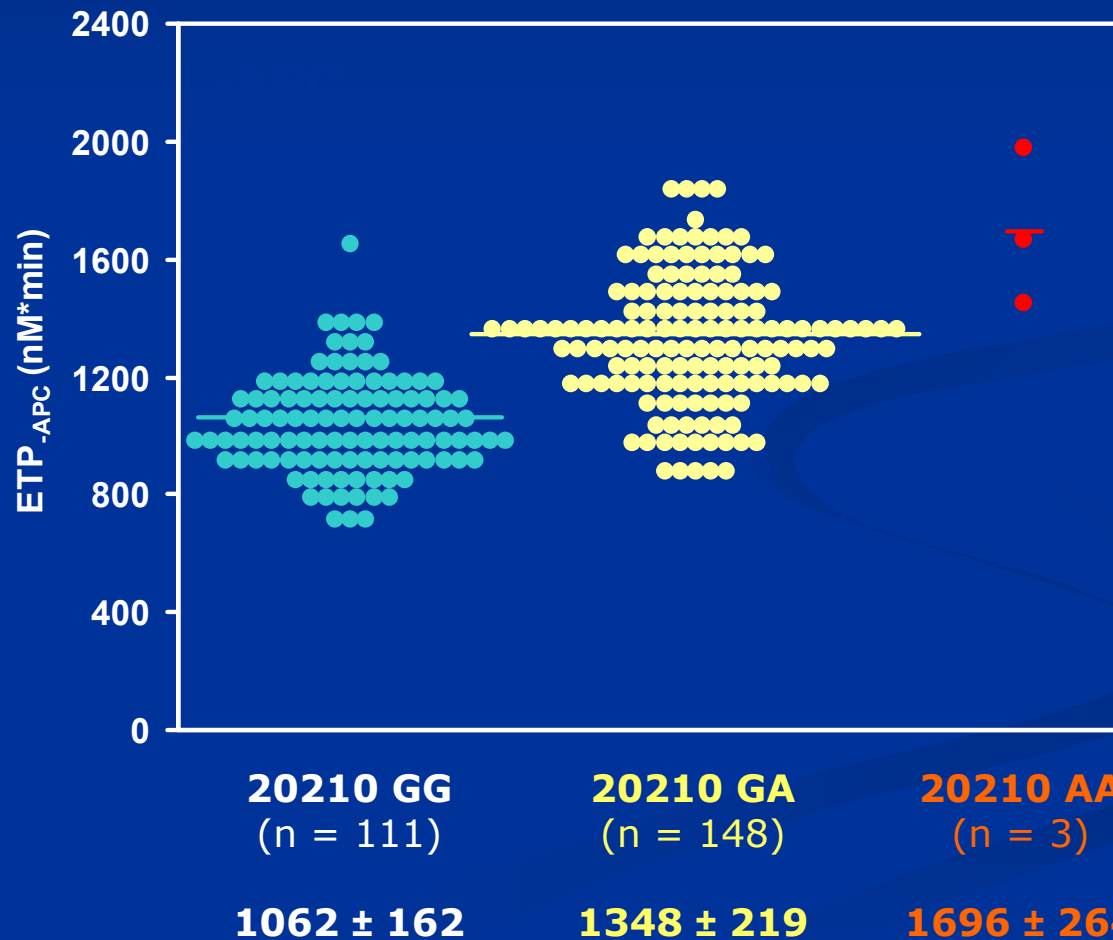
Thrombine generatie in plaatjesrijk plasma



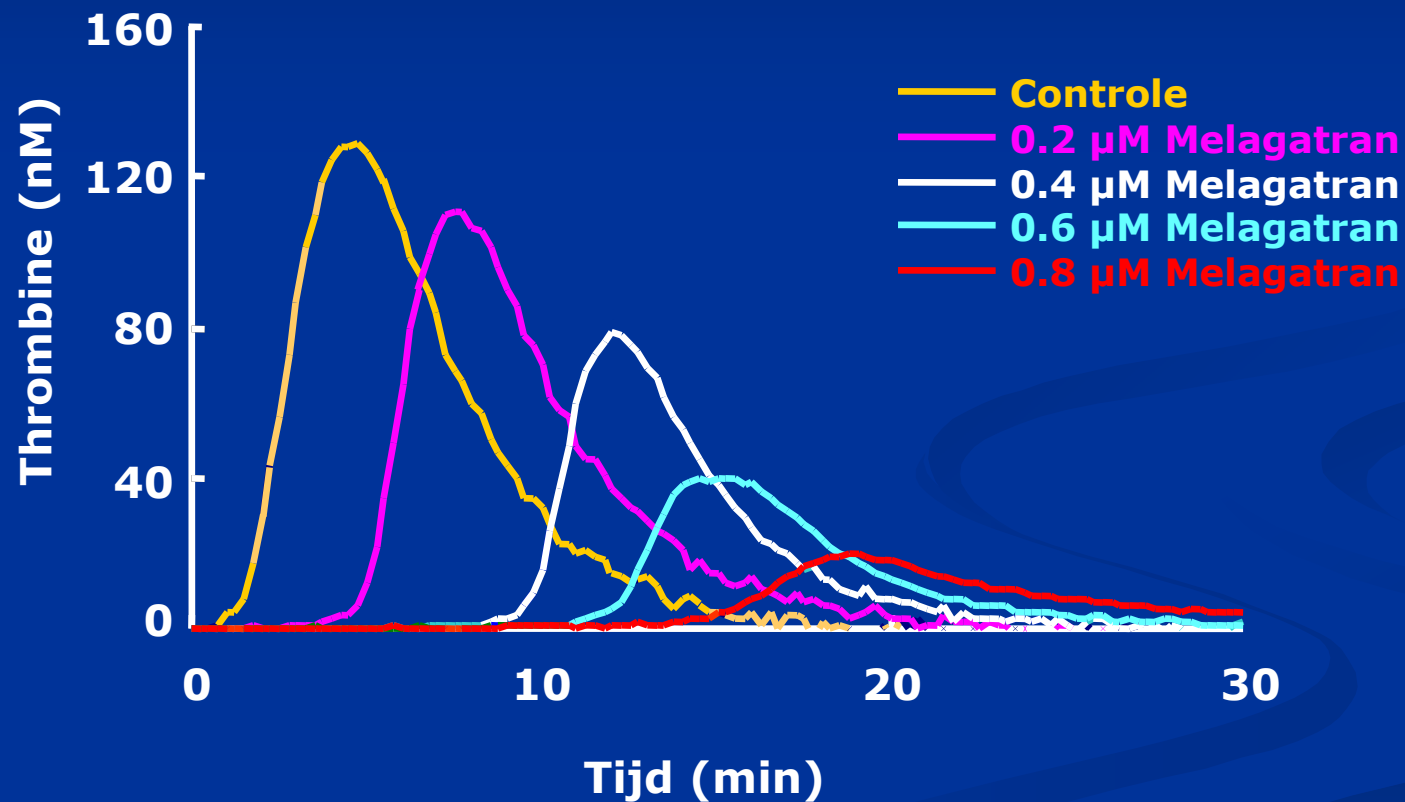
Effect van de Prothrombine Concentratie op de Thrombine Generatie



Effect van de PT 20210 G/A mutatie op de Thrombine Generatie



Effect van Melagatran op de Thrombine Generatie



APC Resistentie

❖ 1993 Dahlbäck

In een groot aantal thrombose patienten verlengt APC de stoltijd van plasma nauwelijks

APC resistentie is een belangrijke thrombose risico factor

~ 5% van de West-Europese bevolking is APC resistent

~ 30% van de VTE patienten is APC-resistent

~ 6-voudige toename van het thrombose risico

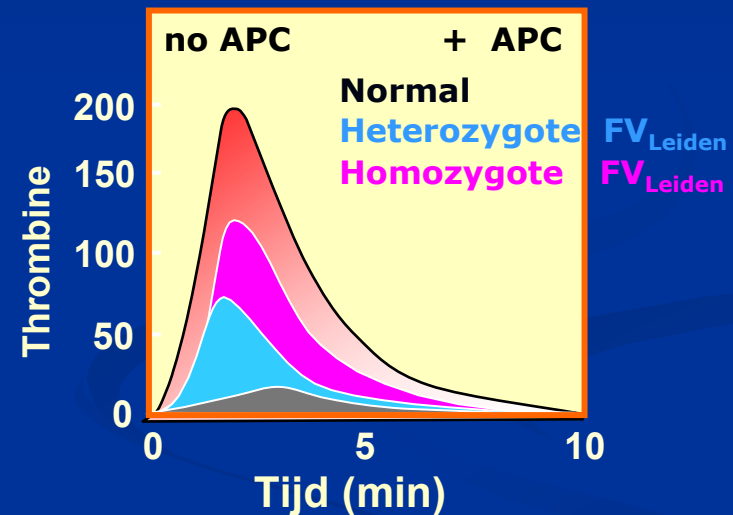
- Erfelijke APC resistentie is een gevolg van een mutatie in FV (FV_{Leiden})
- APC resistentie in afwezigheid van FV_{Leiden} is ook een thrombose risicofactor

APC resistentie test gebaseerd op de Thrombine generatie meting

Tissue factor
+/- APC

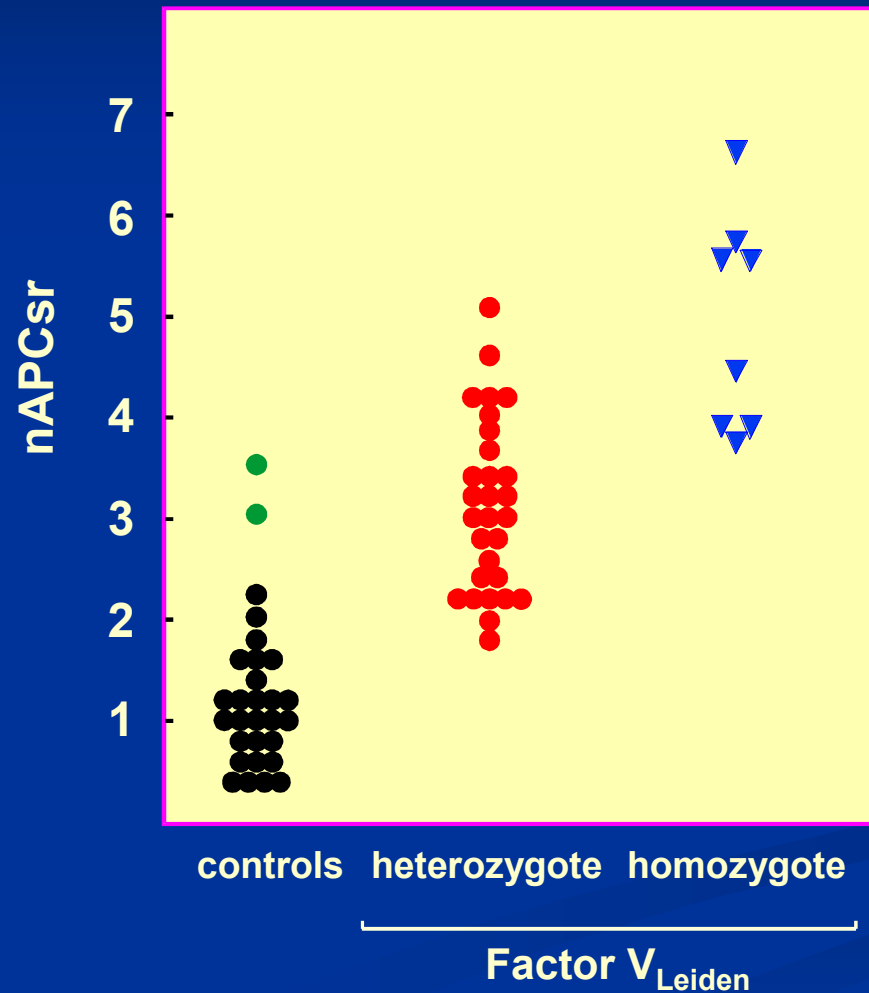


Plasma

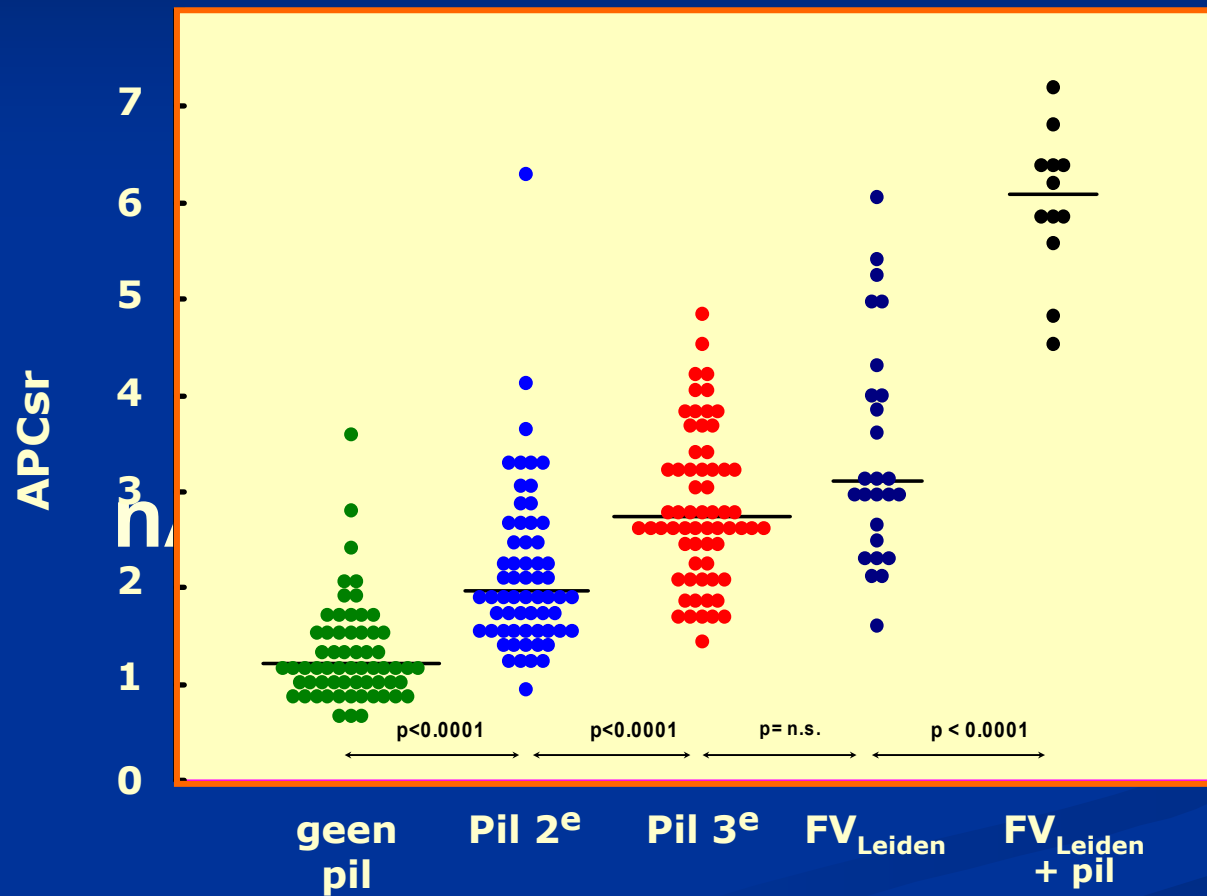


$$nAPCsr = \frac{(\% \text{ rest thrombine vorming}_{+APC})_{\text{plasma monster}}}{(\% \text{ rest thrombine vorming}_{+APC})_{\text{normaal plasma}}}$$

APC Sensitivity Ratio in Normal en in Factor V_{Leiden} Plasma



Verworven APC Resistentie en Pilgebruik



Samenvatting

- **Thrombine generatie is verlaagd in plasma van hemofielen**
- **Thrombine generatie is verhoogd in plasma van patiënten met erfelijke en verworven risicofactoren voor veneuze thrombose**
- **Thrombine generatie is verlaagd in plasma van patiënten behandeld met anticoagulantia**



De thrombine generatie test is een veelbelovend hulpmiddel voor de screening van thrombophilie en bloedingsziekten

The Siemens logo is displayed in a white box on a dark blue background. It consists of the word "SIEMENS" in a bold, teal, sans-serif font.

Laboratory
Diagnostics

DADE BEHRING

Every minute of every day™

Home | Products | Support | Services

 search

PRODUCTS >

Product Search



Catalog #	Product Name (EAN)	Package size	Availability	Product Notes
OPDS05.	Endogenous Thrombin Potential (RUO)	1 Kit / 100 tests	OUS, US	4,12

Notes

- 4. For research use only. Not for use in diagnostic Procedures.
- 12. Please note that products identified as available Outside US (OUS) may not be available in all countries. Please contact your local Siemens Representative for availability in your country.



Haemostasis

- Coagulation assays
- Coagulation Instruments
- Thrombophilia
- Thrombin Generation (TGA)**
- Manual TGA
- Ceveron - Automated TGA**
- Clinical assay kits
- Modular reagents
- Calibrators & Controls
- Buffers
- Microparticles
- Fibrinolysis
- Research
- Platelet Function

Ceveron® TECHNOTHROMBIN® TGA Kit for Thrombophilia

Ceveron® TGA Kit for Thrombophilia screening. CE marked only for the use with Ceveron® alpha.

Available in Autumn 2008 ←

NEW!

Ceveron® TECHNOTHROMBIN® TGA Kit for Haemophilia

Ceveron® TGA Kit for Haemophilia screening. CE marked only for the use with Ceveron® alpha.

Available Spring 2009 ←

NEW!

Product Number	Package Size	CE Status
50 060 12	3 x 16 tests	CE
Safety DS		

Product Number	Package Size	CE Status
50 062 14	3 x 16 tests	CE
Safety DS		



The Calibrated Automated Thrombogram

Home

The method

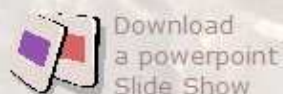
Our products

Our partners

Contact

How to measure the Thrombogram

The Thrombinoscope Software Program



Download
a powerpoint
Slide Show

The Thrombinoscope Software program runs on a Windows PC hooked up to the Thrombinoscope Instrument. With this package it is possible to watch the concentration of thrombin as it develops in clotting (platelet-rich) plasma. All the data collection and reduction is completely automated which makes the method user-friendly and widely applicable. When you are interested to try out the method in practice then please contact us and we will arrange a try-out period in your laboratory and give you, for a limited period of time, a version of the software that has full capabilities.



Add your reagents to the 96-well plate...

...and watch the Thrombin generation on your screen

