Tests of Global coagulation
Thromboelasatometry
Thromboelastography
Thrombin generation

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Tissue Factor Concentration

- **Prothrombin time –**
  - Tissue factor at around 2000pm
  - Sensitive to ‘extrinsic coagulation pathway’
- **Thrombin Generation Tests –**
  - Tissue factor is often used at 1 to 5pm
  - Sensitive to all coagulation factors but *(?XII)* and XIII

Thrombin Generation Tests: Triggering Coagulation

- Low level tissue factor is often the trigger of choice
  - TF-VIIa activates factor IX to IXa
- IXa can be used as trigger of thrombin generation
- Other triggers: e.g. contact factor activators

Value of Thrombin Generation Tests

- May be sensitive to:
  - All clotting factor levels apart from XIII
  - Natural anticoagulants (including TFPI)
  - Pharmacological anticoagulants (e.g. LMWH)
  - Hypercoagulability – predict who will thrombose?
  - Hypocoagulability - prediction of who will bleed?

Thrombin Generation: Pre analytical variable

- Anticoagulant and concentration (+/-CTI)
- Material tested (PPP, PRP, frozen PRP)
- Platelet contamination (including microparticles)
- Contact activation
- Sample quality etc etc

Thrombin Generation Tests: a family of tests that detect thrombin formation

TG assays vary:

- **Trigger** e.g. IXa, TF
- **Phospholipid**
- **Sub sampling or continuous monitoring** to detect thrombin
- **Fibrinogen, chromogenic or fluorogenic** substrate
- **Units to recorded** (e.g. AU, total thrombin, peak thrombin)
- **Calibration system** may be used to measure the thrombin
- **Material tested** (e.g. defibrinated plasma or plasma or PRP)

α2-macroglobulin bound thrombin

- **α2- macroglobulin-thrombin does not cut natural substrates**

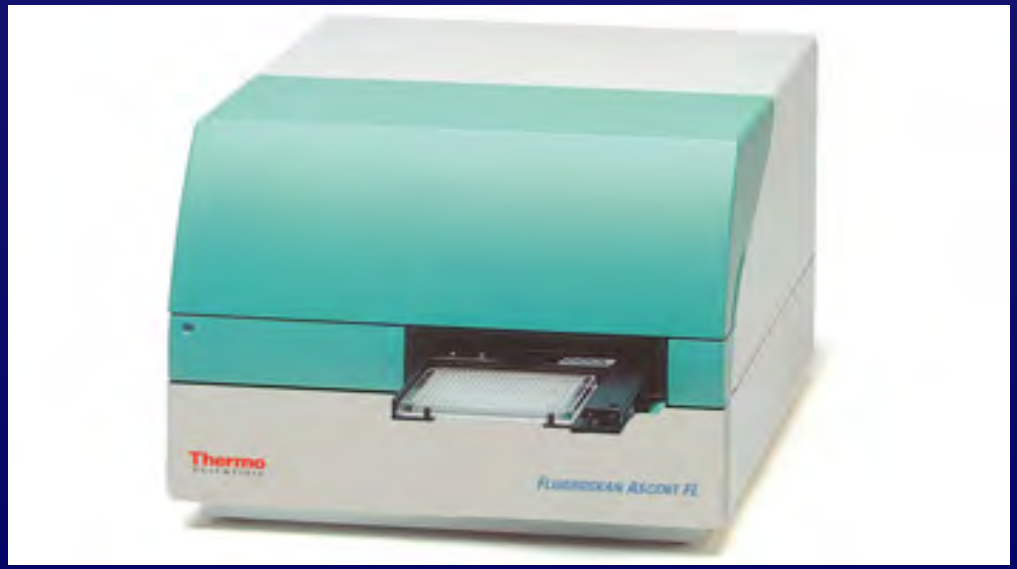
- **But does cleave small chromogenic and fluorogenic substrates**

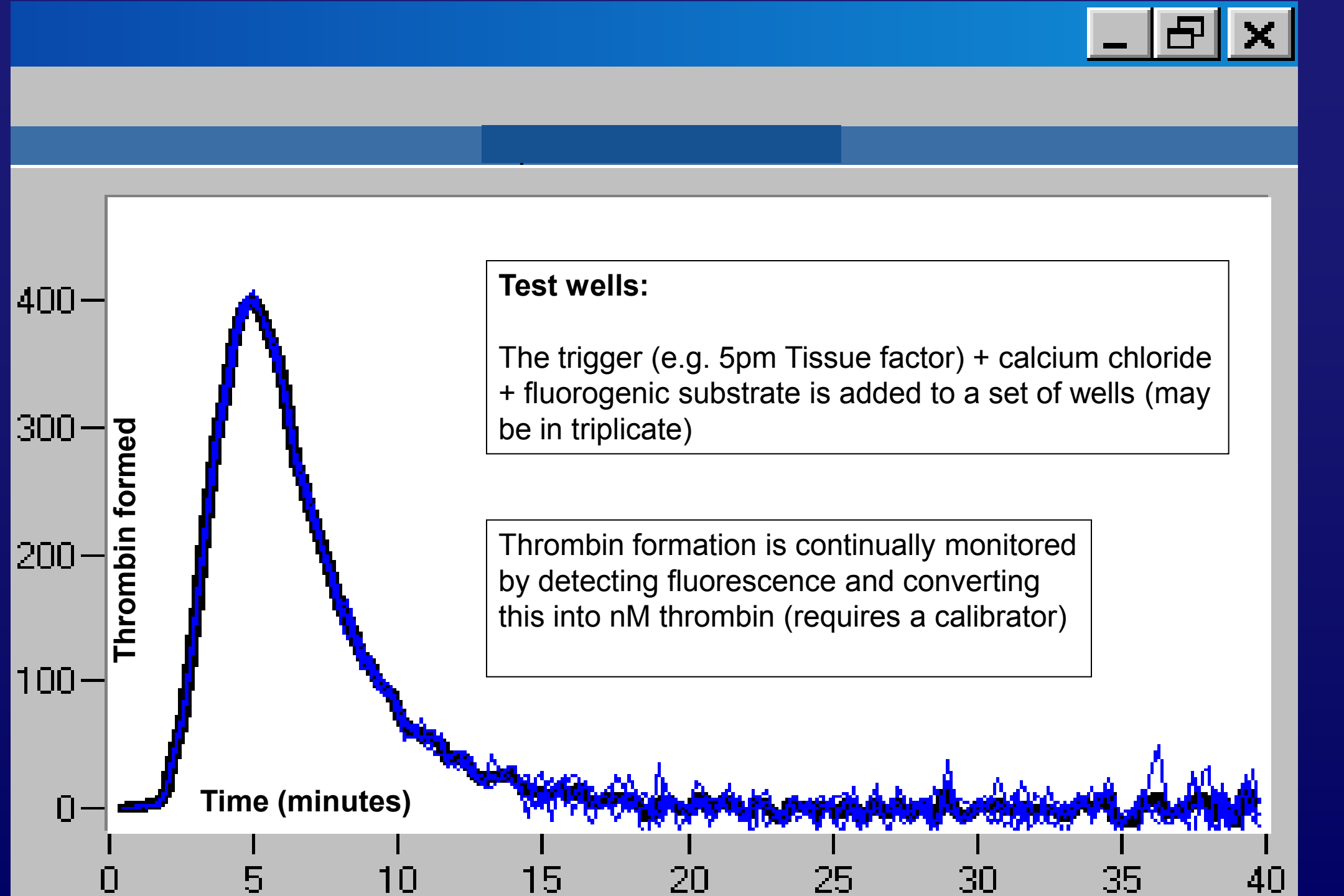
α2-macroglobulin bound thrombin

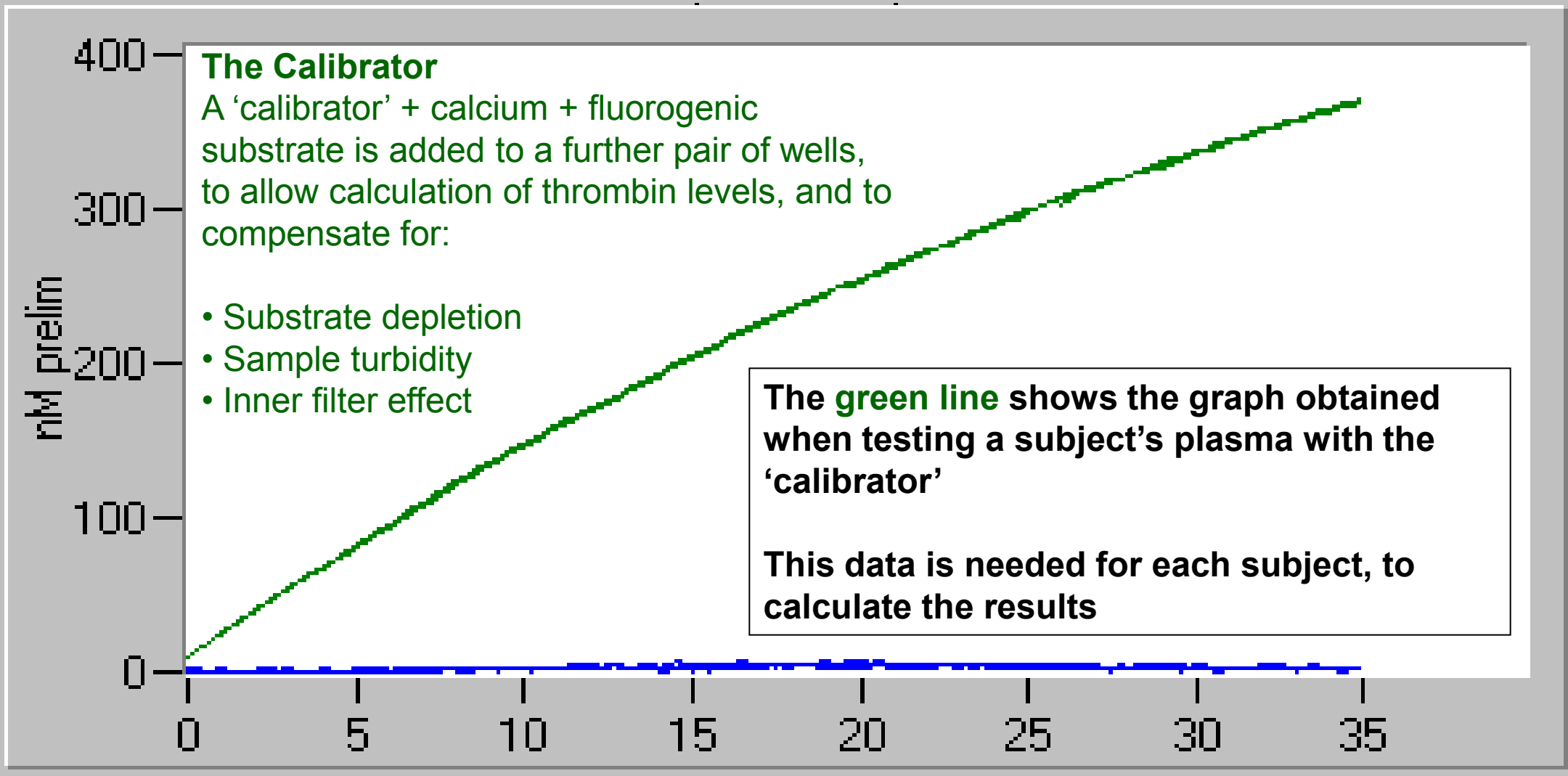
- **The Thrombinoscope assay uses an algorithm to discount this influence, but not all systems do**

- **The Thrombinoscope assay uses α2-macroglobulin-thrombin as reference material to standardise the assay**

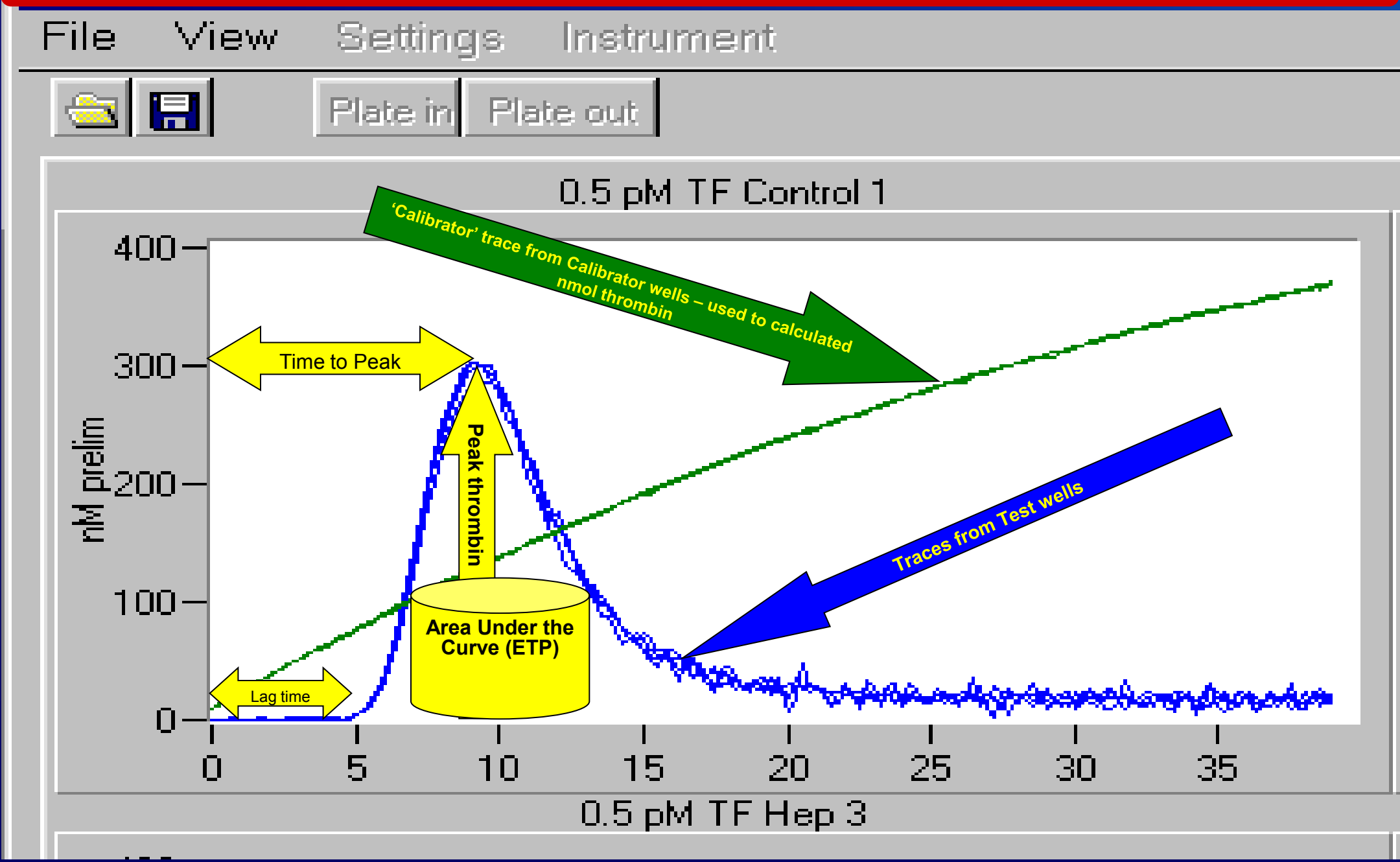
Thrombinoscope TG assay
Is carried out on a Fluorometer




Test wells:
The trigger (e.g. 5pm Tissue factor) + calcium chloride + fluorogenic substrate is added to a set of wells (may be in triplicate)
Thrombin formation is continually monitored by detecting fluorescence and converting this into nM thrombin (requires a calibrator)
Thrombin formed
Time (minutes)
400
300
200
100
0
0
5
10
15
20
25
30
35
40


400
300
200
100
0
nM prelim
0
5
10
15
20
25
30
35
The Calibrator
A 'calibrator' + calcium + fluorogenic substrate is added to a further pair of wells, to allow calculation of thrombin levels, and to compensate for:
• Substrate depletion
• Sample turbidity
• Inner filter effect
The green line shows the graph obtained when testing a subject's plasma with the 'calibrator'
This data is needed for each subject, to calculate the results

Thrombin Generation Test Curve Parameters (Thrombogram)



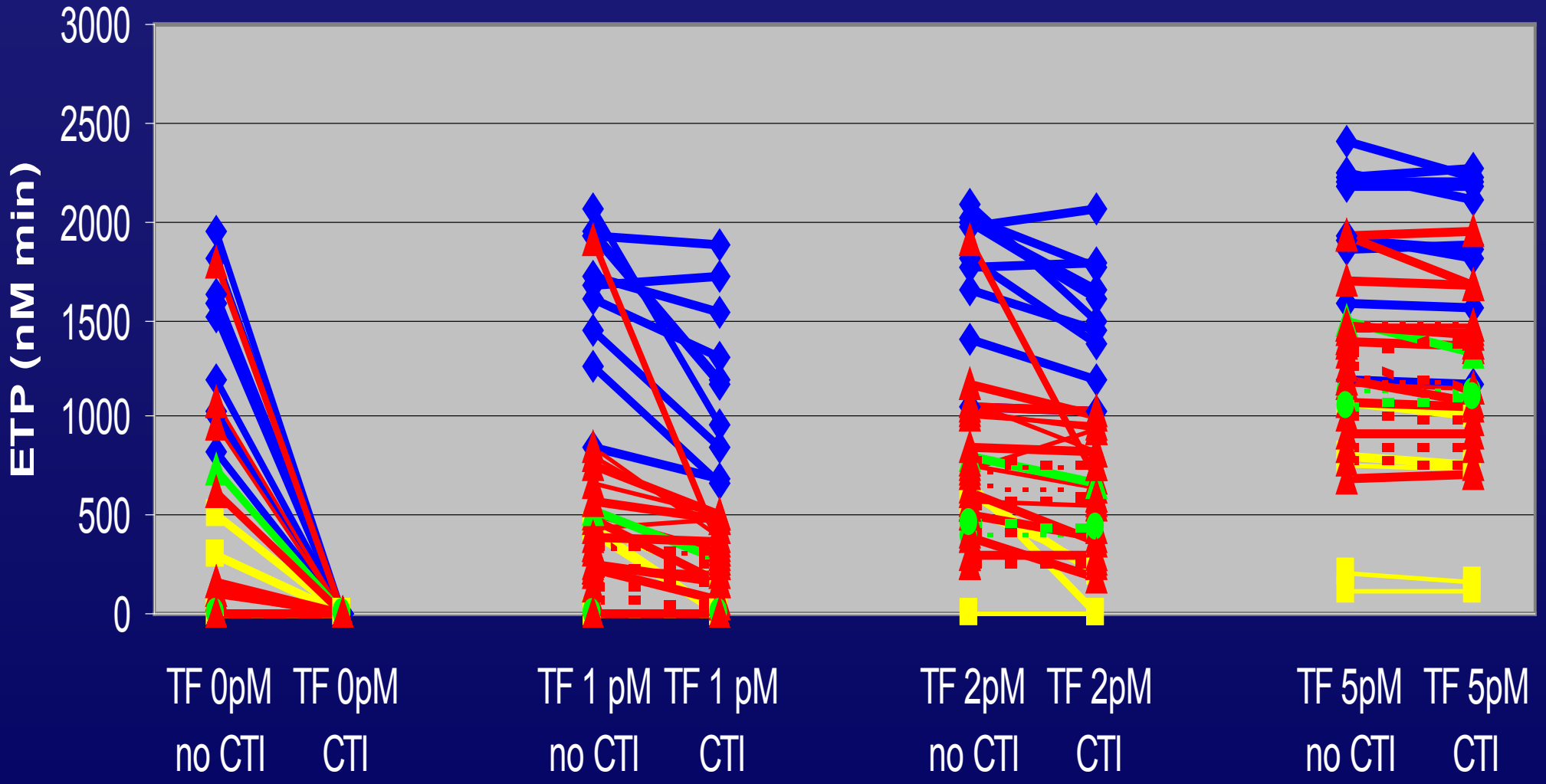
Thrombinoscope TG assay and the calibrator

- Each sample is normally measured in triplicate and the calibrator is tested in duplicate
- The thrombin calibrator (thrombin-α2-M complex) allows calculation of thrombin levels in the sample
- The calibrator compensates for: sample turbidity, the inner filter effect, and substrate depletion

Thrombinoscope TG assay and contact activation (Luddington and Baglin, JTH, 2004)

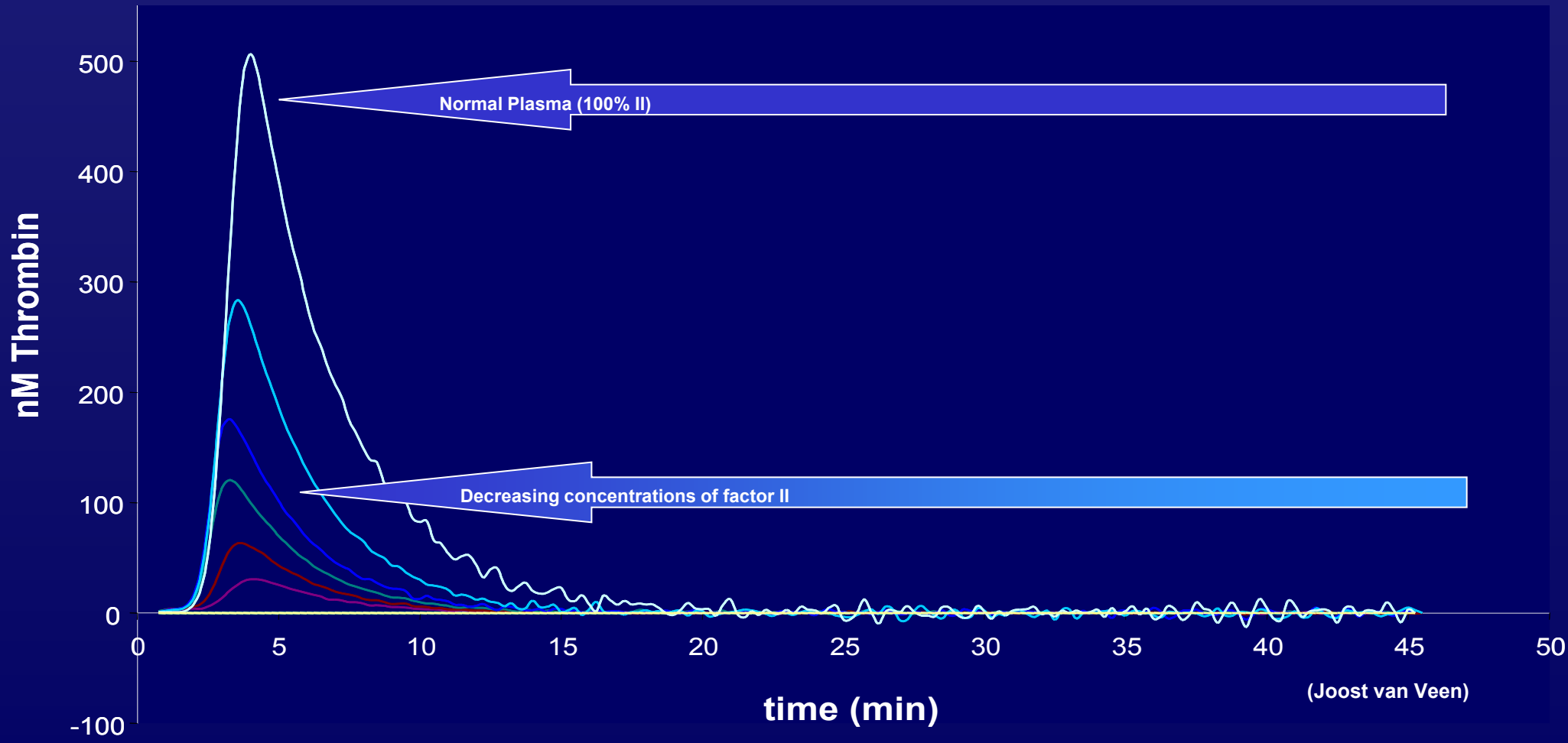
- Contact activation may influence thrombin generation at low TF concentration (=<2pmol)
- Contact activation of XII can occur during venepuncture or sample storage
- Contact activation reduces sensitivity of assay when <=2pm TF is used

Corn Trypsin Inhibitor (CTI) and Tissue Factor concentration in the ETP assay

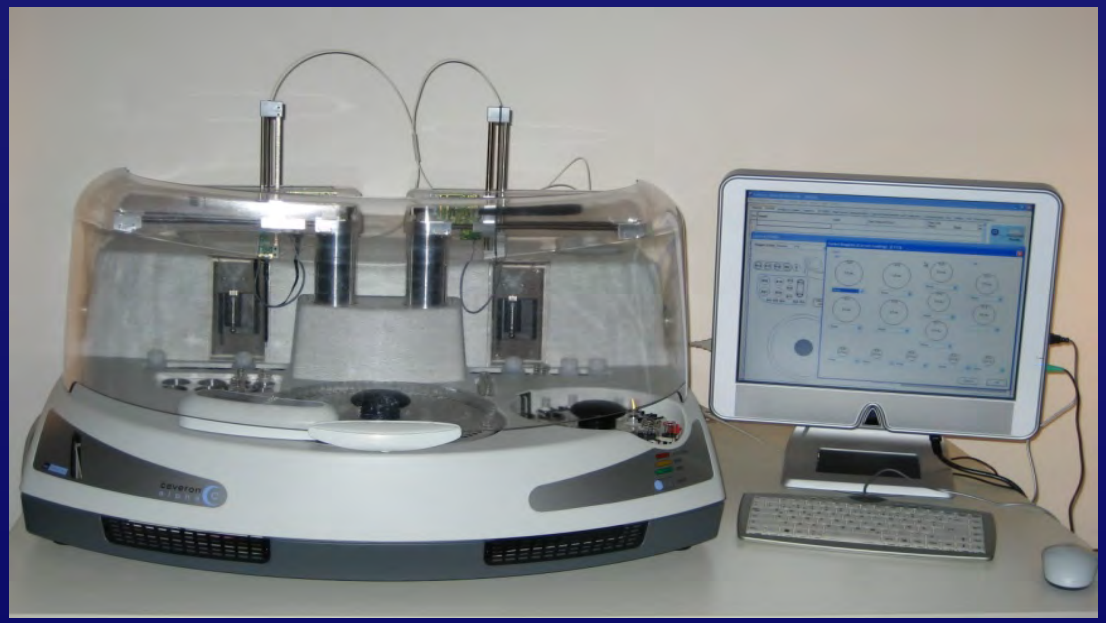


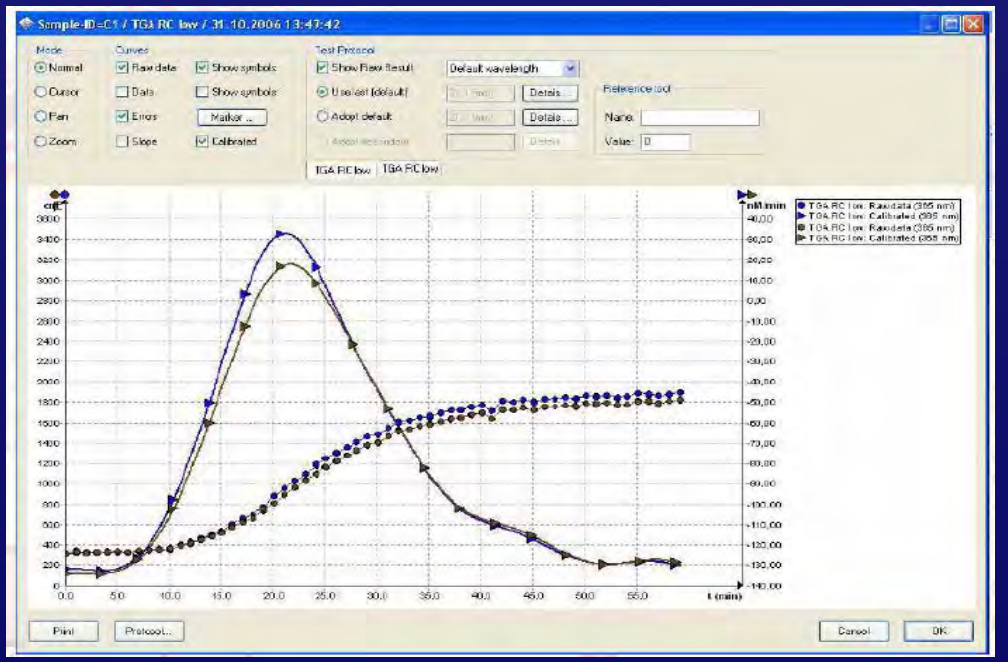
(Joost van Veen, 2005)

Thrombinoscope Thrombin Generation Assay:
Factor II deficient plasma supplemented with normal plasma



Technoclone Ceveron ® alpha





Thrombin generation Assays

- Many assay principles
- Many assay formats
- A lot of publications with in-house assays
- A lot of publications with in-house/hybrid assays
- Not a lot of standardisation
- Is it time to standardise the assays?
  - Working group on thrombin generation

TEG® Analyser

Cup rotates pin is stationary

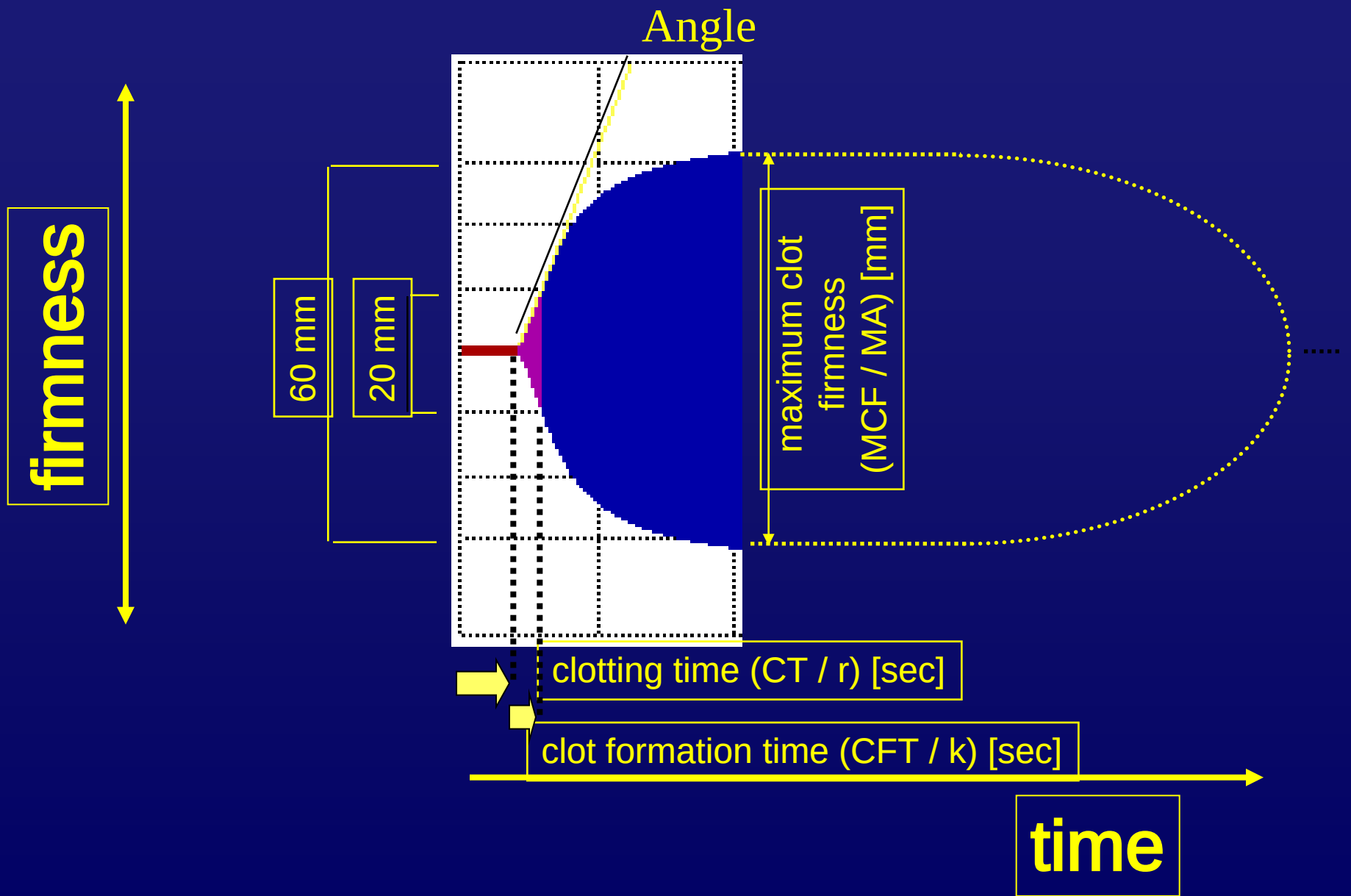
ROTEM ® Device

Pin rotates Cup is stationary

Parameters

	ROTEM	TEG
Time for clotting to begin	CT (sec)	R (min)
Time until clot is 20 mm	CFT (sec)	K (min)
Rate of clot growth	Angle (degrees)	Angle (degrees)
Clot firmness - trace width	MCF (mm)	MA (mm)

ROTEM / TEG analysis



NEQAS Survey data ROTEM® - Intem CT

(n= 7 -10)

Citrated Whole Blood Normal range 100 - 240 sec

	Median	Range	CV
Normal (1)	147 sec	117 - 161	10%
Normal (2)	145 sec	129 - 197	15%
FXI <1 U/dl	950 sec	596 - 1562	37%

NEQAS Survey data TEG® R

(n = 13 - 14)

	Median	Range	CV
Normal (1)	5.6 min	4.8 – 7.6	15%
Normal (2)	5.8 min	4.2 – 7.2	19%
FXI <1 U/dl	No clot	-	-

Agreement between results in different centres - Clot Firmness

	ROTEM (Extem) MCF (mm)		TEG MA (mm)	
	Range	CV	Range	CV
Normal (1)	36 -47	8 %	42-53	8%
Normal (2)	24-27	4.5%	16-61	33%
FXI <1 U/dl	30-36	6%	No clot	-

Fibrinogen in normal 2 2.5 g/l versus 5 g/l in normal 1