A clear view into the future !

TECHNOTHROMBIN TGA

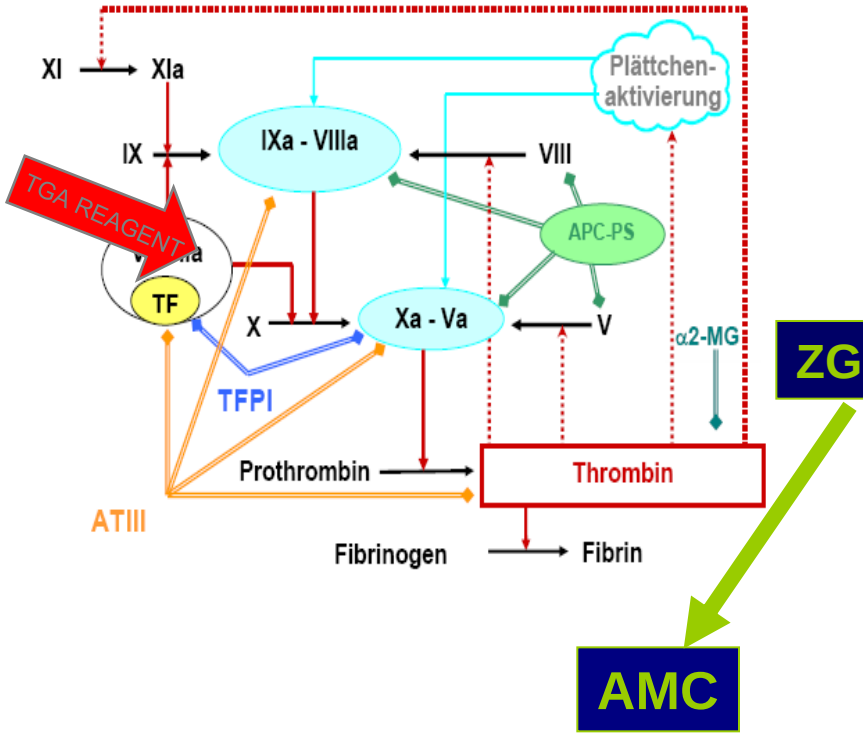
Measurement on Ceveron® alpha TGA

The Ceveron® alpha system
is for Research Use Only
in the US and Canada.

October 2015

technoclone

TEST PRINCIPLE

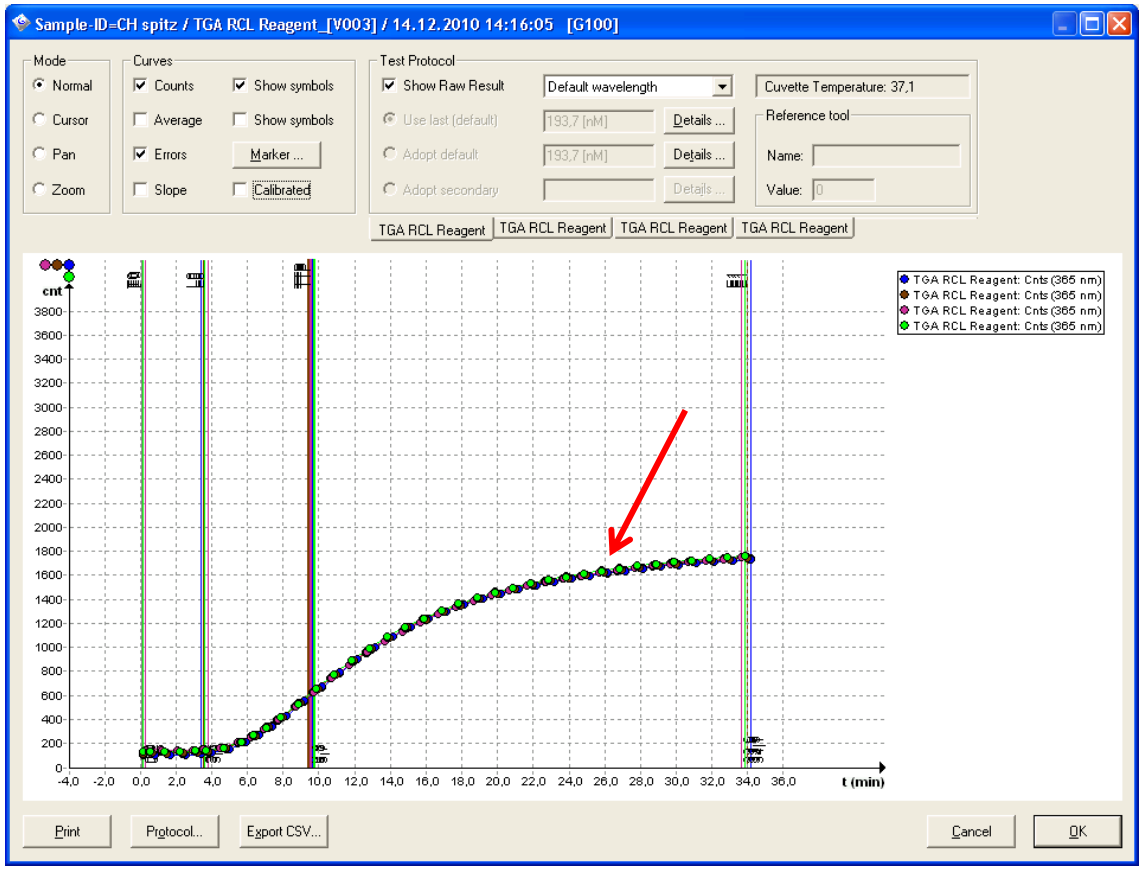


- **The coagulation cascade is activated upon addition of different concentrations of**
  - **tissue factor and**
  - **phospholipids**

The fluorogenic substrate ZGGR-AMC is cleaved by formed thrombin over time.

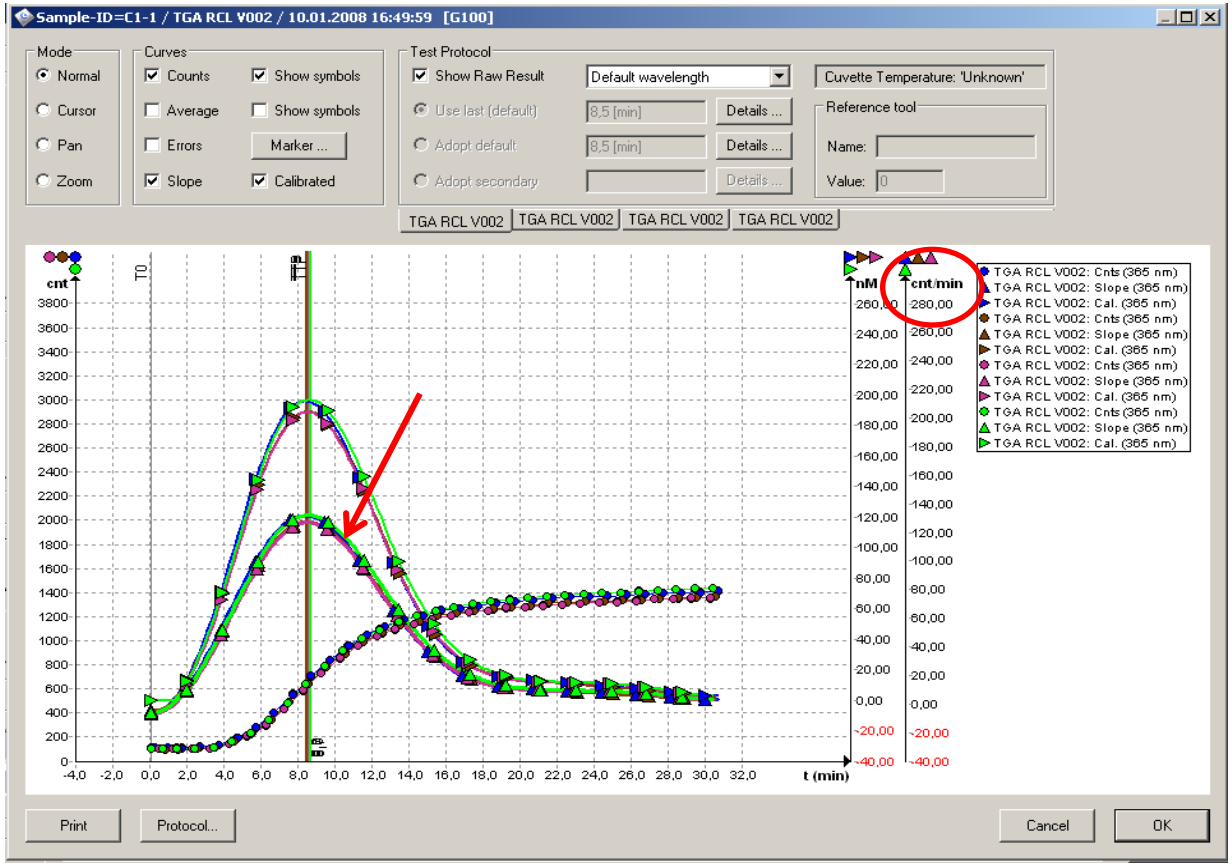
TEST PRINCIPLE

Plotting the fluorescence we obtain the raw data curve of thrombin generation



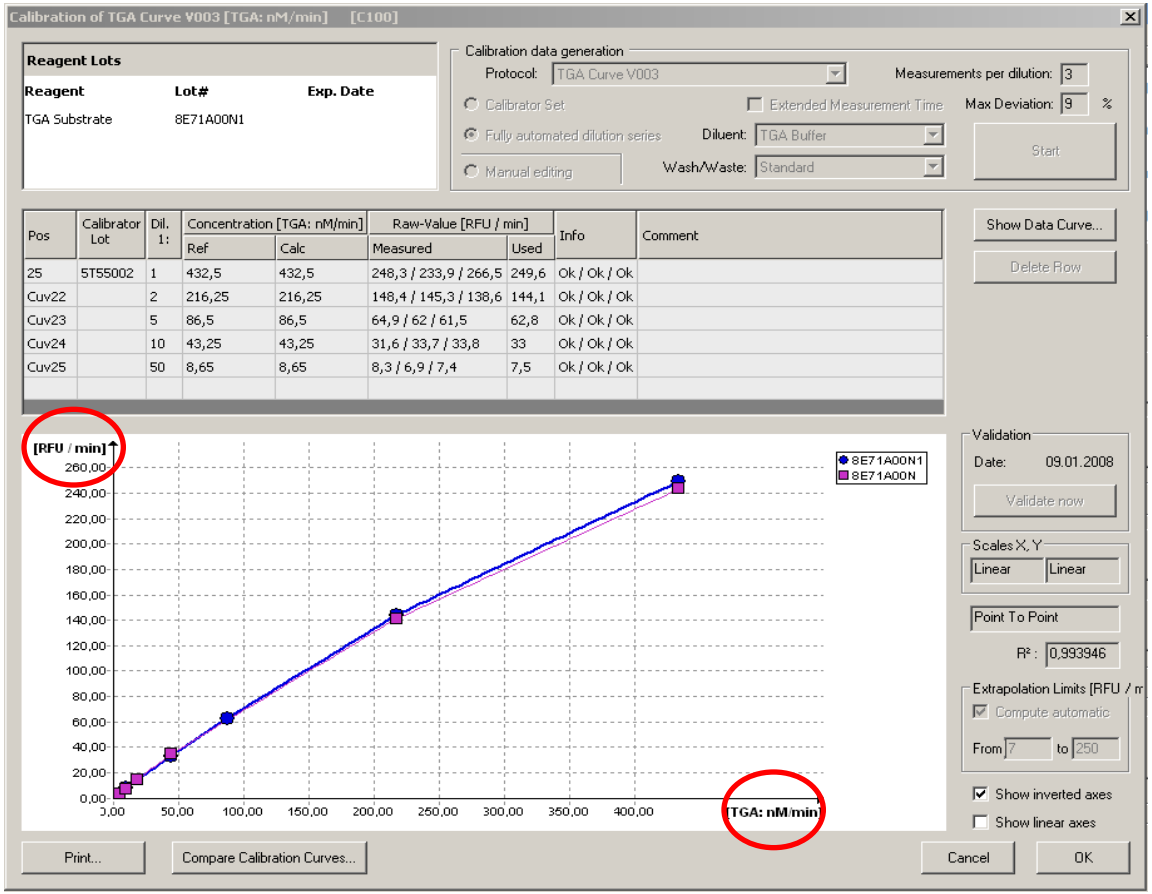
TEST PRINCIPLE

Plotting the changes in fluorescence as a function of time (cnt/min), we obtain a „Thrombin Generation Curve”



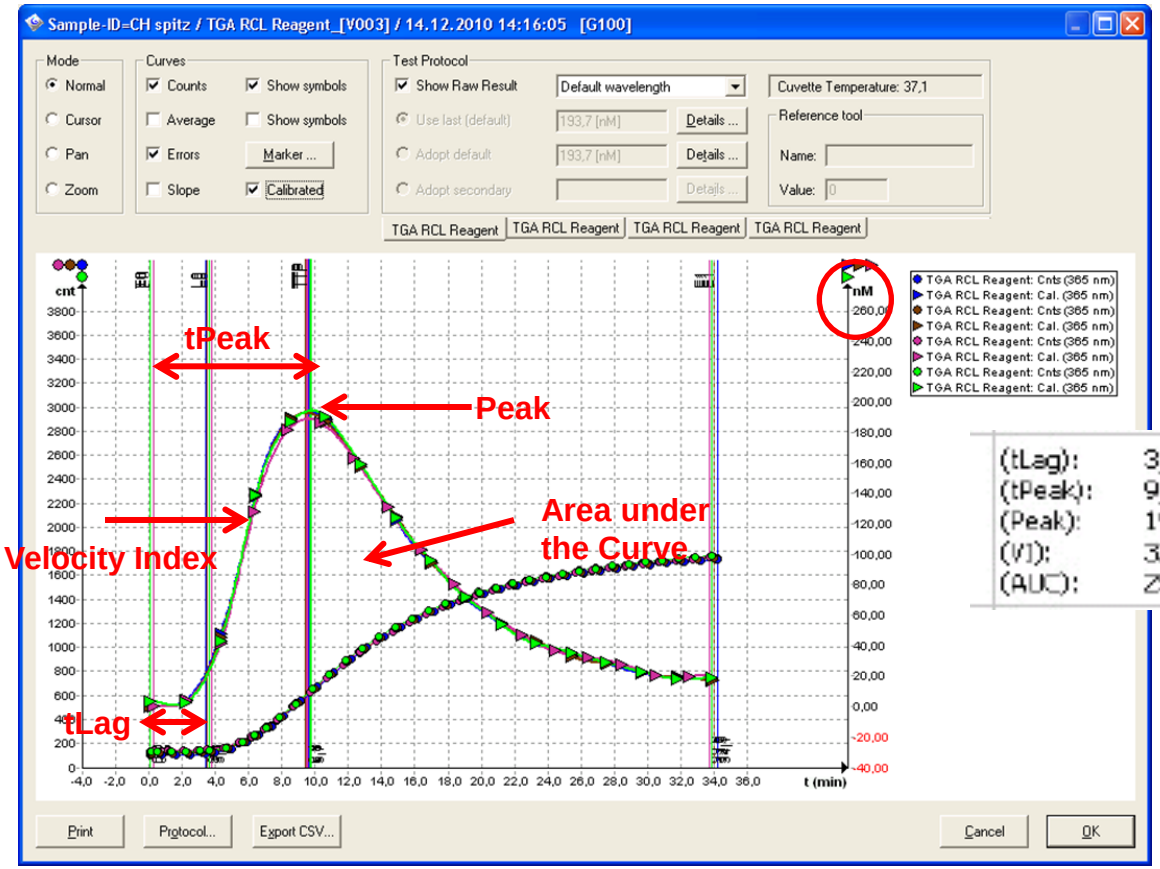
TEST PRINCIPLE

- The calibration curve (thrombin curve) enables conversion of results from cnt/min to nM thrombin.
- The calibration curve is created **separately from sample measurement**.
- For each **lot of substrate** only **one calibration curve** has to be created.



TEST PRINCIPLE

Thrombin Generation Curve in nM Thrombin displays the different phases of the coagulation reaction.



(tLag):	3,5	min (3,4 / 3,5 / 3,8 / 3,6)
(tPeak):	9,5	min (9,6 / 9,4 / 9,5 / 9,7)
(Peak):	192,6	nM (193,7 / 193,0 / 189,1 / 194,6)
(VI):	32,1	nM / min (31,1 / 32,6 / 33,0 / 31,8)
(AUC):	2551,8	nM (2566,3 / 2559,6 / 2521,5 / 2559,8)

We recommend following trigger reagents for the determination of:

Reagent	purpose
TGA RA	- measure the activity of **microparticles**
TGA RB and RC Low	- measure the **thrombophilic tendency** (preferentially with platelet poor plasma PPP) - measure the thrombogenity of microparticles
TGA RC High	- measure the effect of an **anticoagulant**

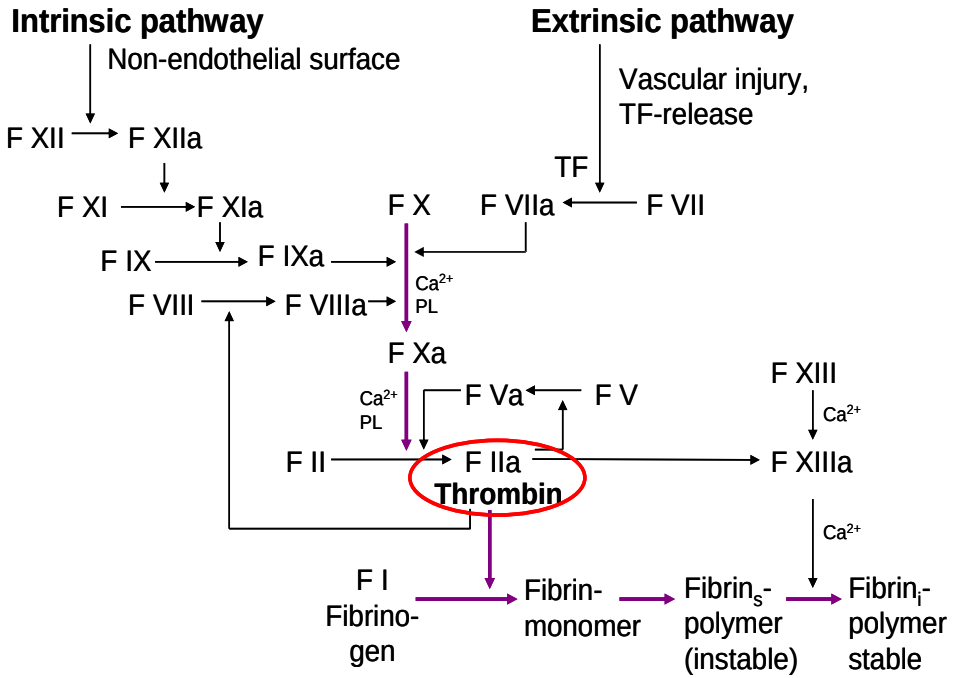
The composition of the different TGA trigger reagents are:

Reagent	Concentration
TGA RA	**Low** conc. of phospholipid micelles containing **no** rhTF Tris-Hepes-NaCl buffer
TGA RB	**Low** conc. of phospholipid micelles containing **low** rhTF in Tris-Hepes-NaCl buffer
TGA RC Low	**High** conc. of phospholipid micelles containing **low** rhTF (same as in RB) in Tris-Hepes-NaCl buffer
TGA RC High	**High** conc. of phospholipid micelles (same as in RCL) containing **high** rhTF in Tris-Hepes-NaCl buffer

Trigger action

RB and RClow are TRIGGERs that

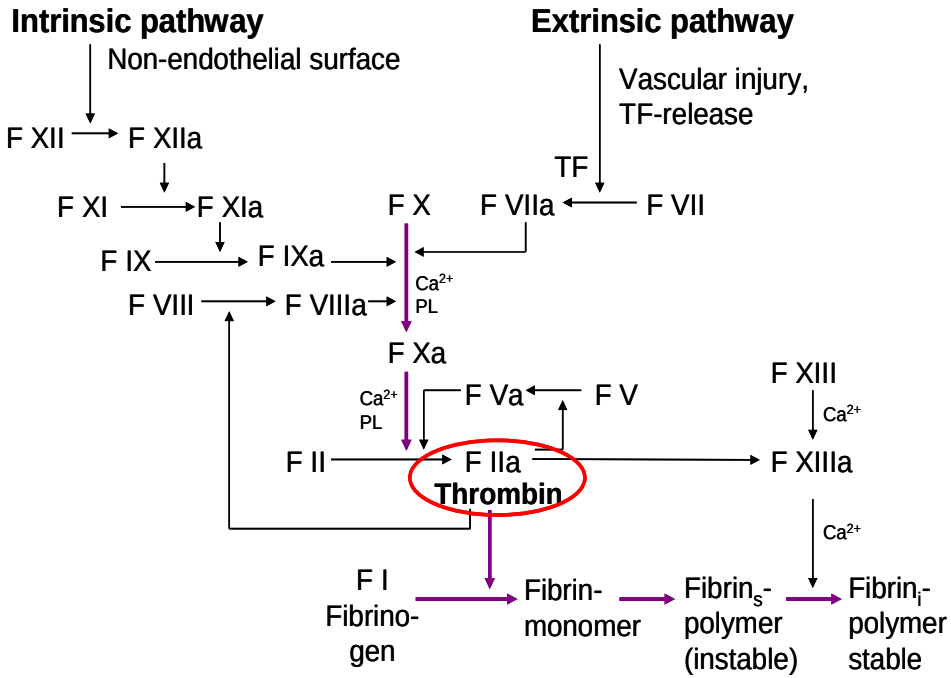
- activate the **extrinsic pathway** and forms a small amount of initial thrombin,
- this leads to formation of fibrin
- it is rapidly inactivated in a TF/FVIIA/FXa complex by TFPI
- activates by positive feedback the **intrinsic system**. This means, via factor XI, IX and VIII more FXa and thrombin are generated.
- when thrombin burst gets too big, differences in e.g., FVIII or FIX can‘t be measured any more.



Trigger action

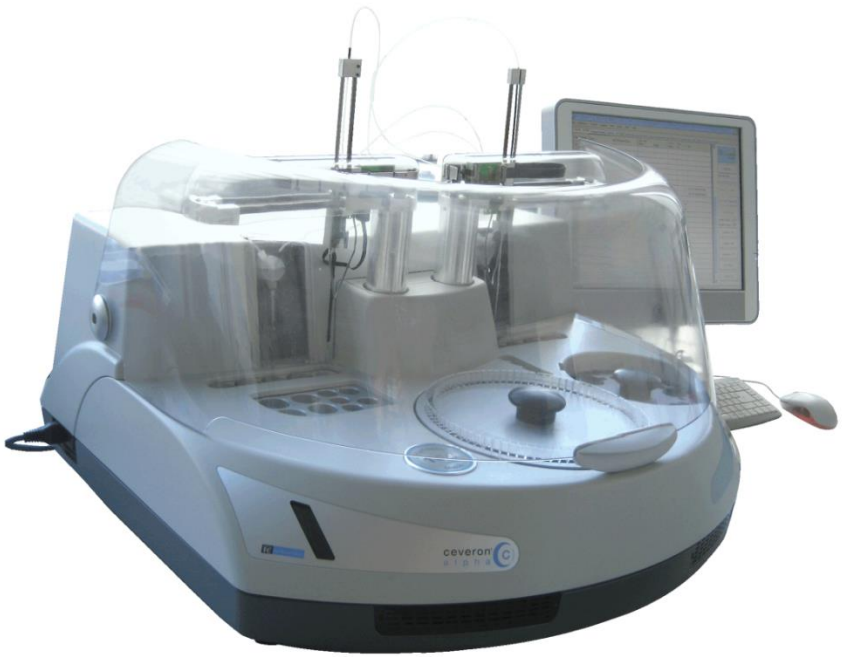
RChigh is a TRIGGER that

- activate the **extrinsic pathway** and forms such a big amount of initial thrombin, this **thrombin burst** gets so big, so that differences in e.g., FVIII or FIX can‘t be measured any more.



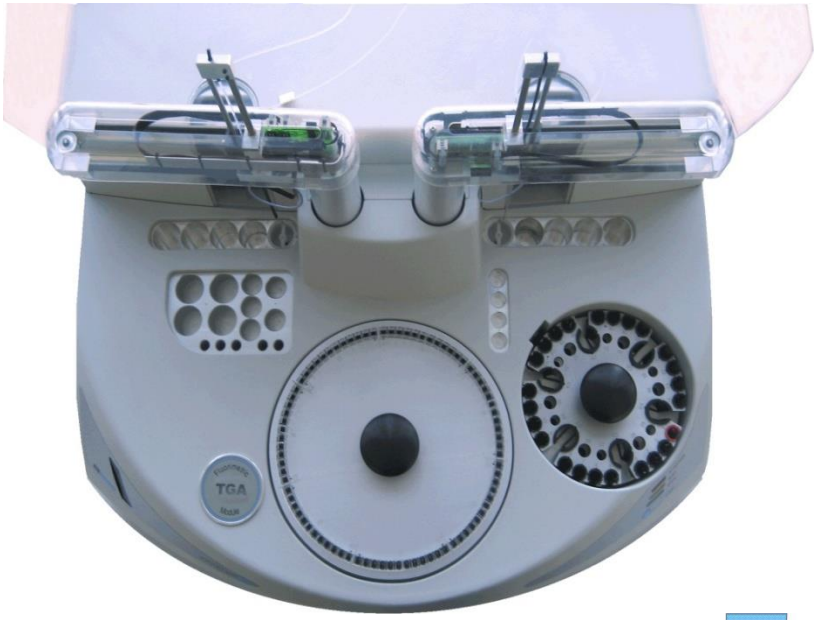
Reader/analyzer implication on thrombin generation measurement

Advantages of TGA Measurement on Ceveron alpha TGA

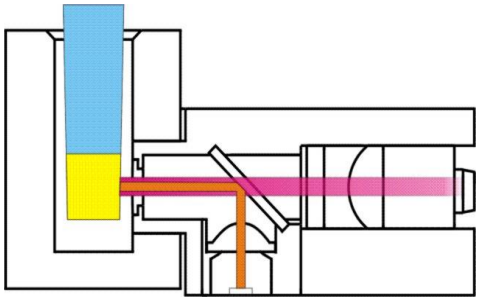


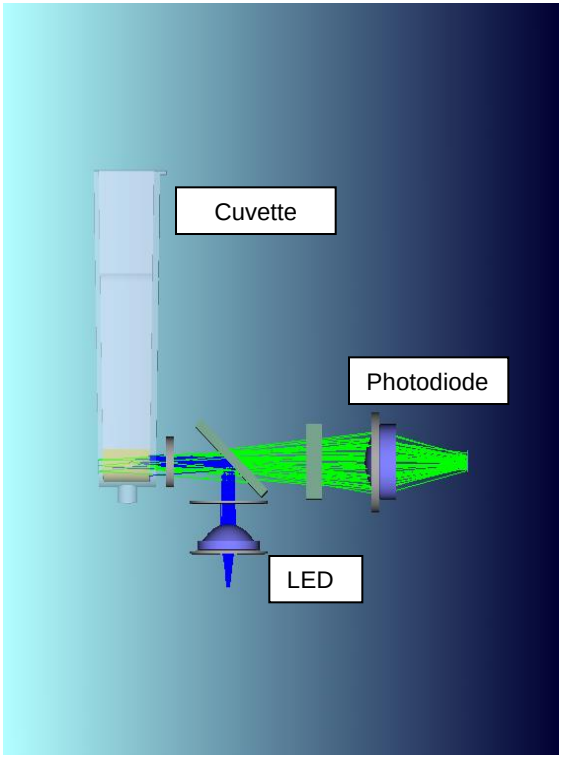
- ✓ Cuvette temperature is constant at 37° C
- ✓ Reagents are preheated in the tip and pipetted at 37° C
- ✓ LED temperature is constant resulting in standardized excitation intensity
- ✓ No influence of meniscus due to side measurement
- ✓ Check of fluorogenic channels with F-Standard
- ✓ Short assay time due to possibility of stopping at peak thrombin
- ✓ Possibility to run routine assays and TGA from the same sample in same run

Reader/analyzer implication on thrombin generation measurement



For thrombin generation measurement 4 channels with special fluorometric TGA modules consisting of an UV LED (365nm) for excitation and a photodiode for measurement of the emitted signal.



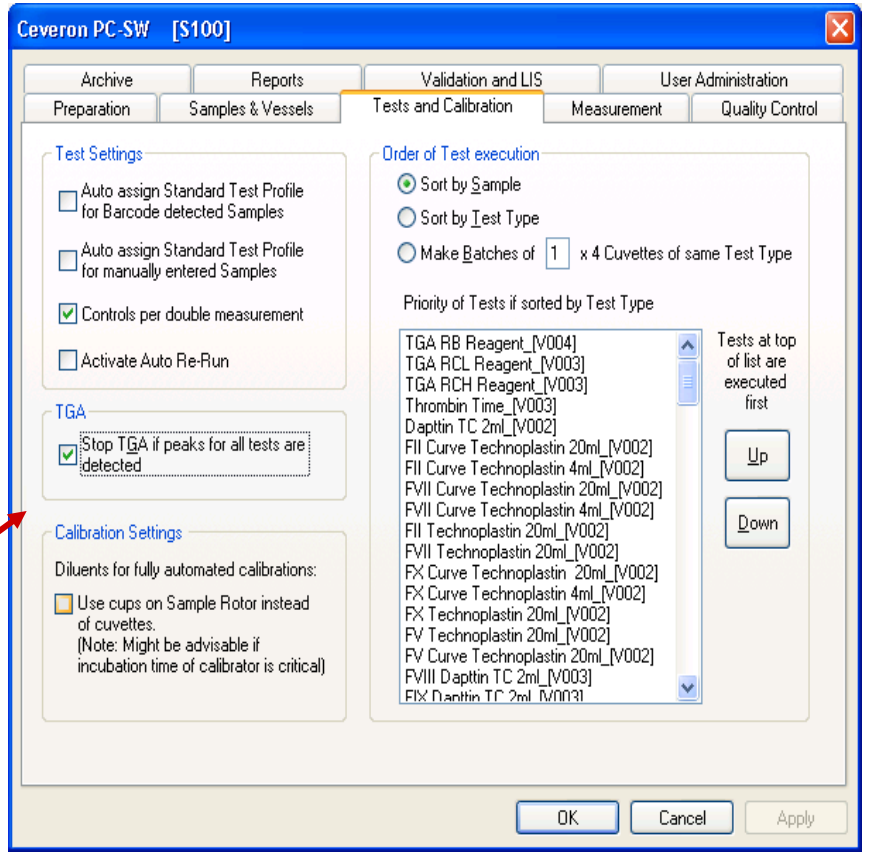


Reader/analyzer implication on thrombin generation measurement

UNIQUE: Measurement can be stopped after Peak Thrombin has been reached

ADVANTAGE:

> Shorter Assay time **20 min**



Reader/analyzer implication on thrombin generation measurement

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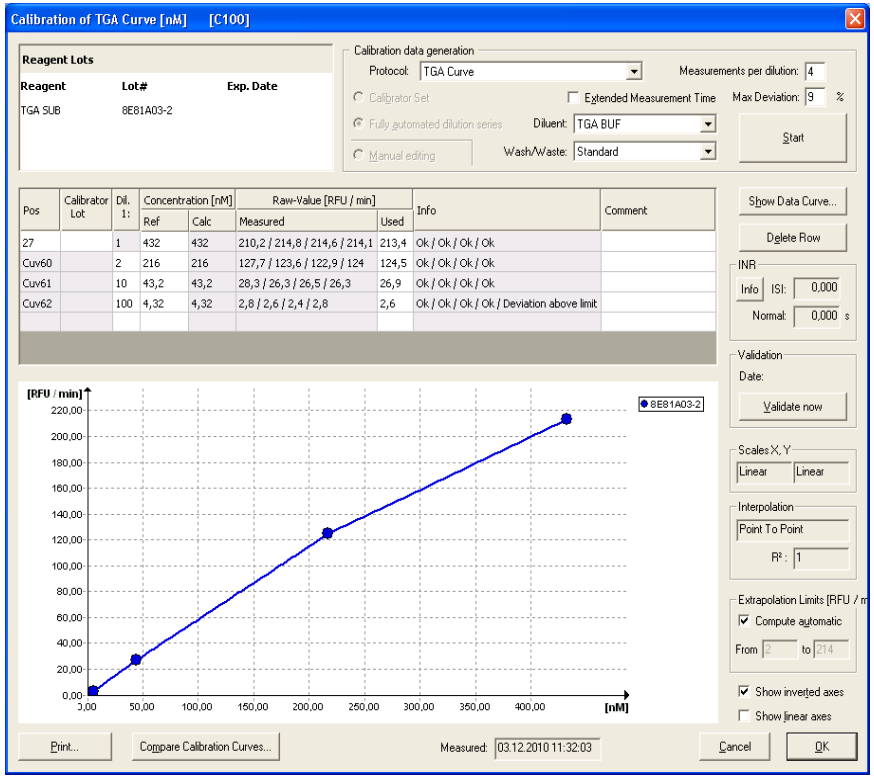
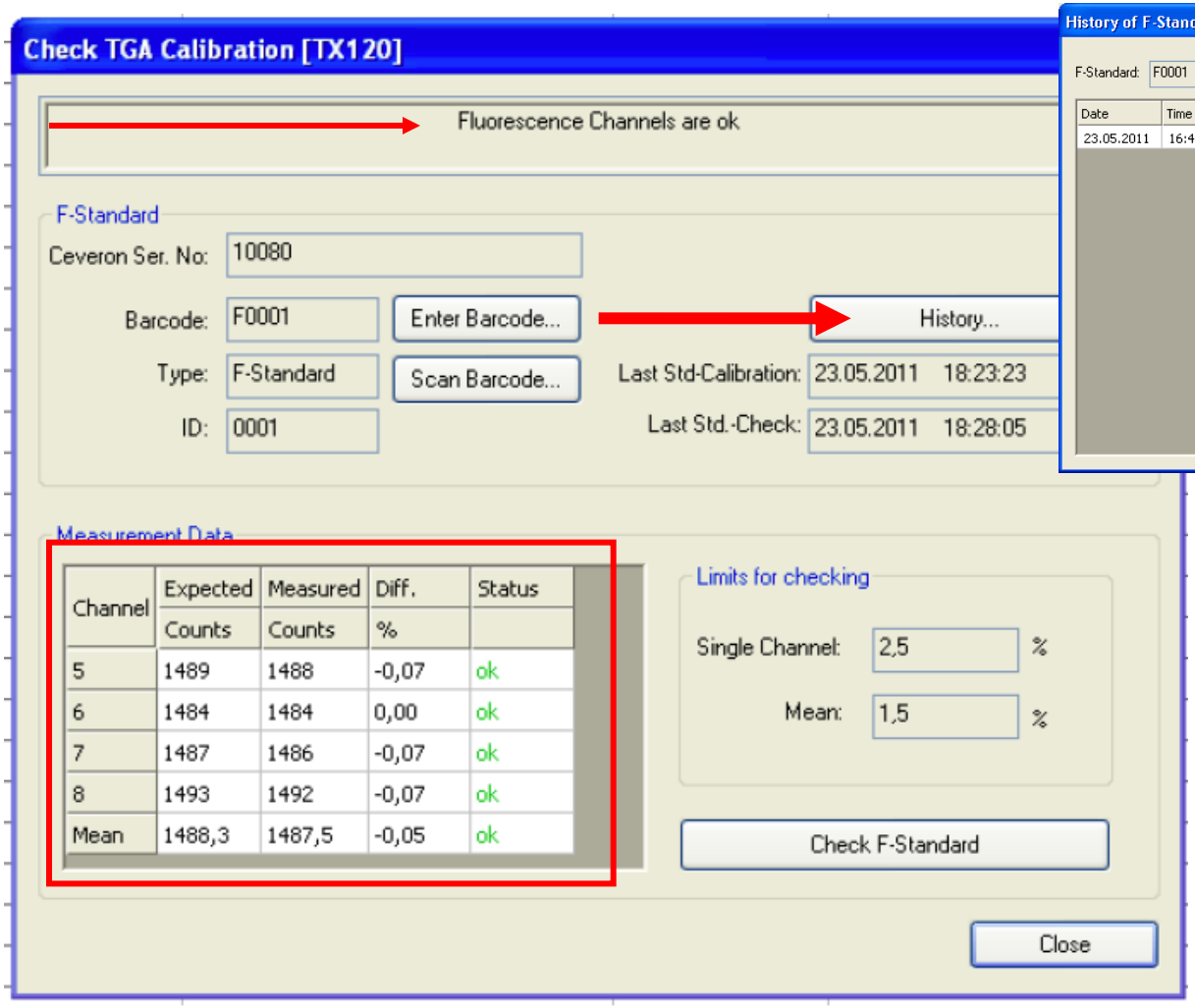
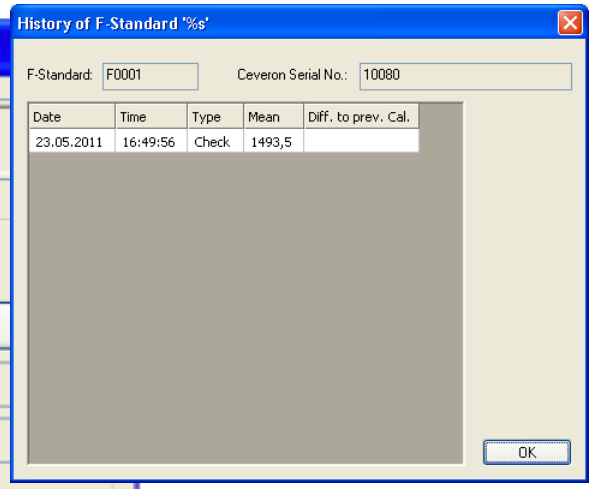


Reader/analyzer implication on thrombin generation measurement





F-Standard – for target value verification - barcoded