

Test and equipment evaluation a practical approach

ECAT

November 6th 2008

An Stroobants

Evolution of coagulation tests





Evaluation Centre

- Extensive evaluation in one laboratory avoids time consuming and costly evaluations in other laboratories

Evaluated



Evaluation of coagulation tests

- Analyser evaluation
- Reagent evaluation
- Clinical evaluation

Analyser evaluation



Analyser

General evaluation

- Properties of analyser, reagents, manufacturer
- Literature
- Experiences of other users

Analyser

Analytical evaluation

- Familiarisation
- Within assay variation
- Carry-over
- Capacity
- Imprecision

Capacity test

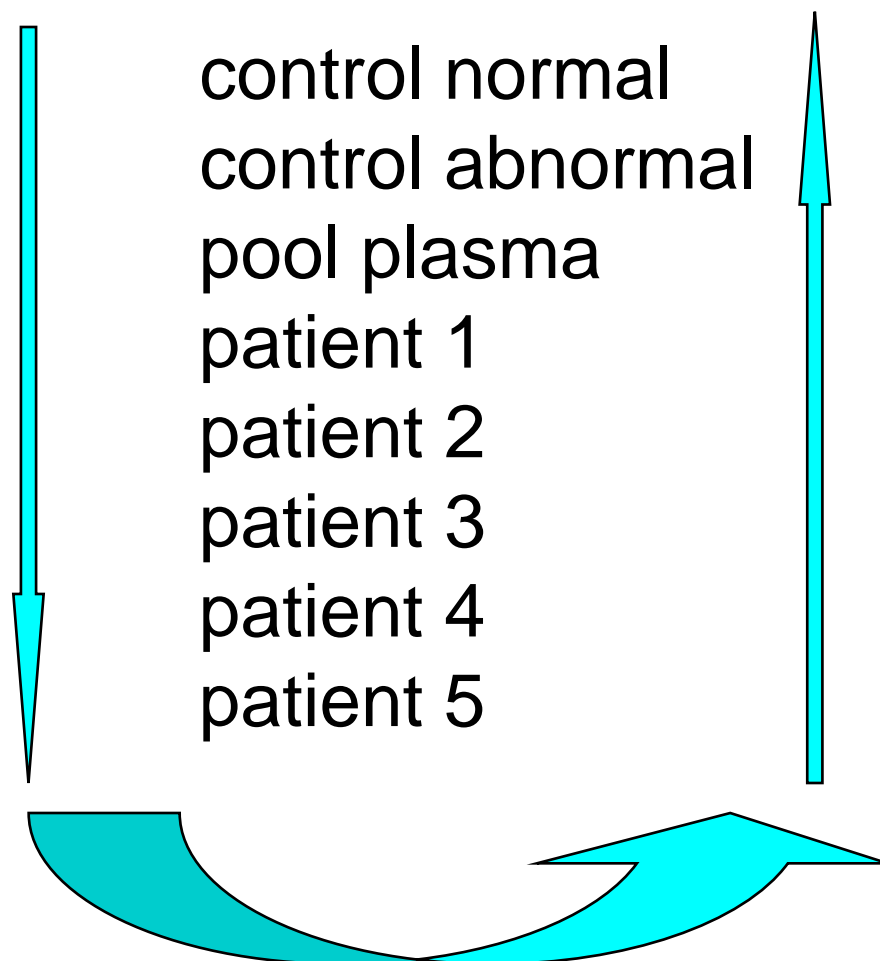
- 50 samples in duplicate:
APTT, PT, fibrinogen, AT, factor VII
- 40 routine (8 micro, 32 normal)
- 10 stat (2 micro, 8 normal)

Capacity test results

	CA-7000	ACL-TOP	STA-R Evolution
50 samples	2h 28'	3h 24'	3h 15'
1 STAT	10' 24"	23' 12"	9' 21"
5 STAT	27' 34"	43' 9"	29' 15"

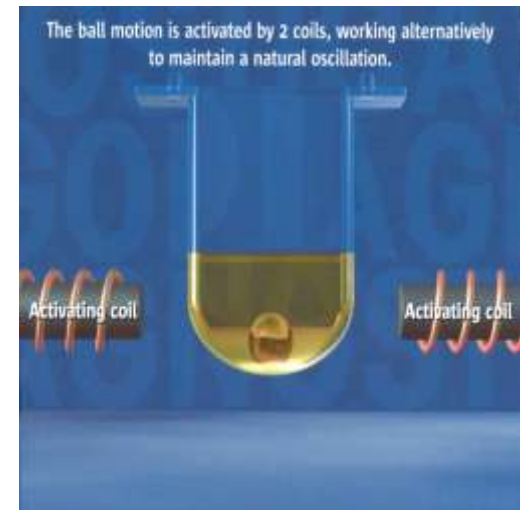
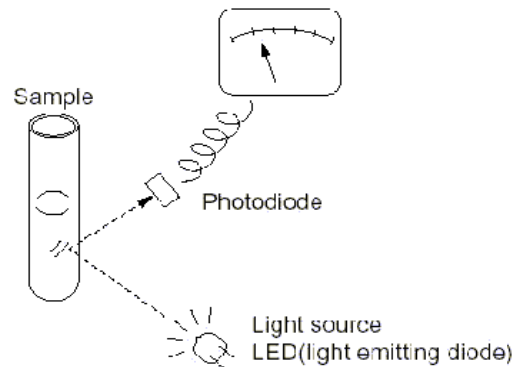
Imprecision test

2 runs during 10 days



Measuring principles

- Coagulation
- Chromogenic
- Immunochemical



Routine

APTT
PT/INR
Fibrinogen
AT
D-Dimer

Special

Factor VII
Factor VIIIc
Factor VIII chromogenic
LAC screen and confirm
Protein C activity
Protein S activity
vWF activity
vWF antigen
Anti-Xa
PCP

Materials

- Control materials manufacturer
- Pool healthy volunteers
- Fresh or frozen patient samples

Example imprecision

PT	Mean sec	Within run CV %	Total CV %
Control 1	14.4	1.0	1.3
Control 2	36.2	1.7	2.9
Pool	12.9	0.9	1.8
Factor VIII	Mean %	Within run CV %	Total CV %
Control 1	119	8.6	11.8
Control 2	37	12.7	14.3
Pool	129	5.6	7.6

Example improved method

Factor VIII	Mean %	CV %	CV %
Control 1	83	9.7	6.0
Control 2	47	11.5	7.8
Pool	100	9.8	5.7

Enhanced dispence of CaCl_2

Evaluation checklist

50 questions:

- Analytical performance
- Handling
- General properties
- Manufacturer
- Hardware
- Software
- Reagents



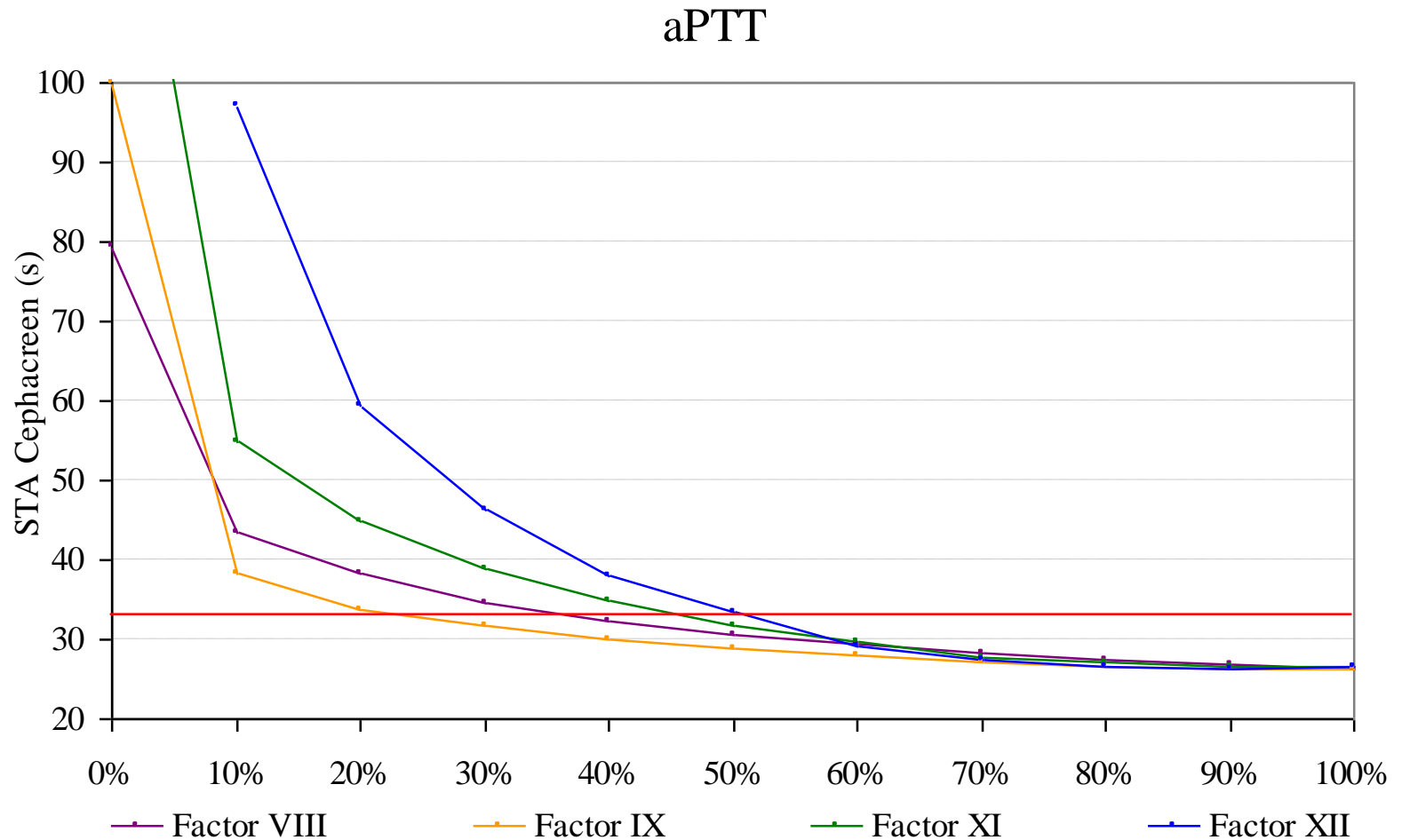
Reagent evaluation



Reagent evaluation

- Reference value
- Sensitivity to
 - factor deficiencies
 - therapeutic anticoagulants
 - vitamin K deficiency
 - lupus anticoagulant

Sensitivity to factor concentration

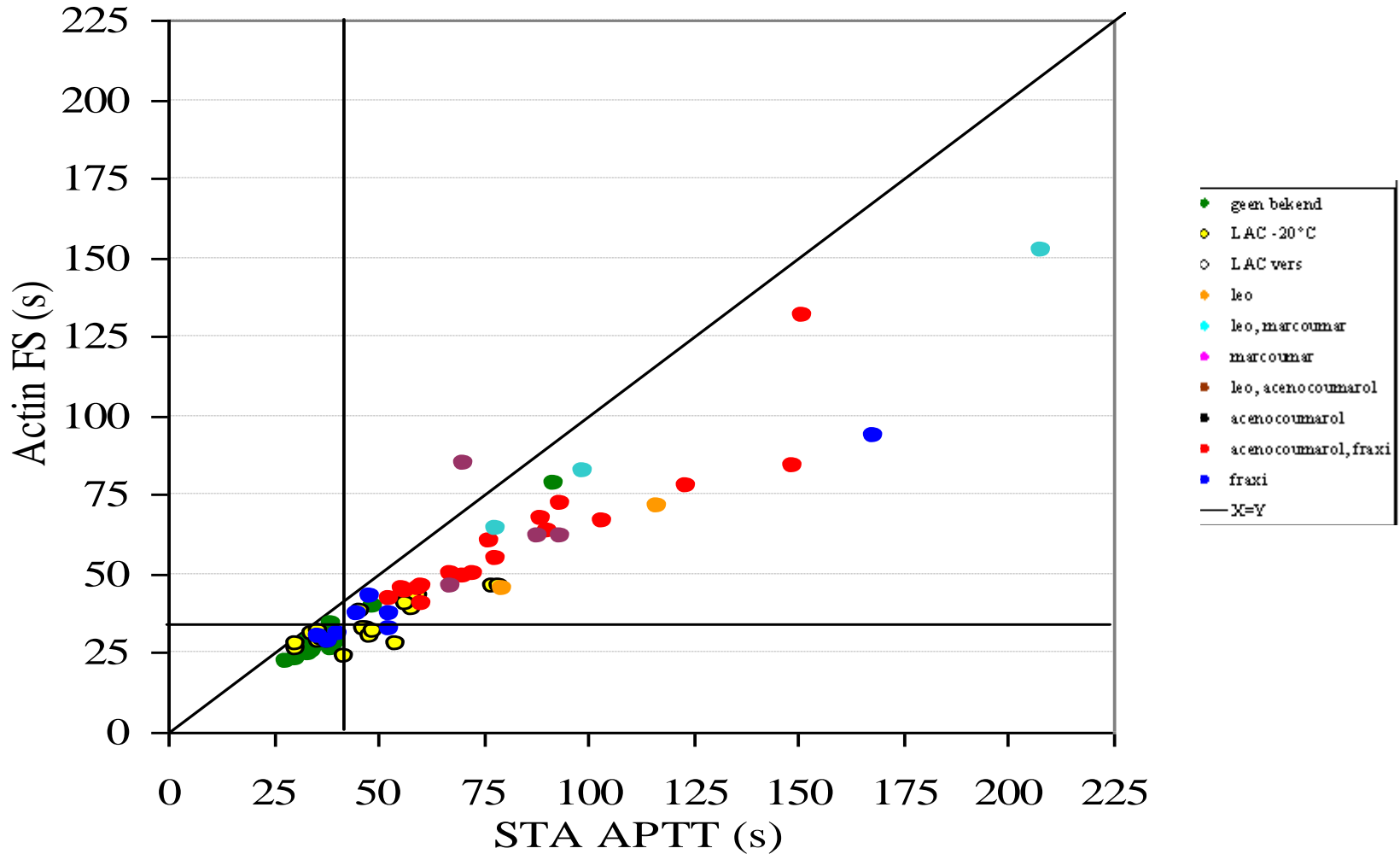


Sensitivity to factor concentrations

	STA APTT	STA Cephascreeen	Actin FS	SynthAsIL	SynthAfAX
F VIII	20	35	30	60	30
F IX	20	25	20	60	20
F XI	25	45	50	80	50
F XII	30	50	50	85	50

LAC and anticoagulant

aPTT



Reference values

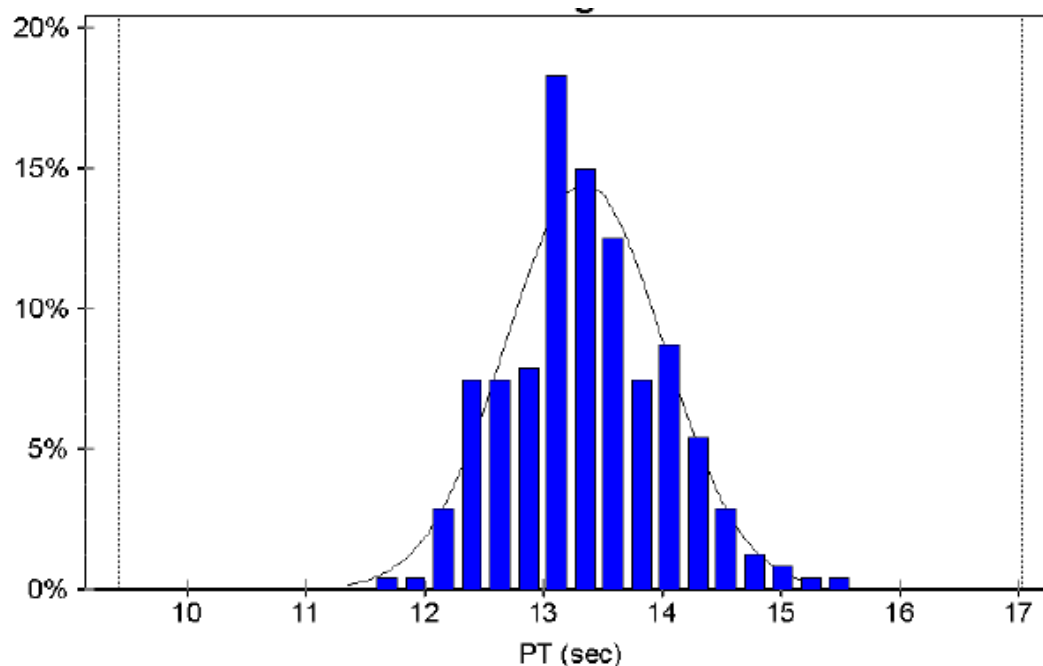
N: 240

Mean: 13.3 sec

SD: 0.6

min.: 11.7 sec

max.: 15.4 sec



Reference values: 12.1 - 14.7 sec.

APTT reagents

Reagent	STA APTT	STA Cephascreeen	Actin FS	Synthasil	Synthafax
Activator	Silica	ellagic acid	ellagic acid	colloidal silica	ellagic acid
Upper reference value (seconds)	42.0	33.0	33.0	38.0	30.4
Reproducibility (VC, 21 measurements)	< 1.1%	< 1.0%	< 0.9%	< 0.8%	< 1.1%
Factor deficiency detectable	< 20-30%	< 25-50%	< 20-50%	< 60-85%	< 20-50%
Unfractionated heparin sensitivity	very sensitive	sensitive	sensitive	sensitive	not very sensitive
LAC sensitivity	very sensitive	sensitive	not very sensitive	not very sensitive	sensitive
User friendliness	- 30 min. preparation 5 mL bottles 24 h expiry	+ ready to use 10 mL bottles 240 h expiry	+ ready to use 10 mL bottles 168 h expiry	+ ready to use 10 mL bottles 240 h expiry	+ ready to use 10 mL bottles 120 h expiry

Correct concentration?

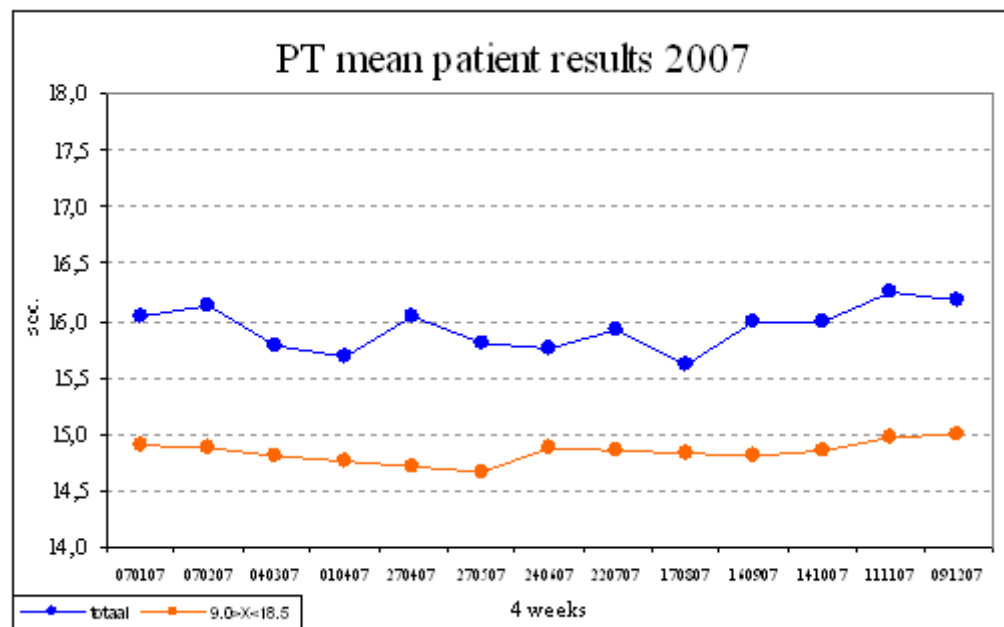
- Comparison with former method
- Internal QC
- External QC (ECAT, SKML)
- WHO standard

- Mean of patient results
- Batch to batch comparison of reagents

Mean of patient results

Every 28 days

- aPTT 20 – 240 s
- aPTT 20 – 80 s
- PT 9 – 40 s
- PT 9 – 18.5 s



Batch to batch comparison

- Masterbatch: 42 sec
- Next batch daily production with both reagents
- $Y = aX \pm b$

Date	Upper limit of normal (s)
May 03	42.0
Feb 04	39.8 -> 42.0
Dec 04	41.3
Aug 05	40.0
Apr 06	40.0
Nov 06	40.1
May 07	40.4
Sep 07	40.9

Clinical evaluation



Clinicians desire

- Reliable results
- Clinical value
- Fixed reference values
- Fixed clinical decision limit
- Fixed therapeutic ranges
- Known sensitivities reagents

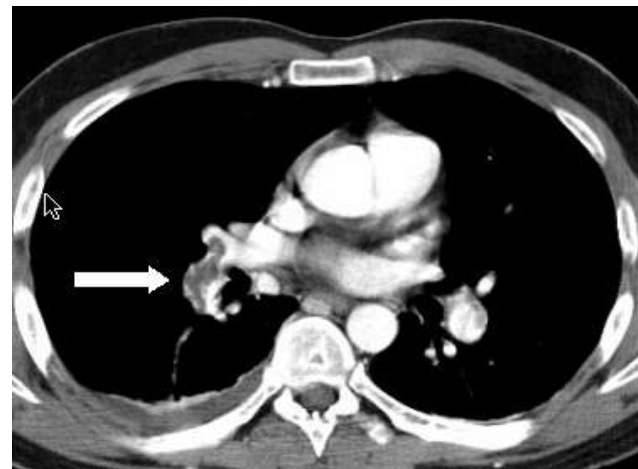


Fixed clinical decision limit

	Max CV %	Decision limit
D-dimer Plus	14.7	0.15 mg/L
Innovance D-dimer	3.4	0.5 mg/L

Clinical evaluation of D-dimer test

Gold standard available
for DVT and PE



D-dimer test

	Scan positive	Scan negative
D-dimer > 0.5 mg/L	TP	FP
D-dimer \leq 0.5 mg/L	FN	TN

sensitivity	$TP / (TP + FN)$	99.4
specificity	$TN / (FP + TN)$	38.3
NPV	$TN / (FN + TN)$	99.5

How to evaluate other assays?

- Prolongation aPTT/PT in case of factor deficiency?
- Prolongation aPTT heparin treatment-> aPTT versus anti-Xa
- Clinical awareness

Conclusions

- Perform correct tests with correct material
- Establish properties of analyser + reagents
- Check results when in use
- Communicate with clinicians

Thanks!

- Siemens Healthcare solutions
- Roche Diagnostics/Stago
- Instrumentation Laboratory

