Monitoring anti-IIa

Inhibitors

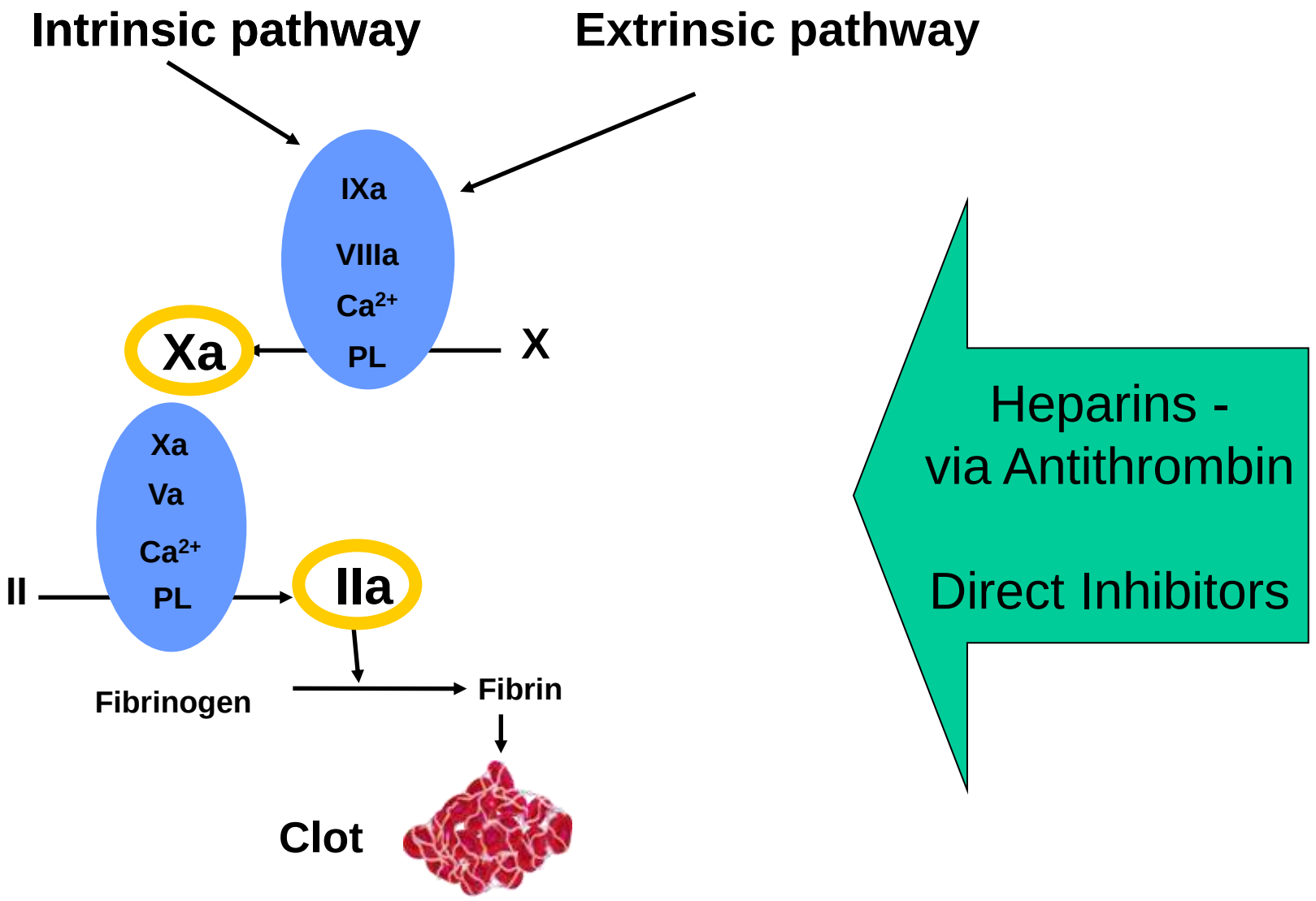
how to monitor a plurality of substances?

specific anti-Xa and anti-IIa methods in every lab

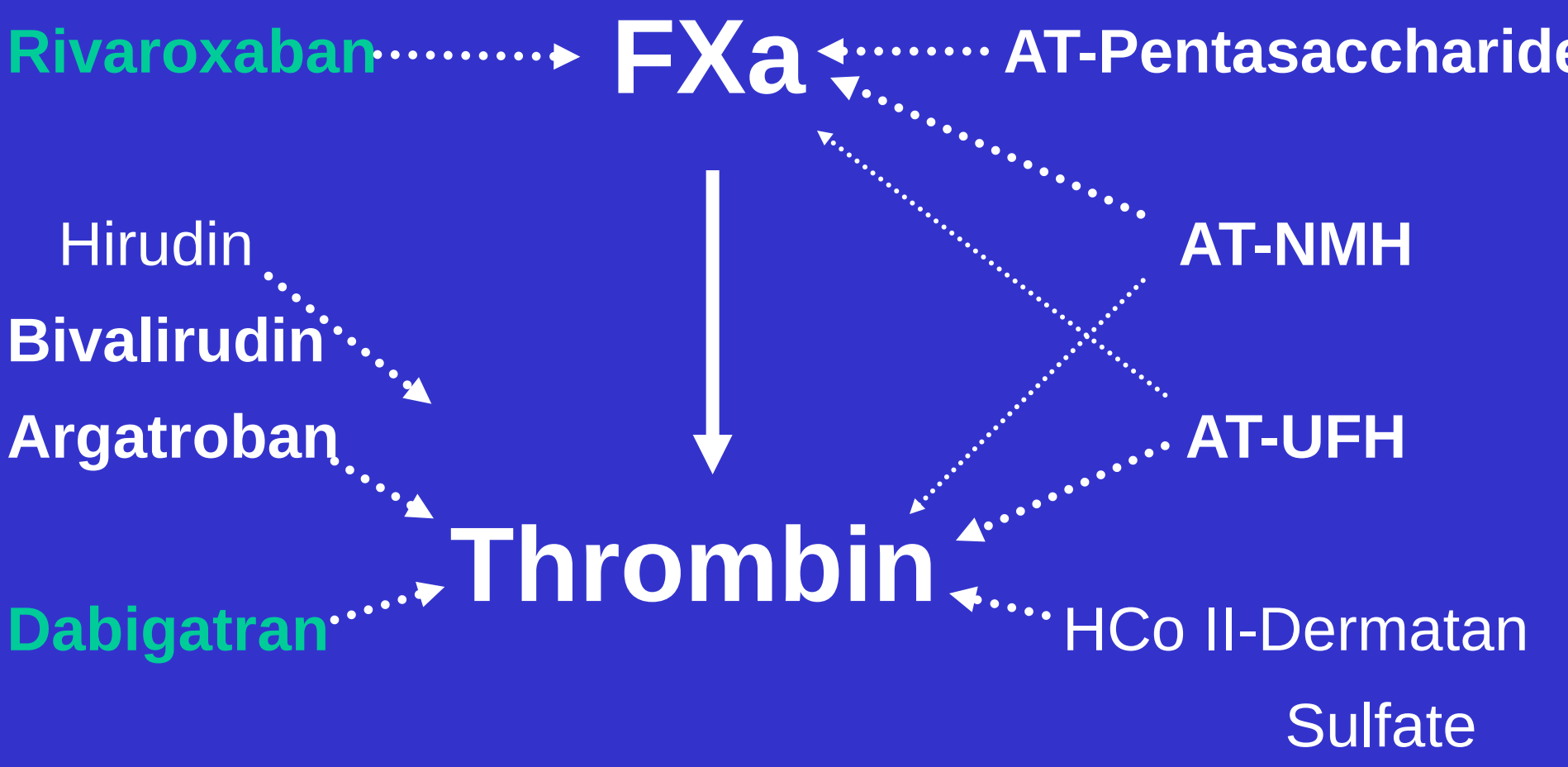
in addition to global clotting tests?

michael spannagl

LMU muenchen


Intrinsic pathway
Extrinsic pathway
IXa
VIIIa
Ca2+
PL
Xa
X
Xa
Va
Ca2+
PL
II
IIa
Fibrinogen
Fibrin
Clot
Heparins -
via Antithrombin
Direct Inhibitors

direct **anticoagulants** indirect



the good news:

routine monitoring required:

only

COUMADIN®
(Warfarin Sodium Tablets, USP) Crystalline

all the other substances require monitoring only in **special** situations

Monitoring Anticoagulants

Routine

Special Situations

RARE??

COUMADIN®
(Warfarin Sodium Tablets, USP) Crystalline

Only INR
Standardized by International Consensus

Monitoring LMWH and Thrombin Inhibitors

THE BAD NEWS: There are many special situations

Routine

Compliance ?

Bleeding Copmlpcations during Anticoagulation

Comedication: Antiplatelets…

PREGNANCY?

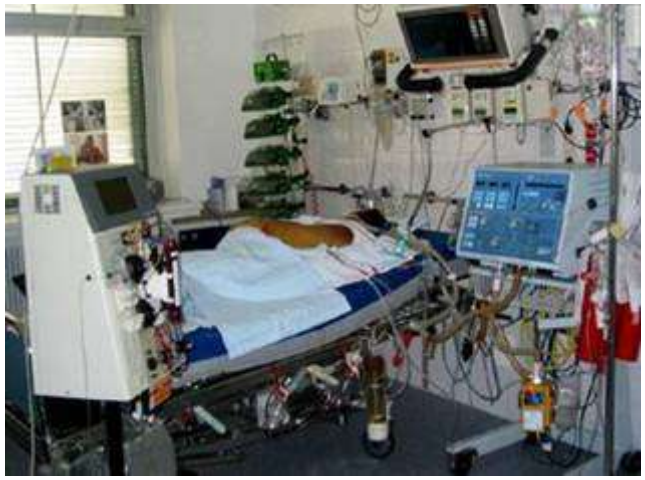
Children

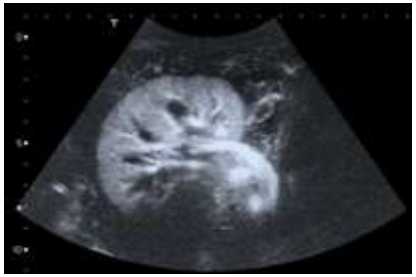
Comorbidity: Hepatology, Hematology …

Alter

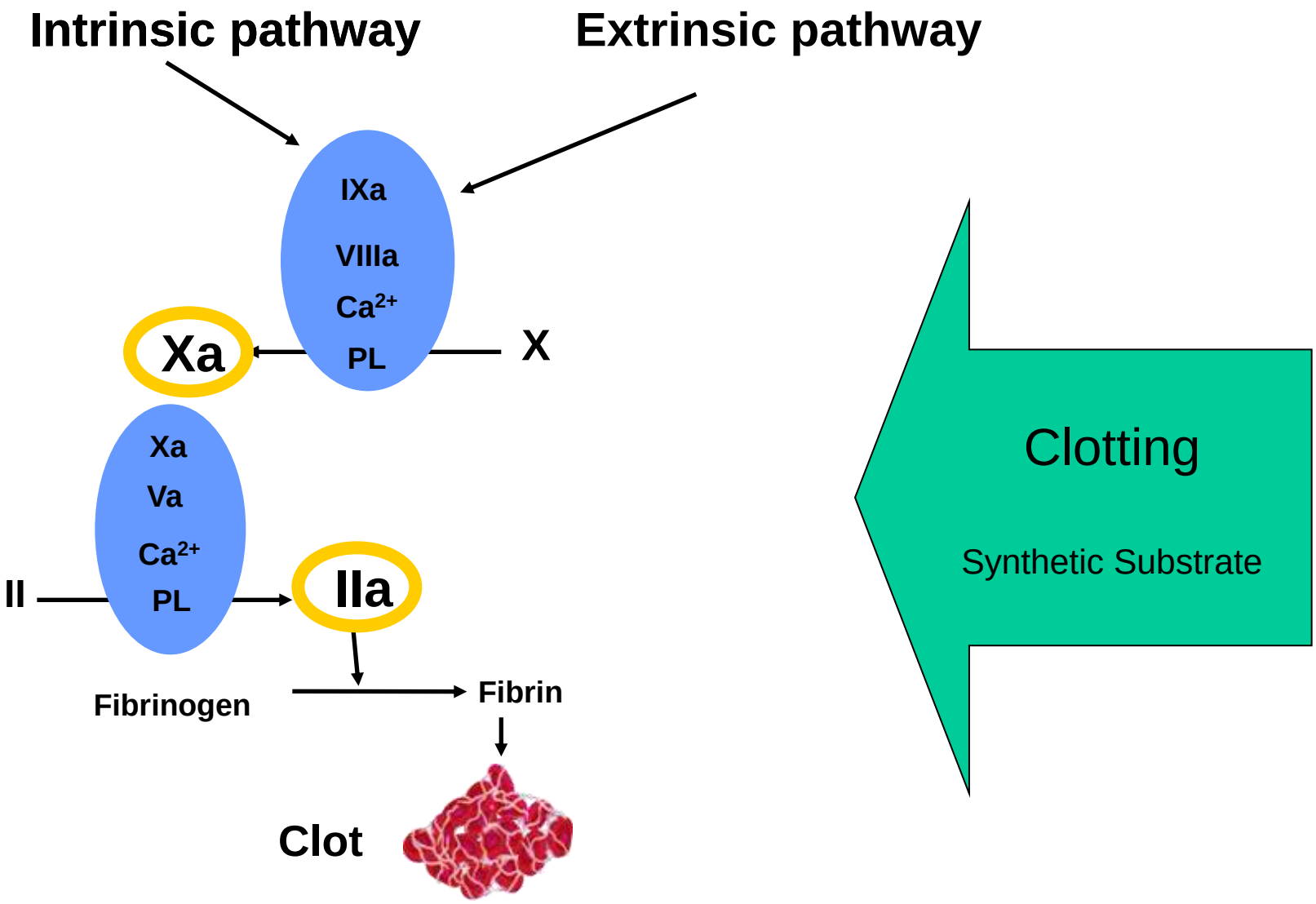
? Accumulation ?

Intensive Care





Body Weight


Intrinsic pathway
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IXa
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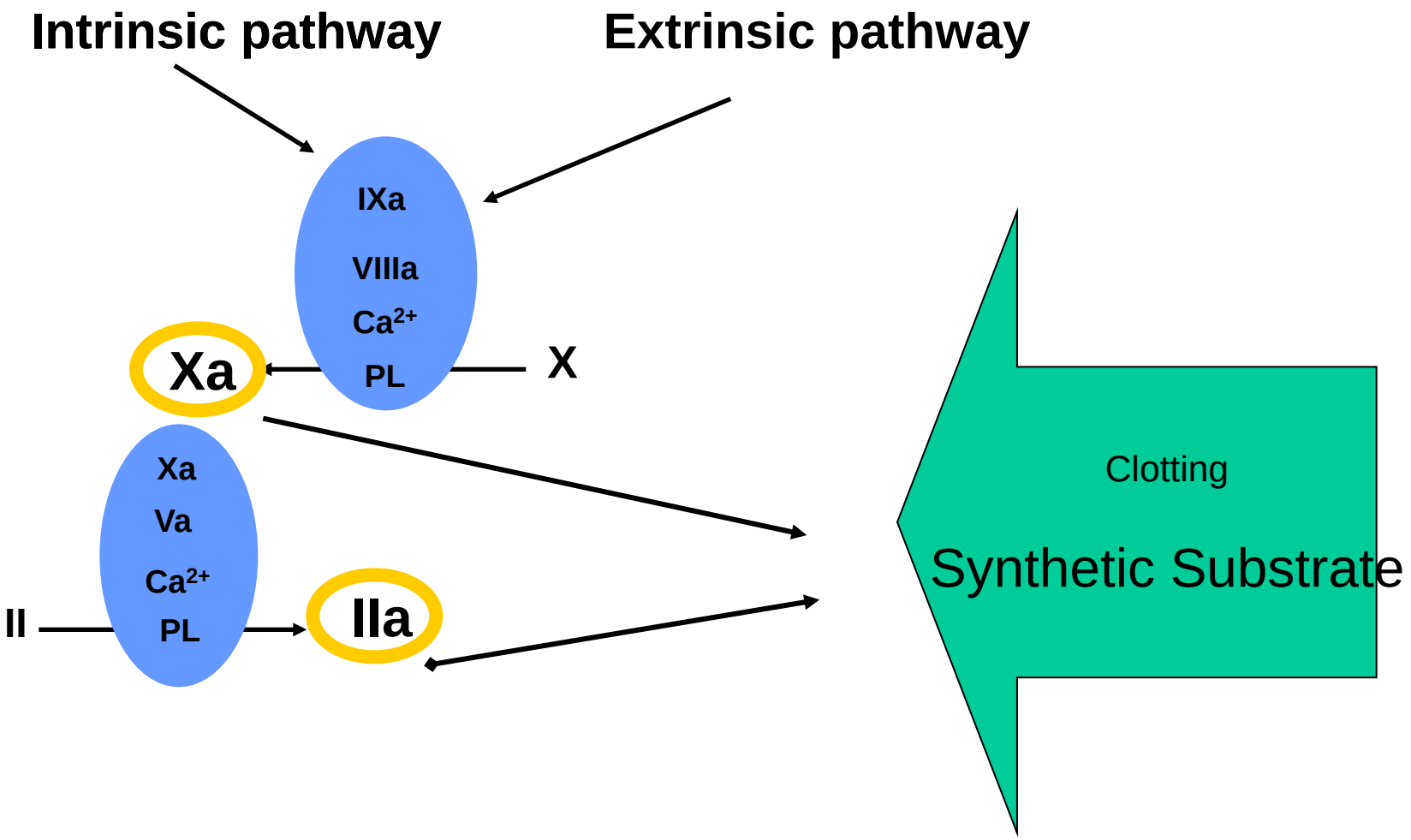
Ca2+
PL
Xa
X
Xa
Va
Ca2+
PL
II
IIa
Fibrinogen
Fibrin
Clot
Clotting
Synthetic Substrate


Intrinsic pathway
Extrinsic pathway
IXa
VIIIa
Ca2+
PL
Xa
X
Xa
Va
Ca2+
PL
II
IIa
Clotting
Synthetic Substrate

Introduction: aPTT: physiological basis of the monitoring of anticoagulants

contact activator

standardization

not part of hemostasic process

FXII, prekallikrein, kininogen

FXIa

phospholipids - Ca^{++} - FIXa -FVIIIa

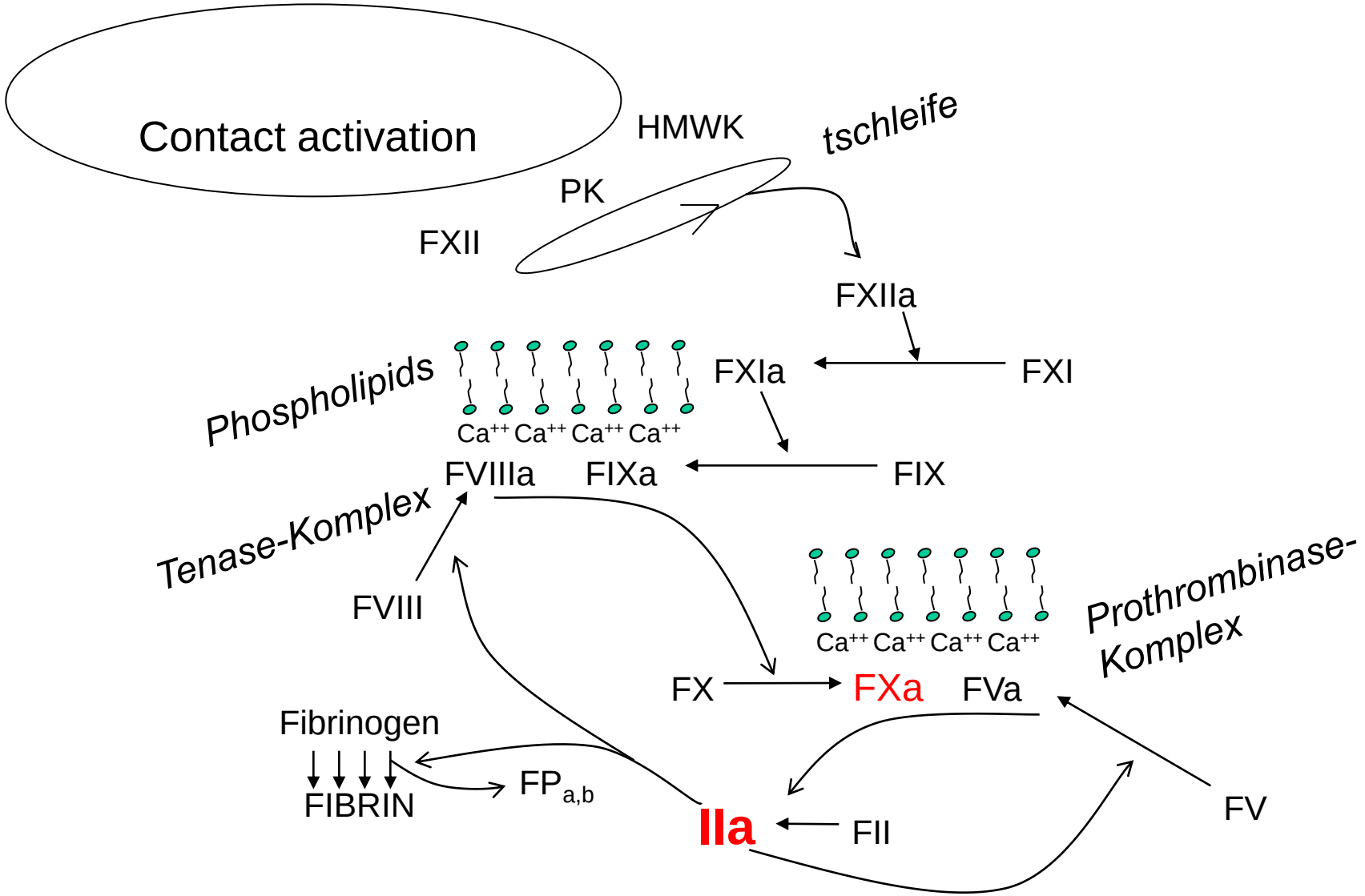
acute phase reaction

phospholipids - Ca^{++} - **FXa** - FVa

FIIa ☒ fibrin ☒ detection of clotting

main action of direct / indirect anticoagulants

aPTT in concert

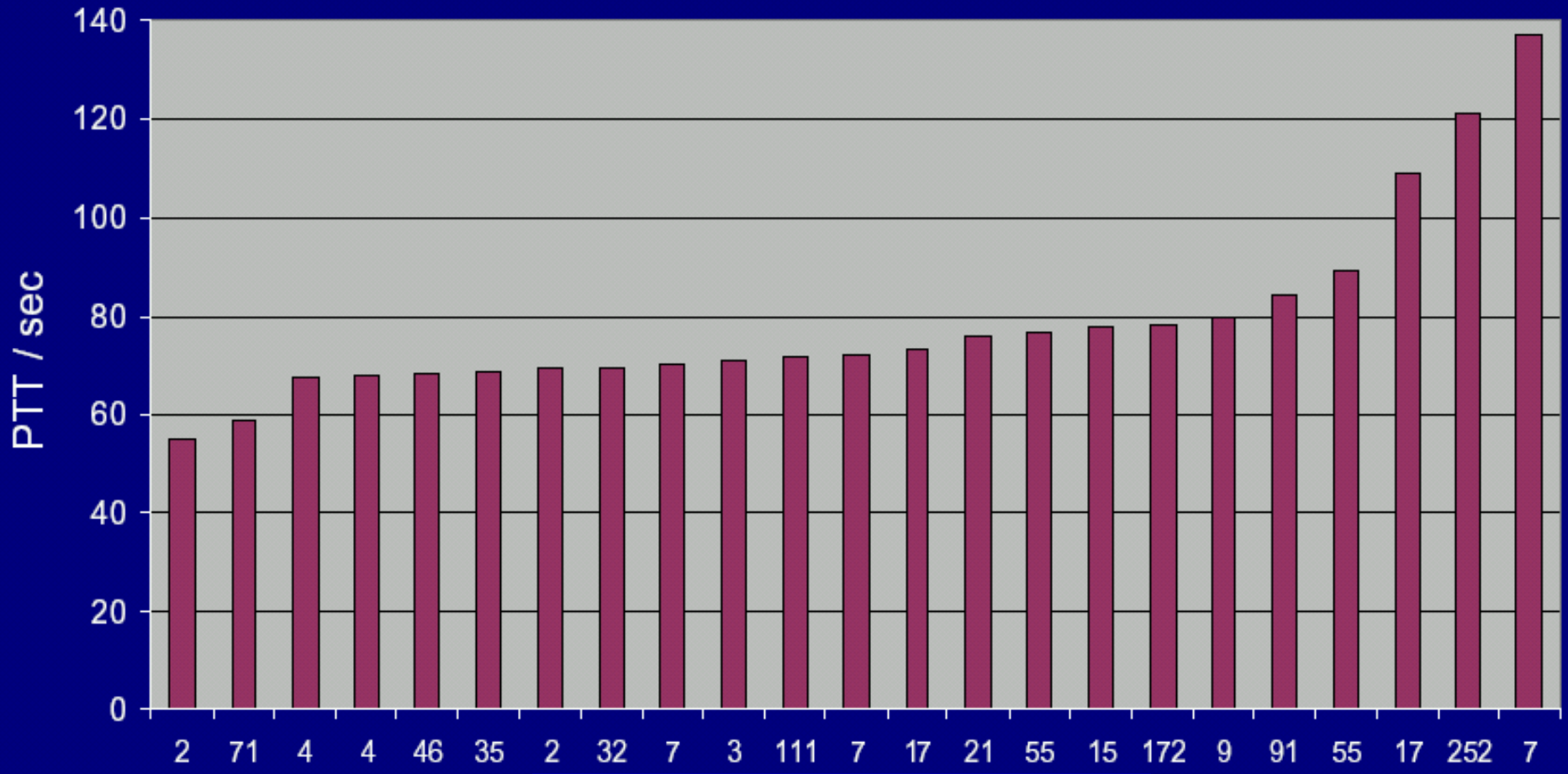


aPTT: standardization: literature

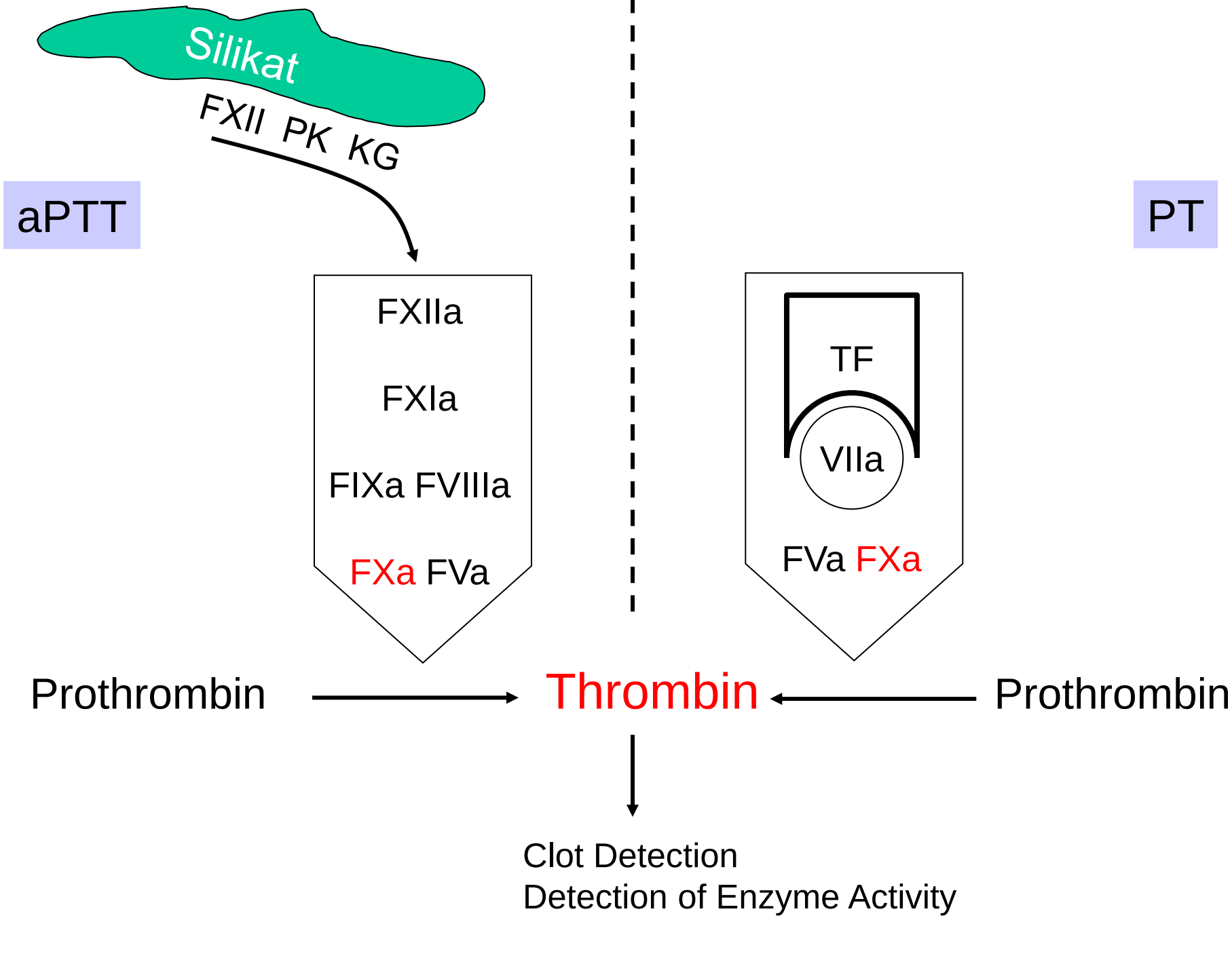
- poor standardization between different reagents:
- attempts to enhance standardization using strategies comparable to the INR / ISI systems for the PT have failed:
- poor standardization even between different lots of the same reagent
- roughly the same situation with ACT

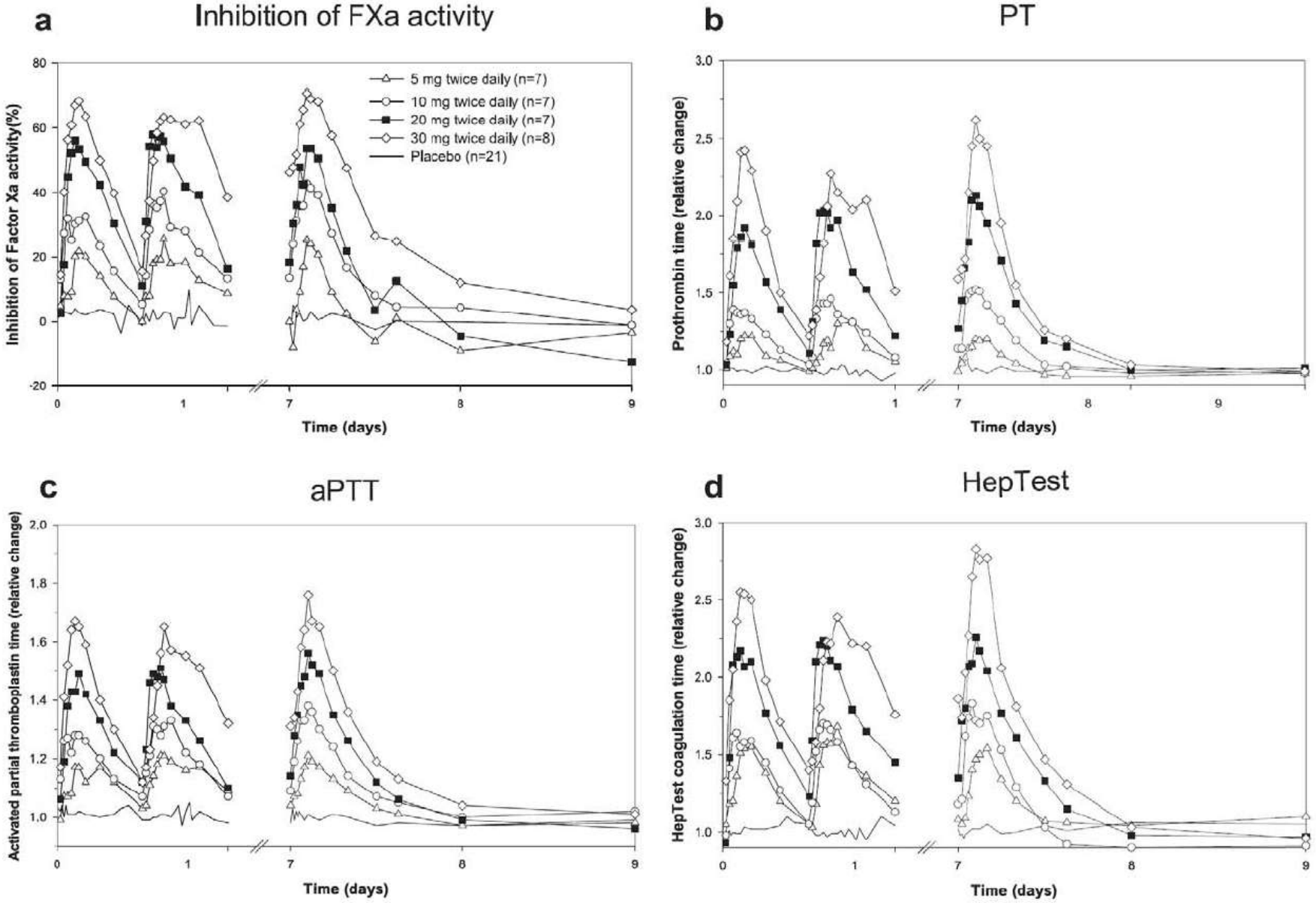
MEDIAN / REAGENT

0,8 U/ml UFH in normal plasma



Dr. Spannagl


Silikat
FXII PK KG
aPTT
PT
FXIIa
FXIa
FIXa FVIIIa
FXa FVa
TF
VIIa
FVa FXa
Prothrombin
Thrombin
Prothrombin
Clot Detection
Detection of Enzyme Activity

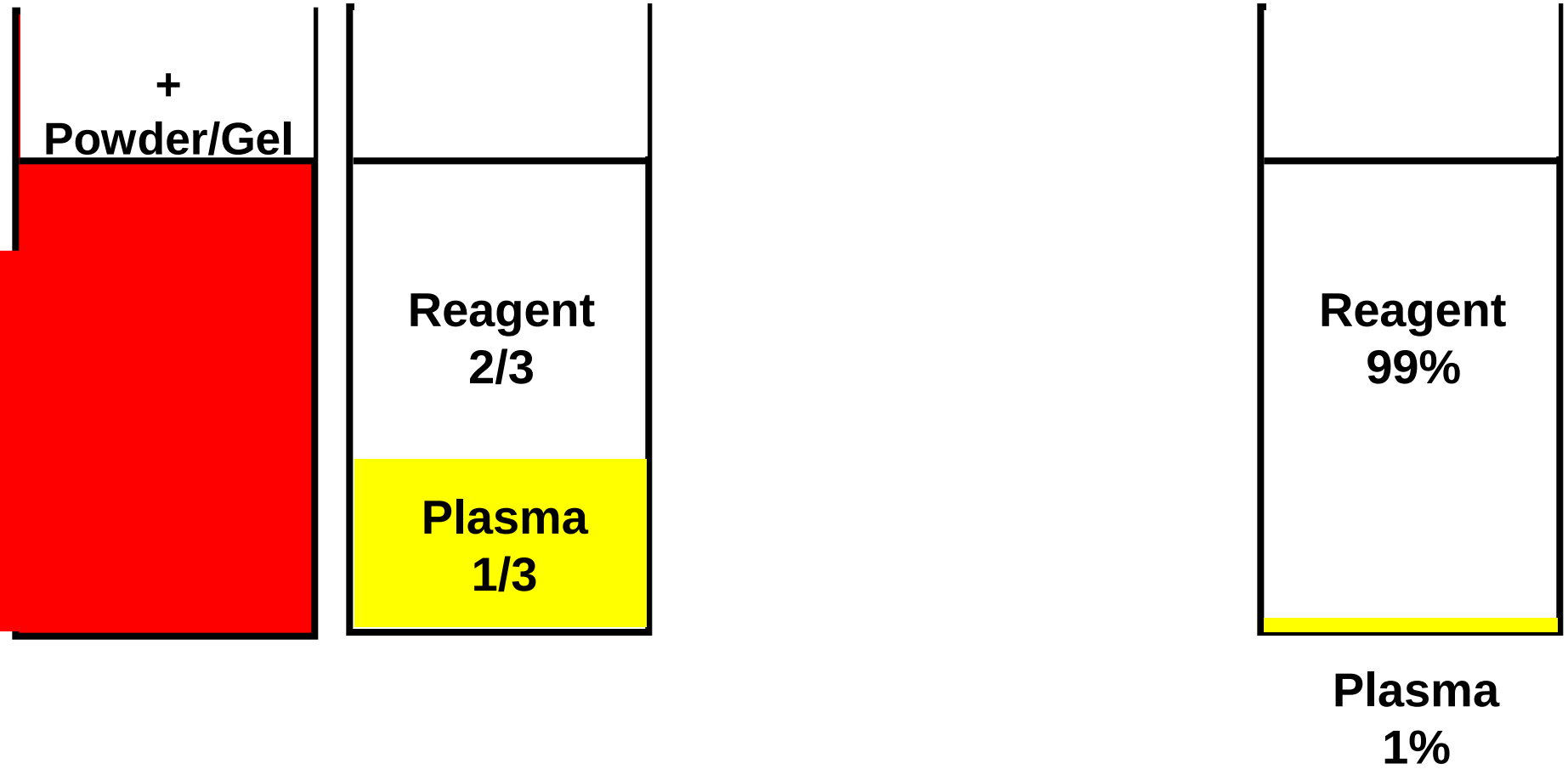

a
Inhibition of FXa activity
Inhibition of Factor Xa activity(%)
5 mg twice daily (n=7)
10 mg twice daily (n=7)
20 mg twice daily (n=7)
30 mg twice daily (n=8)
Placebo (n=21)
Time (days)
b
PT
Prothrombin time (relative change)
Time (days)
c
aPTT
Activated partial thromboplastin time (relative change)
Time (days)
d
HepTest
HepTest coagulation time (relative change)
Time (days)

PREANALYTICS - MATRIX

Whole Blood: ACT

aPTT

Anti- IIa/Xa Inhib.: Synth. Substrate



Introduction: Monitoring of anticoagulants: ***requirements***

- analysis in emergency laboratory with short turn-around-time
- high standardization between centers for applying the experience of studies

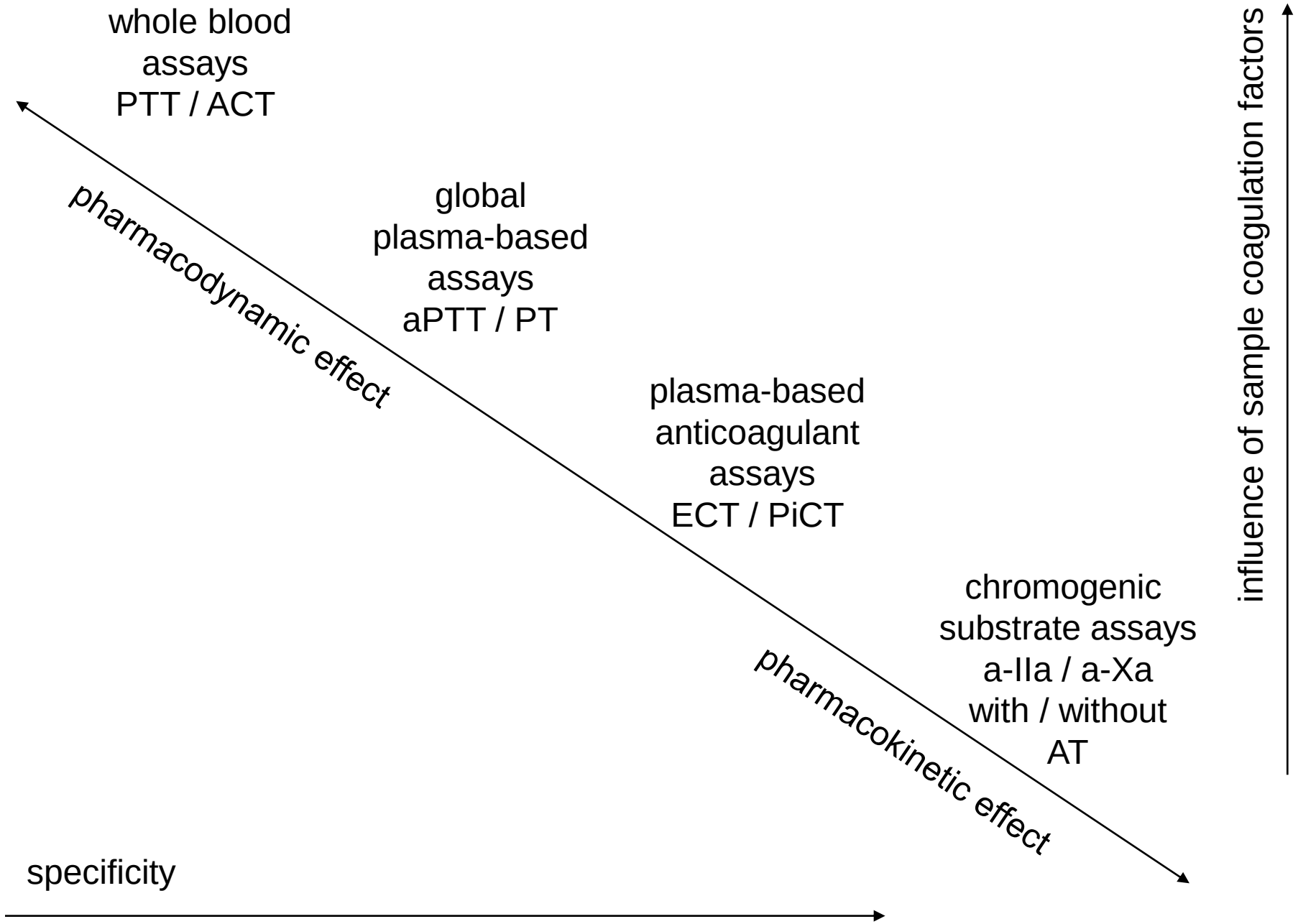
Monitoring of anticoagulation during invasive procedures:

- either POC analysis
- or reliable and fast logistics for emergency laboratory analysis (transport-analysis-communication)

Hemochron® Cuvette Tests

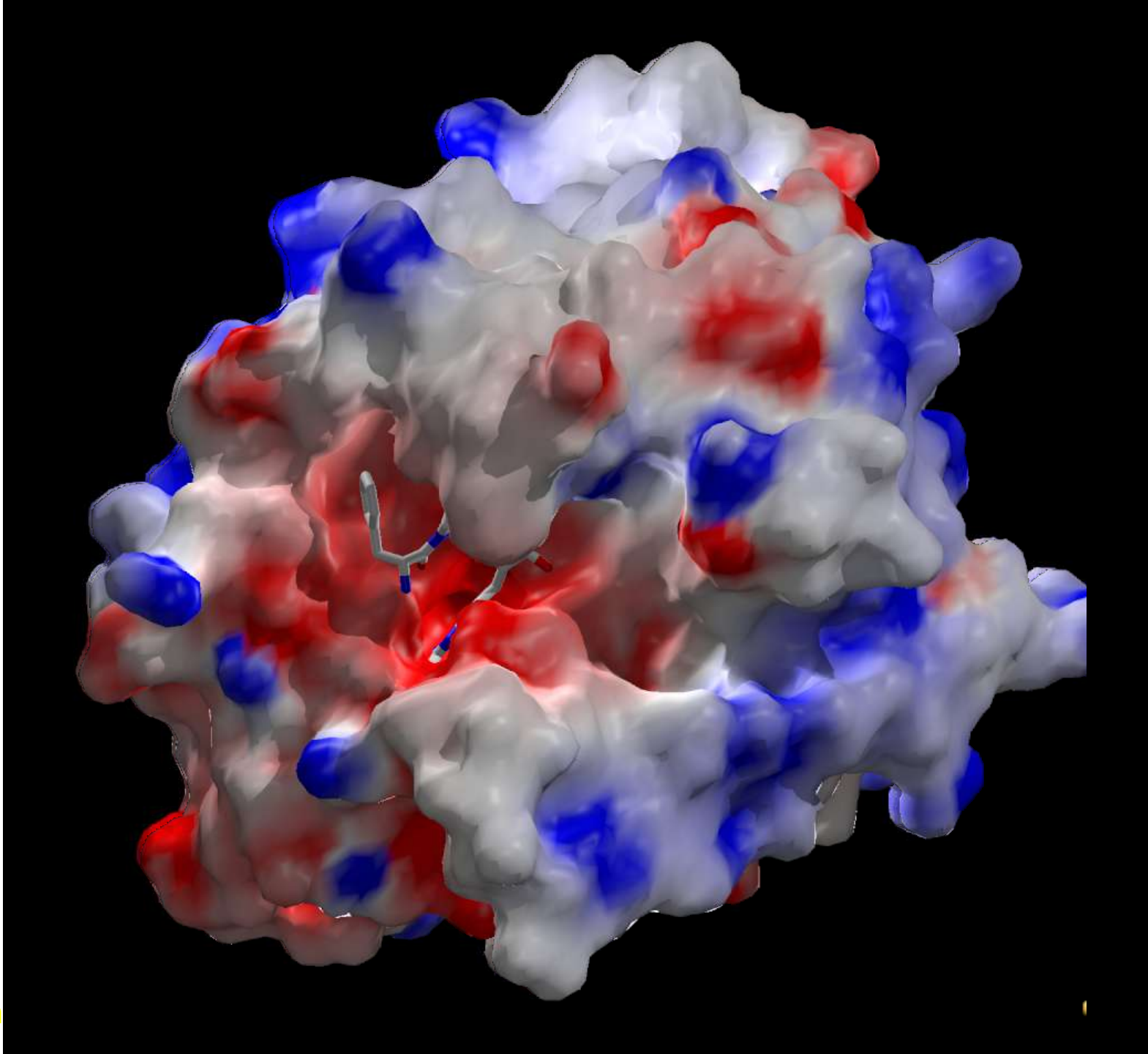
THE **POINT** OF CARE

- ACT+
- ACT-LR
- APTT
- Citrate APTT
- PT
- Citrate PT
- Anti Xa


whole blood
assays
PTT / ACT
global
plasma-based
assays
aPTT / PT
plasma-based
anticoagulant
assays
ECT / PiCT
chromogenic
substrate assays
a-IIa / a-Xa
with / without
AT
pharmacodynamic effect
pharmacokinetic effect
influence of sample coagulation factors
specificity

Bode, Mayr, Baumann, Huber, Stone, Hofsteenge (1989) EMBO J. 8, 3467 - 3475.

Bode, Turk & Karshikov (1992) Prot. Science 1, 426-471



PPACK-thrombin

1: Blood Coagul Fibrinolysis. 1991 Feb;2(1):121-7.

Development of a chromogenic substrate assay for the determination of hirudin in plasma.

Spannagl M, Bichler J, Birg A, Lill H, Schramm W.

Department of Medicine, Ludwig-Maximilians-University, Munich, Germany.

Hirudin is a potent and specific thrombin inhibitor. Since recombinant hirudin is being considered for anticoagulant and antithrombotic therapy we developed a fast and sensitive chromogenic substrate assay for its determination in plasma. The plasma samples (20 microliters) were incubated with 1 ml reagent mixture (0.2 M Tris buffer, 0.025 M NaCl, pH 8.1, containing 0.833 M urea, 0.7 trypsin inhibitor U/ml aprotinin, 100 ng/ml Polybrene and 0.31 NIH U/ml bovine thrombin) for 1 min. Thereafter 100 microliters Chromozym TH (Tos-Gly-Pro-Arg-pNA, 1.9 mM) was added. The change in absorbance/min (delta A/min) was recorded at 405 nm. delta A/min was linear for at least 3 min. The calibration curve was linear at least up to 800 ng hirudin/ml plasma. Intra-assay and inter-assay coefficients of variation were 2.8-3.1% and 5.3-5.8% respectively. The influence of progressive thrombin inhibitors can be neglected because of the short incubation time. Plasma samples can be assayed directly if aprotinin, polybrene and urea are added to the reagent mixture.

PMID: 1772979 [PubMed - indexed for MEDLINE]

Display AbstractPlus Show 20 Sort By Send to

Now we have polymerisation inhibitors!

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Question remains: +/- Antithrombin
Complexed therapeutic agents may be dissolved under in vitro conditions ?

Pergamon

Thrombosis Research 100 (2000) 55–60

THROMBOSIS RESEARCH

ORIGINAL ARTICLE

Hirudin Determination in Plasma Can Be Strongly Influenced by the Prothrombin Level☆

Edelgard Lindhoff-Last[1], Gerd Paul Piechottka[2], Fritz Rabe[2] and Rupert Bauersachs[1]

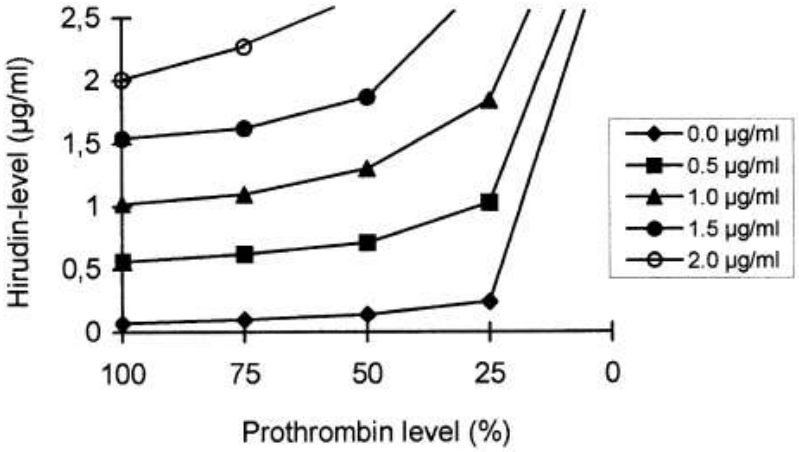

Fig. 1. Influence of decreasing prothrombin levels on hirudin-determination in vitro using the ecarin clotting time (ECT); mean of $n=4$ measurements.

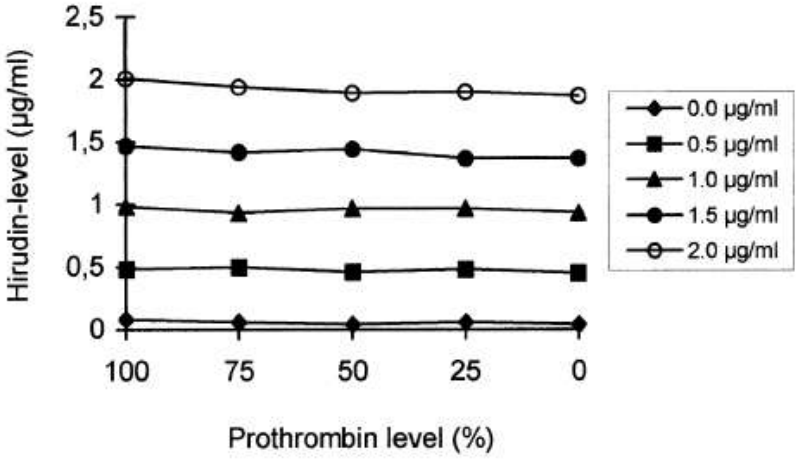

Fig. 2. Influence of decreasing prothrombin levels on hirudin determination in vitro using the chromogenic substrate assay (CSA); mean of $n=4$ measurements.

Pergamon

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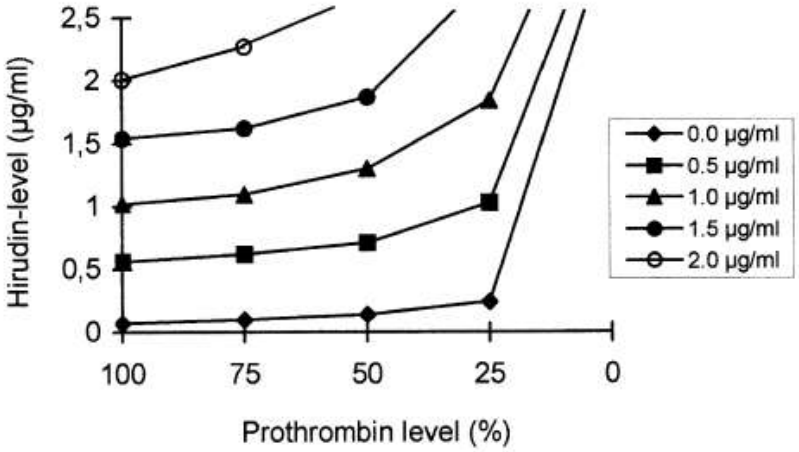

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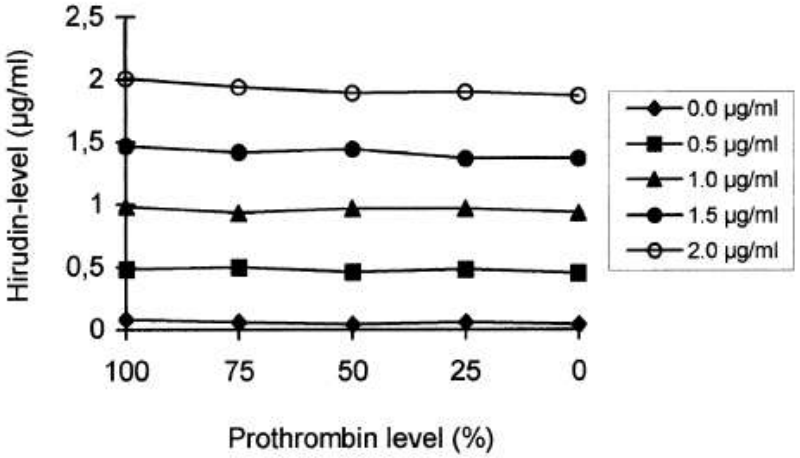


Fig. 2. Influence of decreasing prothrombin levels on hirudin determination in vitro using the chromogenic substrate assay (CSA); mean of $n=4$ measurements.

PIVKA Influence?

Ex vivo samples:

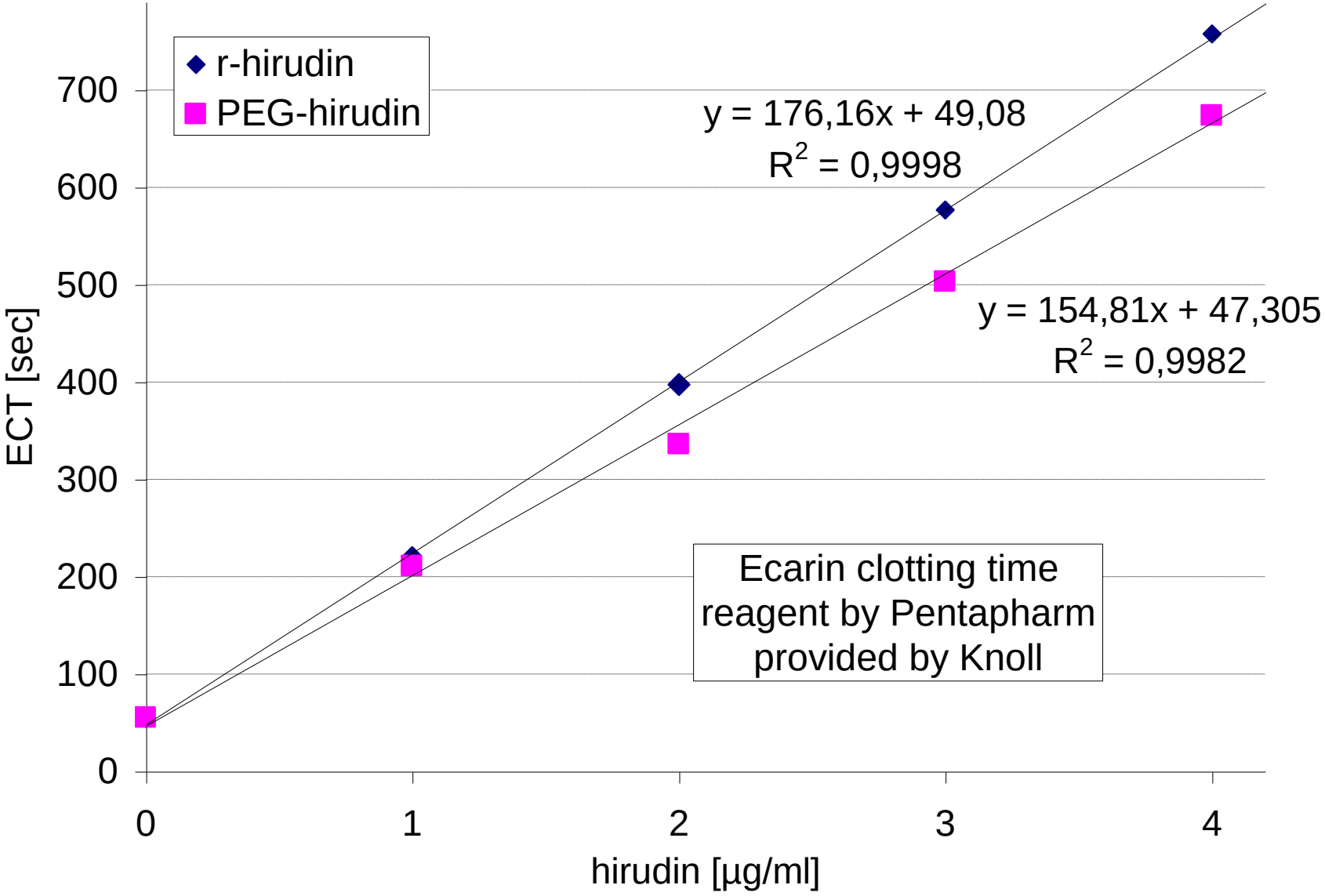
Samples from 36 volunteers receiving i.v.
r-hirudin (Lepirudin) or PEG-hirudin
bolus + infusion

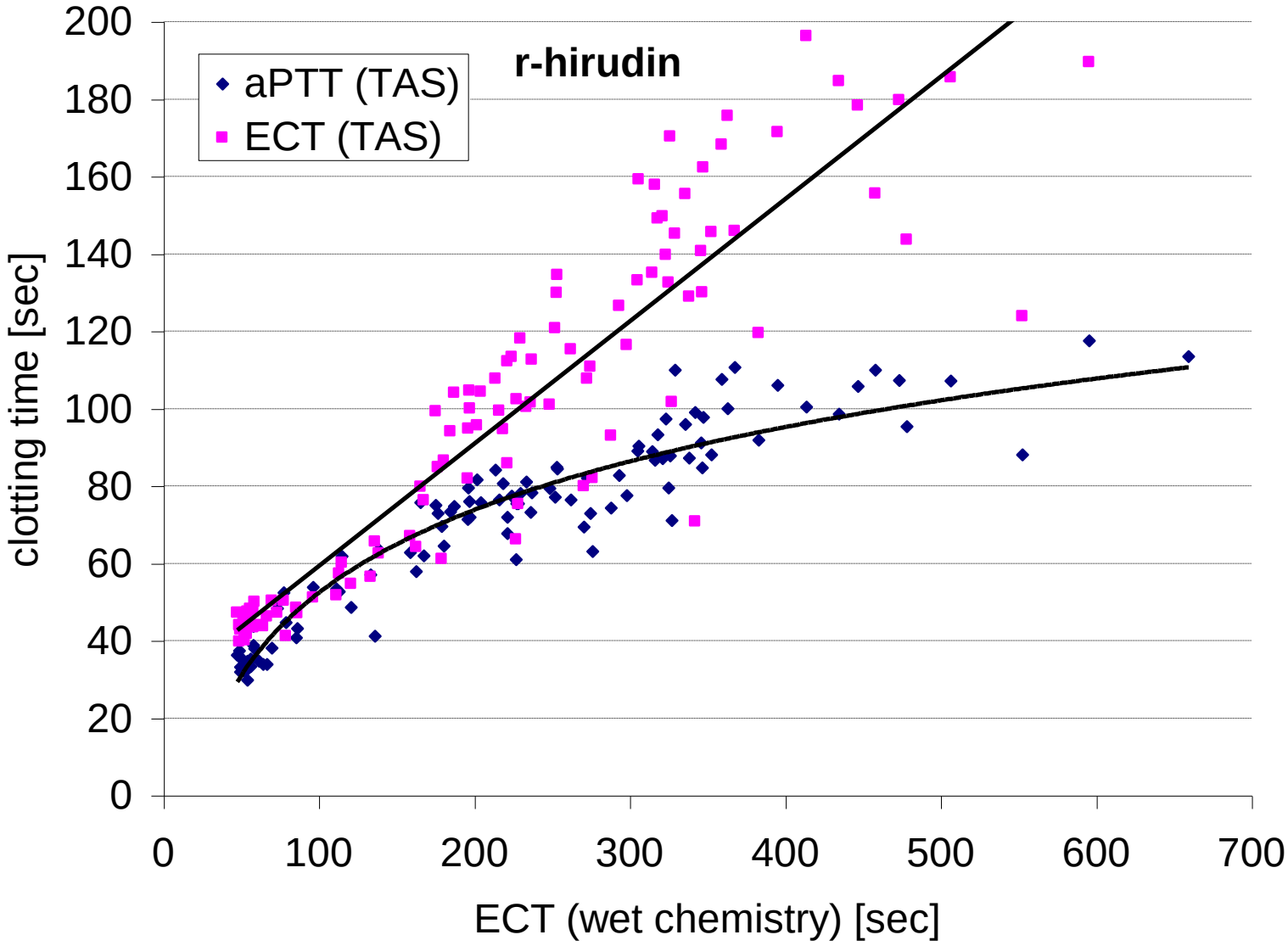
Tests:

aPTT:
Actin (Dade-Behring): Ellagic Acid + Soya PL
Dapttin (Immuno): Sulfatid + Kaolin + Rabbit Brain Cephalin
Synth-A-Sil (Hemoliance): Silica + synthetic phospholipids
aPTT-SP (IL): Silica + synthetic phospholipids

ECT:
Pentapharm: 0,4 U /ml
100 µl reagent + 50 µl PPP

determination of hirudin concentration using the ecarin clotting time: calibration curves (ACL 300R)




r-hirudin
aPTT (TAS)
ECT (TAS)
clotting time [sec]
ECT (wet chemistry) [sec]
0
20
40
60
80
100
120
140
160
180
200
0
100
200
300
400
500
600
700

Results r-hirudin - aPTT

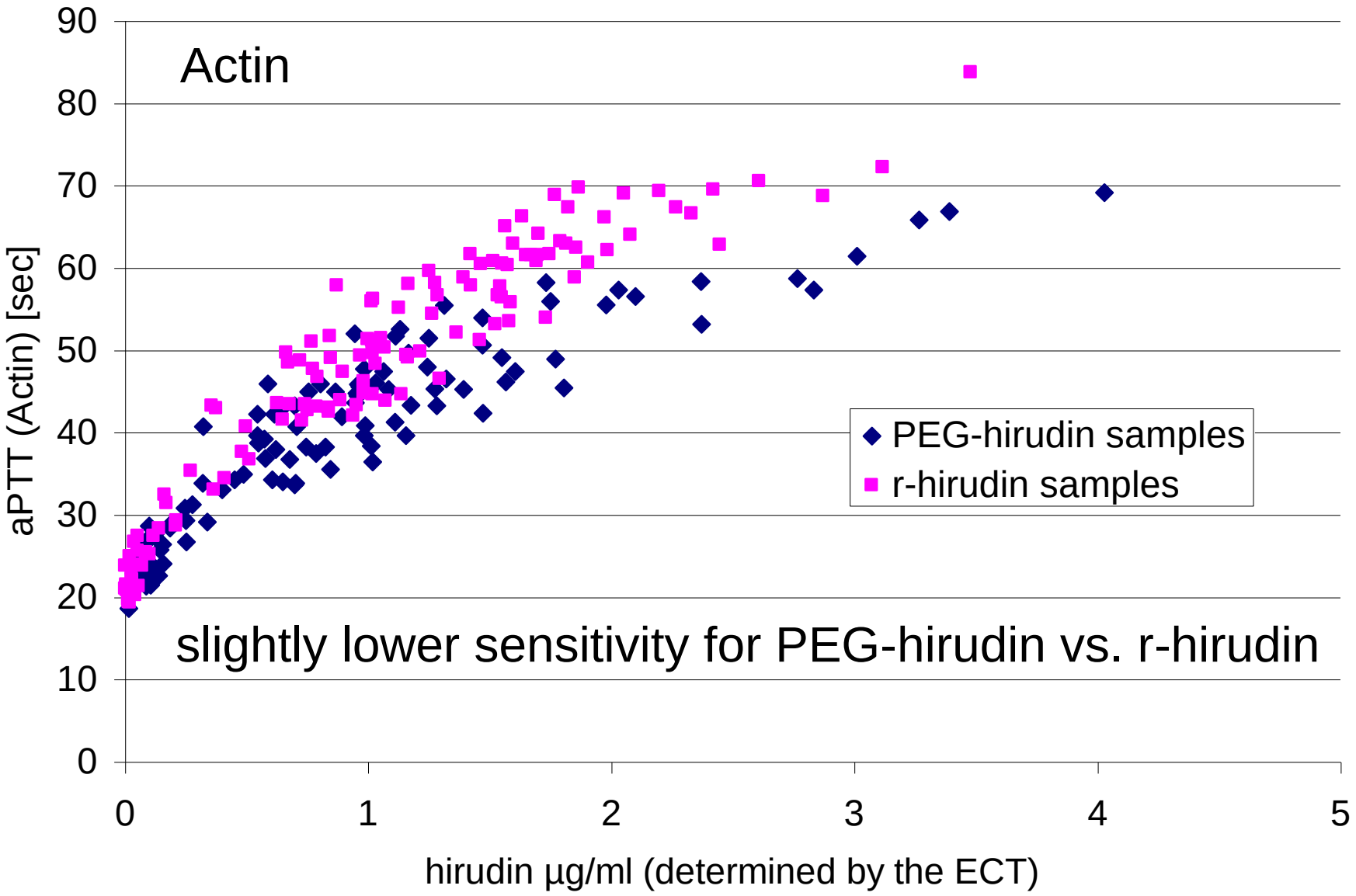
even in healthy volunteers (comparable matrice effects) the aPTT provides only a relatively rough estimation of hirudin concentration

low and medium hirudin dosages are sensitively detected

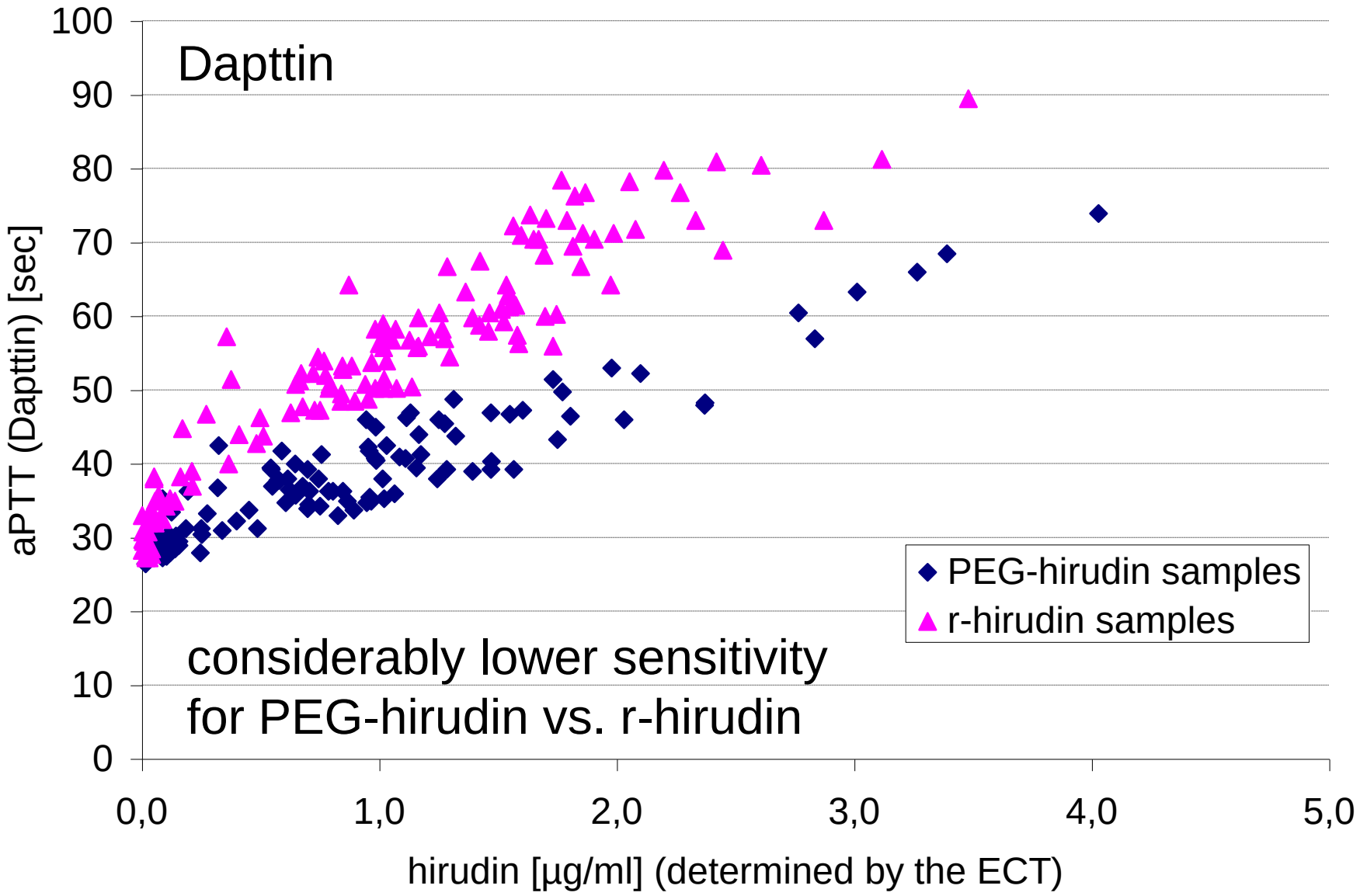
there is no clear cut-off for detecting overdosages due to the parabolic dose-responce relationship

variability among different reagents (+instruments) must be taken into account

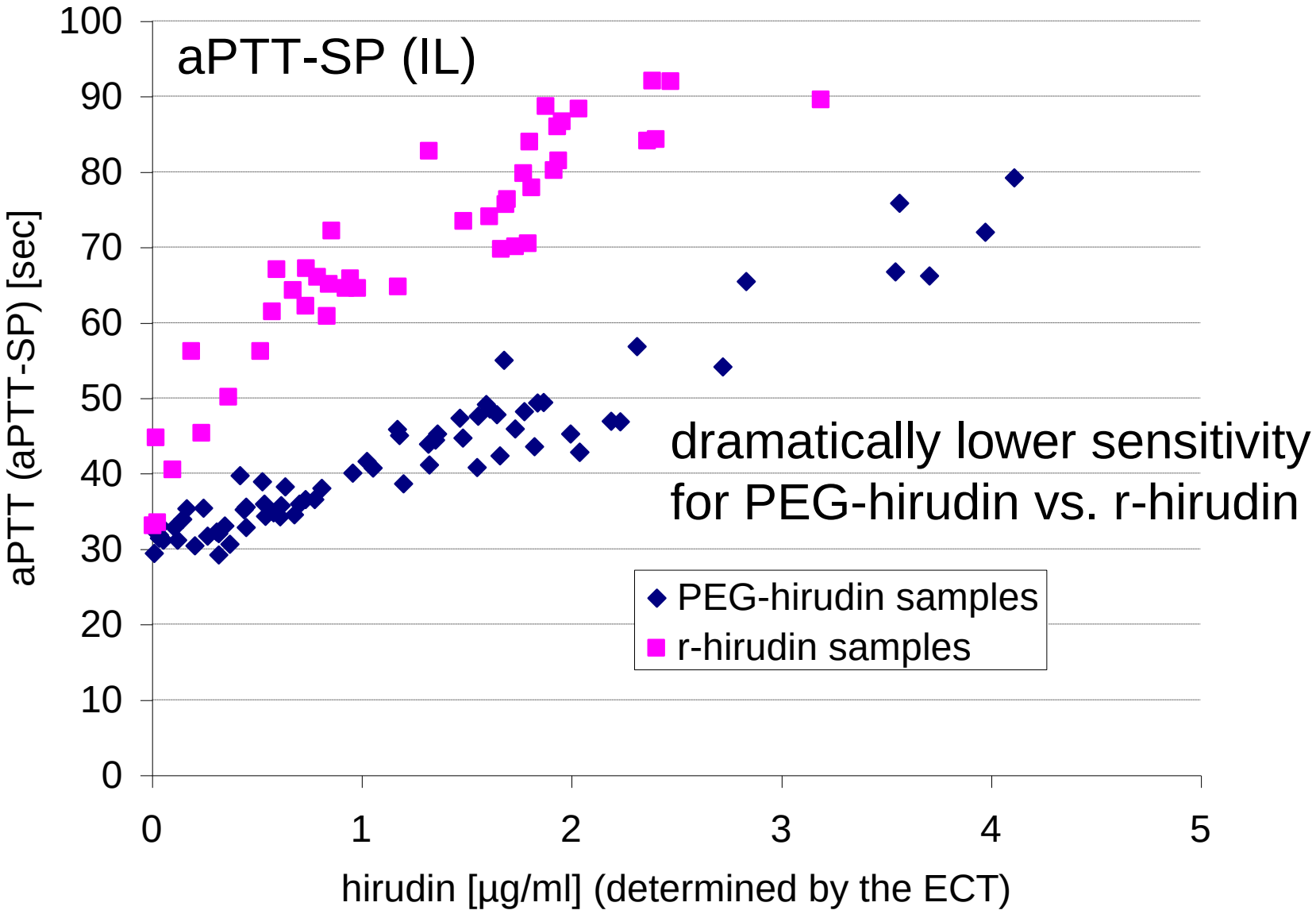
Actin: Ex vivo samples: r- and PEG-hirudin



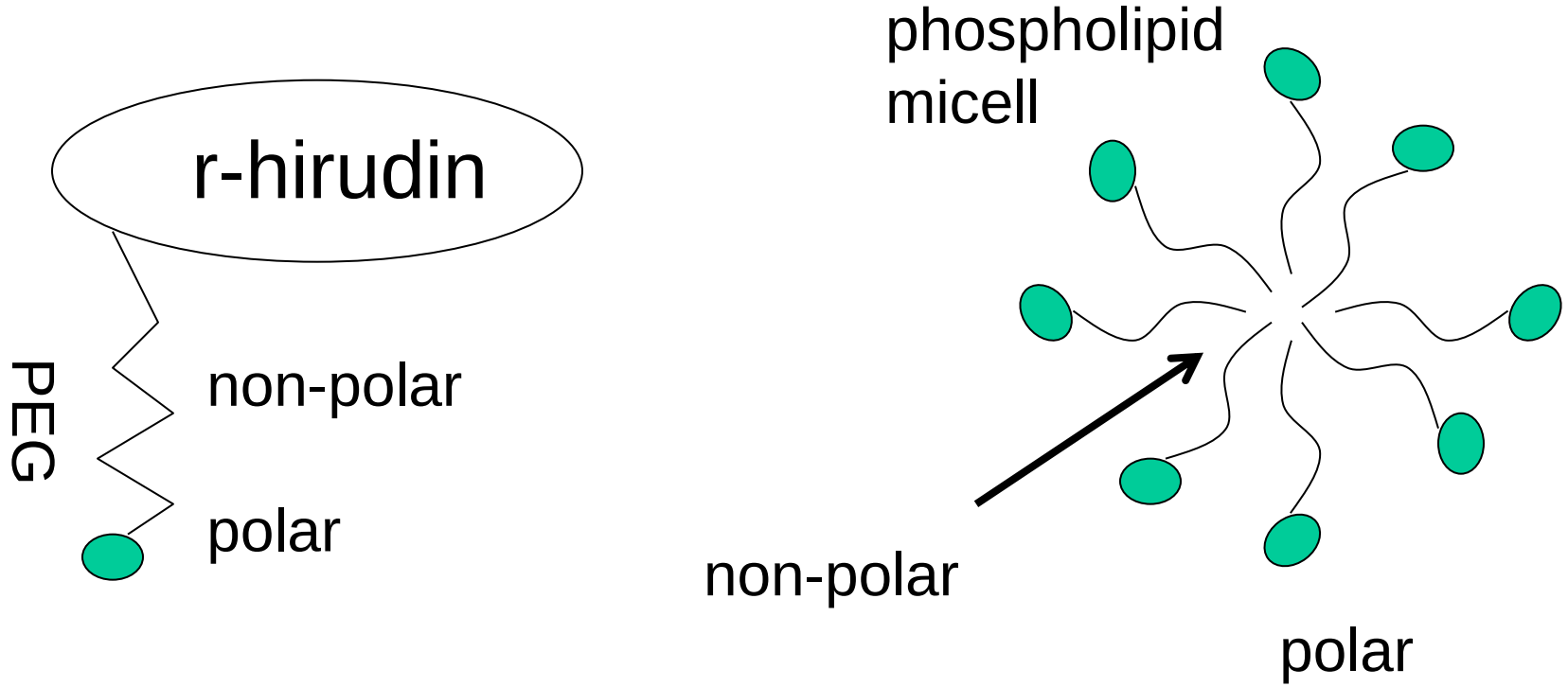
Dapttin: Ex vivo samples: r- and PEG-hirudin



aPTT-SP: Ex vivo samples: r- and PEG-hirudin



possible mechanism of decreased sensitivity of certain aPTT for PEG-hirudin



interaction PEG - phospholipids?

PEG-hirudin

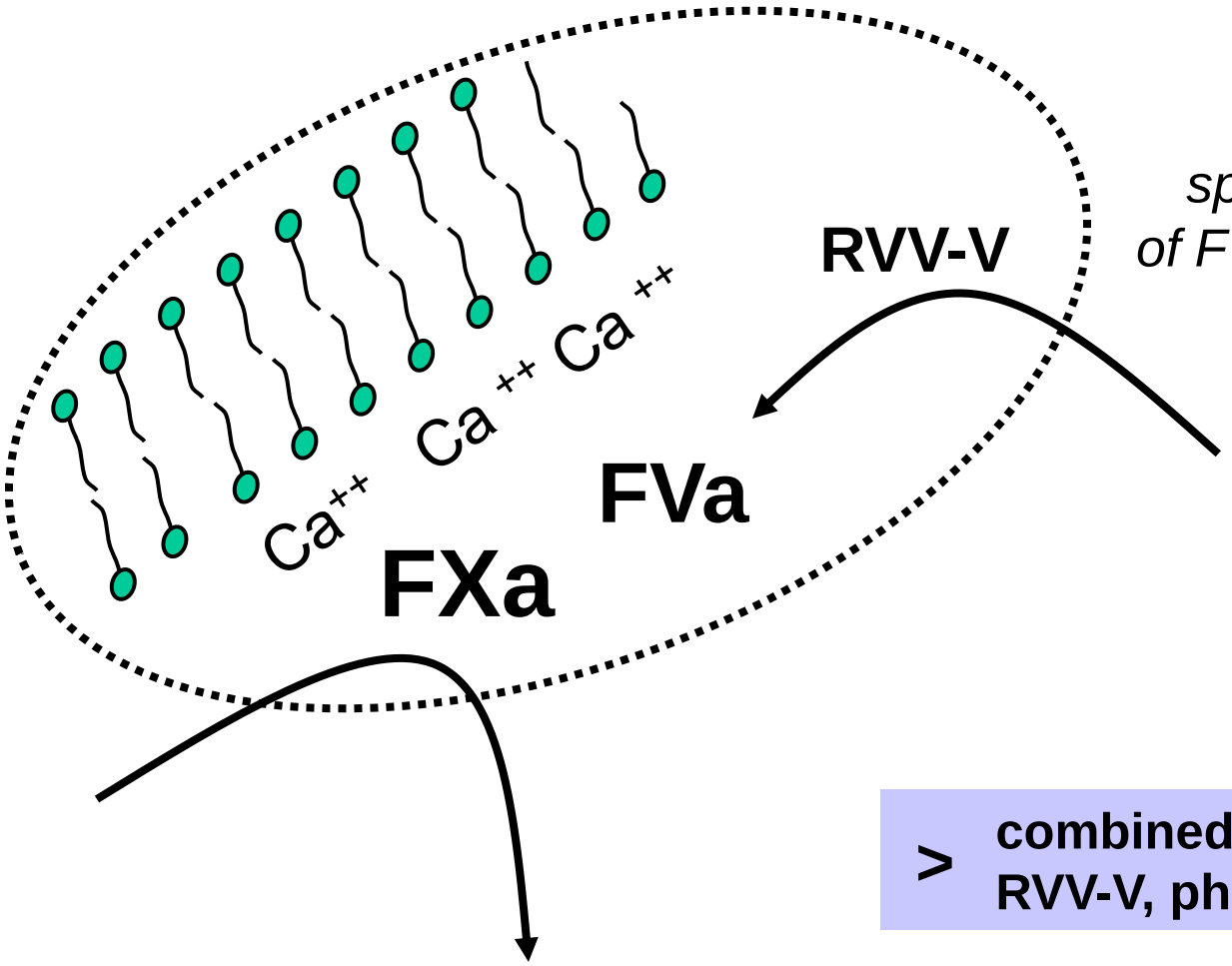
we found dramatic differences in the sensitivity of widely applied aPTT reagents to PEG-hirudin

every aPTT reagent used for monitoring of PEG-hirudin must be tested in respect to its sensitivity to PEG-hirudin

Other example:

Contact Activator may react with therapeutic substances
(Aprotinin Story in heart surgery)

> prothrombinase-induced clotting test: principle



> **combined activation by FXa, RVV-V, phospholipids and $CaCl_2$**

> **method focuses on FXa and thrombin inhibition**

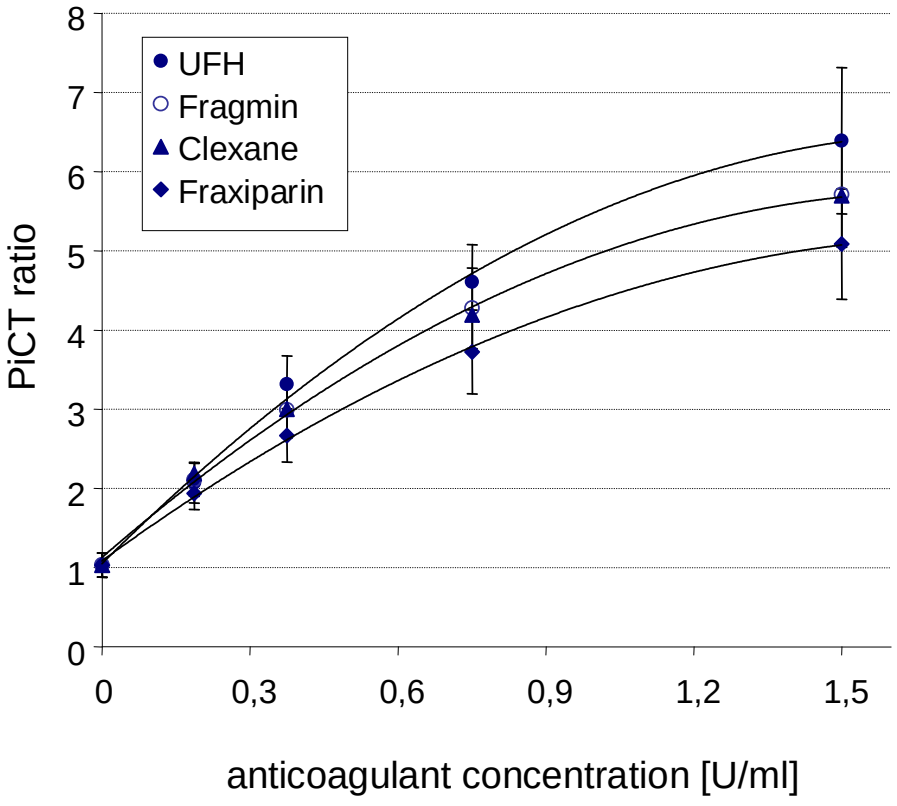
PiCT – results:

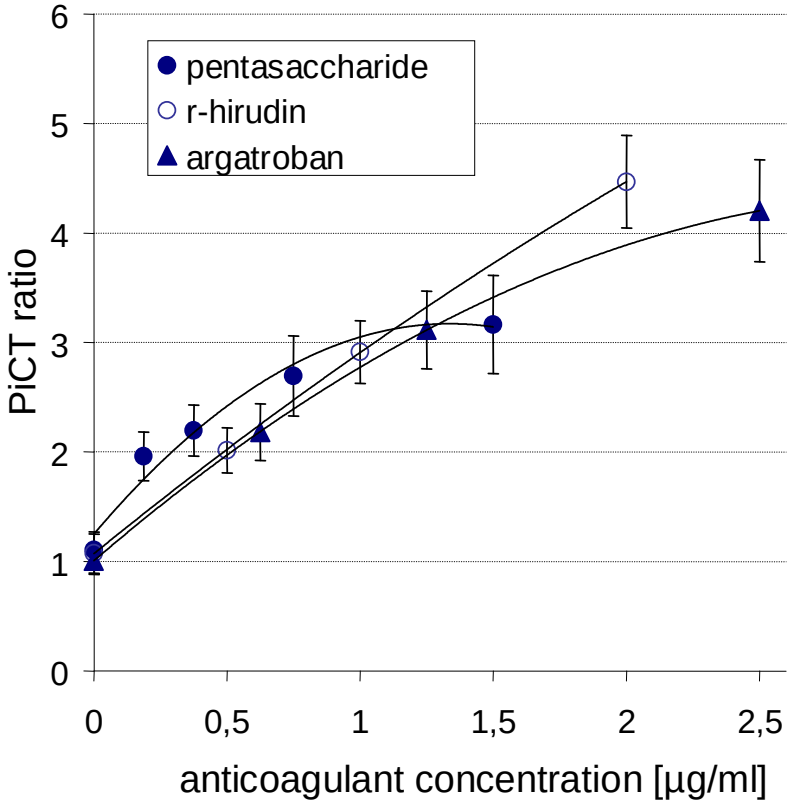
a) Clotting time in sec

b) PiCT Ratio = $\frac{\text{PiCT (patient)}}{\text{PiCT (control plasma = plasma pool)}}$

c) Calibration against anticoagulant concentration possible

> PiCT®: dose-response relationships: UFH, LMWH and Hirudin





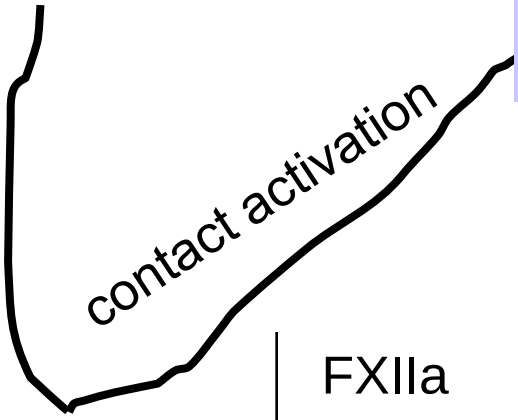
PiCT

FXa-FVa-PL-Ca^{++}

Thrombin

Fibrinogen → Fibrin

aPTT



FXIIa

FXIa

FIXa-**FVIIIa**-PL-Ca^{++}

FXa-FVa-PL-Ca^{++}

Thrombin

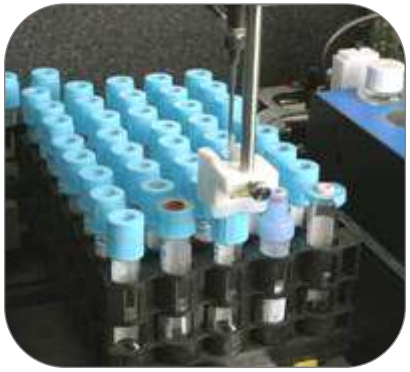
Fibrinogen → Fibrin

detection of clotting

Less influence of patient clot.factors than aPTT and PT ?

Monitoring

Preanalytics



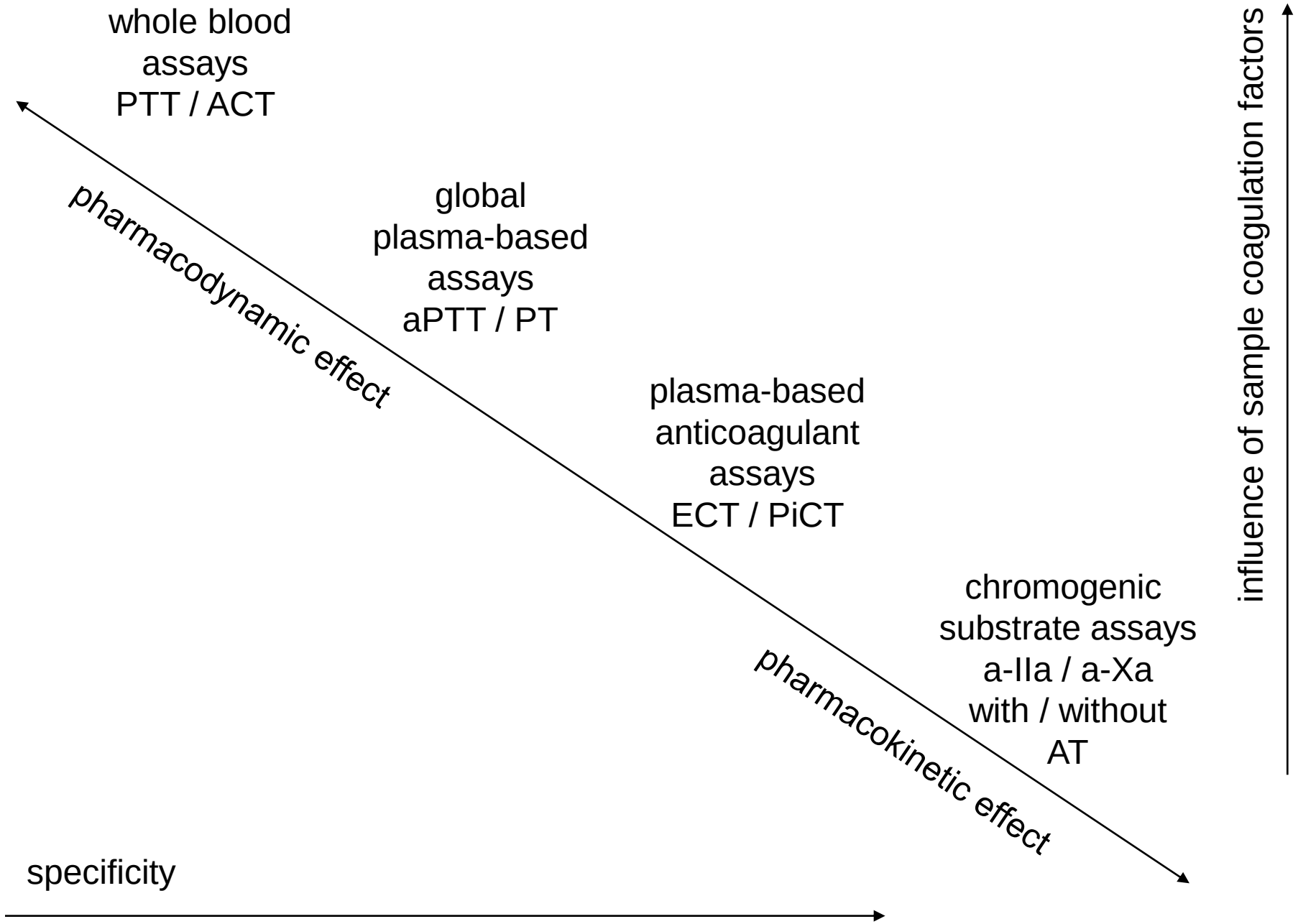
Reagents /
Kalibration

Analysis

Seconds

Transformation
I
Anticoagulant Concentration

Result


whole blood
assays
PTT / ACT
global
plasma-based
assays
aPTT / PT
plasma-based
anticoagulant
assays
ECT / PiCT
chromogenic
substrate assays
a-IIa / a-Xa
with / without
AT
pharmacodynamic effect
pharmacokinetic effect
influence of sample coagulation factors
specificity

Recommendations

Identify all anticoagulants applied to the patients in your hospital

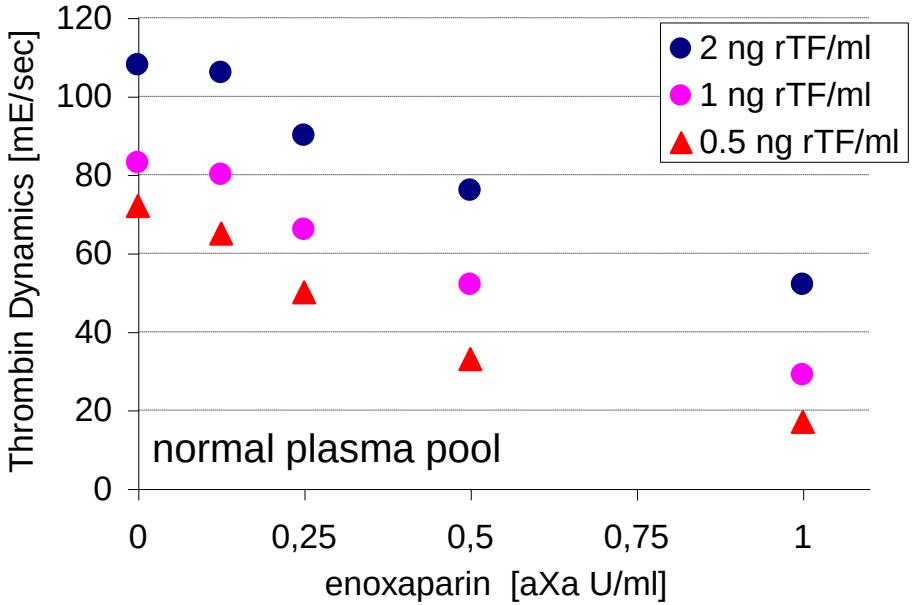
Identify commercially available assays with acceptable performance data (also own experience ex vivo!) (Consider pharmacokinetics , -dynamics)

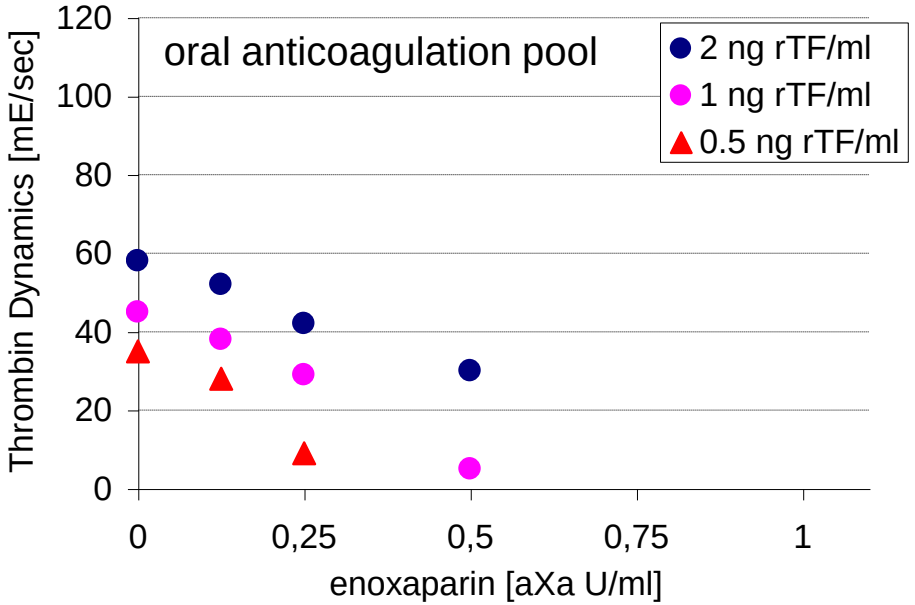
USE commercially available CALIBRANTS
Spiking normal plasma is nice, BUT you need ex vivo plasma material produced under standardized conditions

Be sure that changing anticoagulant concepts is communicated with the lab IN ADVANCE

Educate your clinicians to send samples for anticoagulantS monitoring regularly (you need measuring- they need interpretation experience)

TDT: Antikoagulantieneffekte 1/2: Heparine

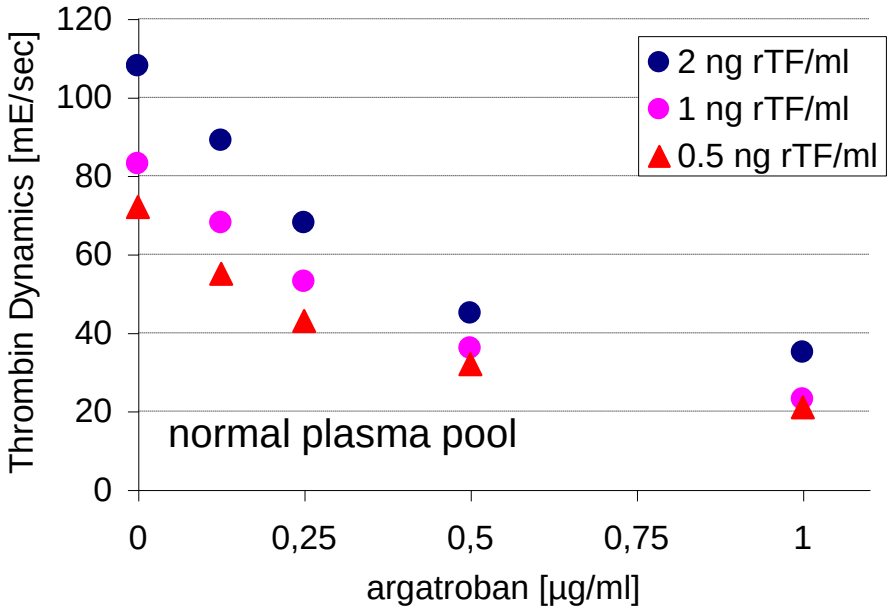


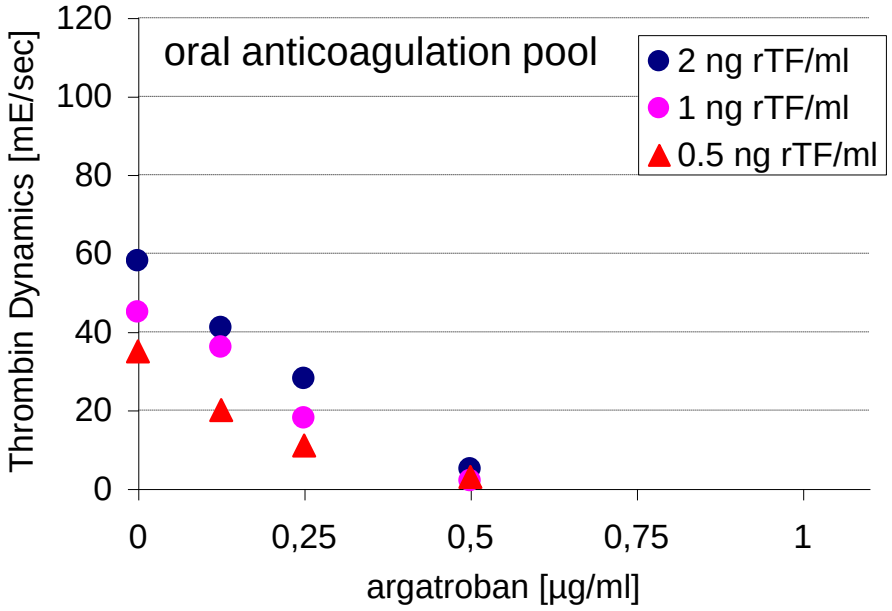


- clear dose response for levels of the tissue factor activation and the enoxaparin levels on the thrombin formation dynamics
- **50% inhibition of thrombin formation at approximately 0.6-0.7 aXa U/ml**
- approximately 40% inhibition of the thrombin formation dynamics by the oral anticoagulation
- additive effect of oral anticoagulation and enoxaparin

applicability of the TDT method in anticoagulation

TDT: Antikoagulantieneffekte 2/2: Direkte Thrombininhibitoren





- clear dose response for levels of the tissue factor activation and the argatroban levels on the thrombin formation dynamics
- **50% inhibition of thrombin formation at approximately 0.3 µg/ml**

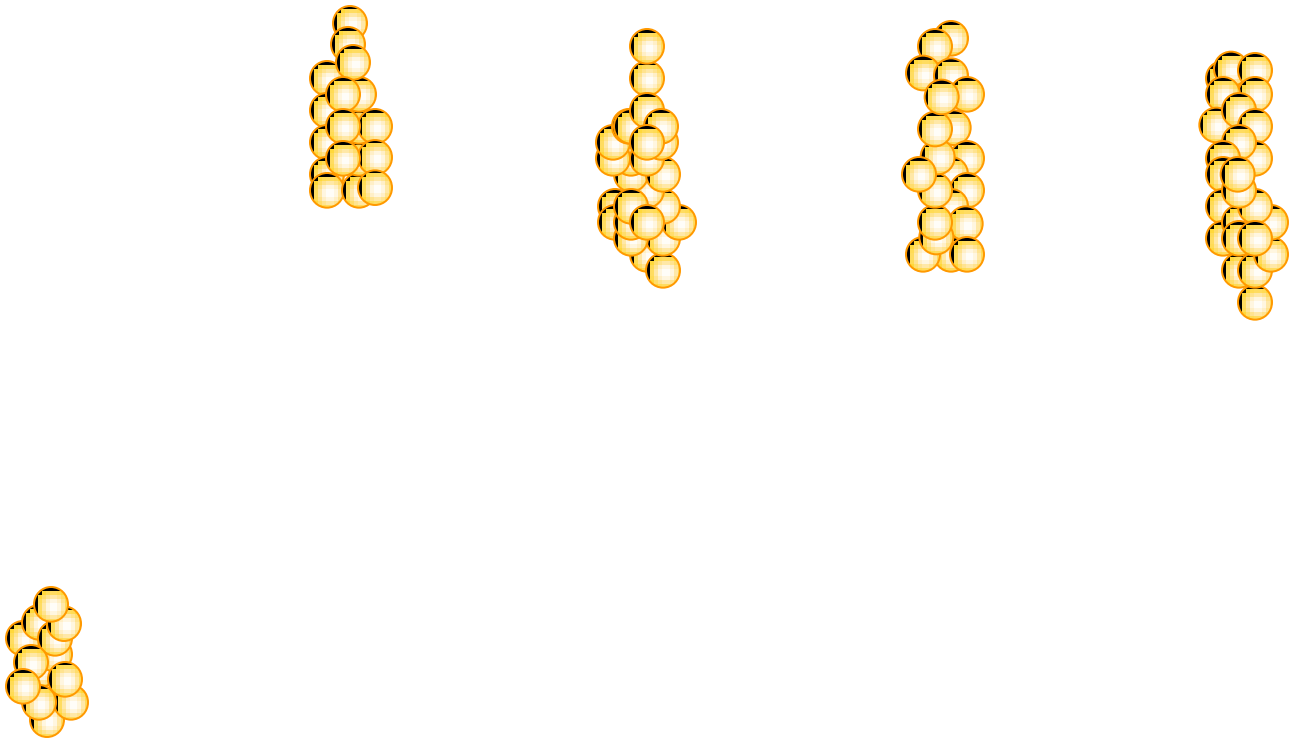
applicability of the TDT method in anticoagulation

Gegenwärtig verfügbare Präparate mit niedermolekularen Heparinen

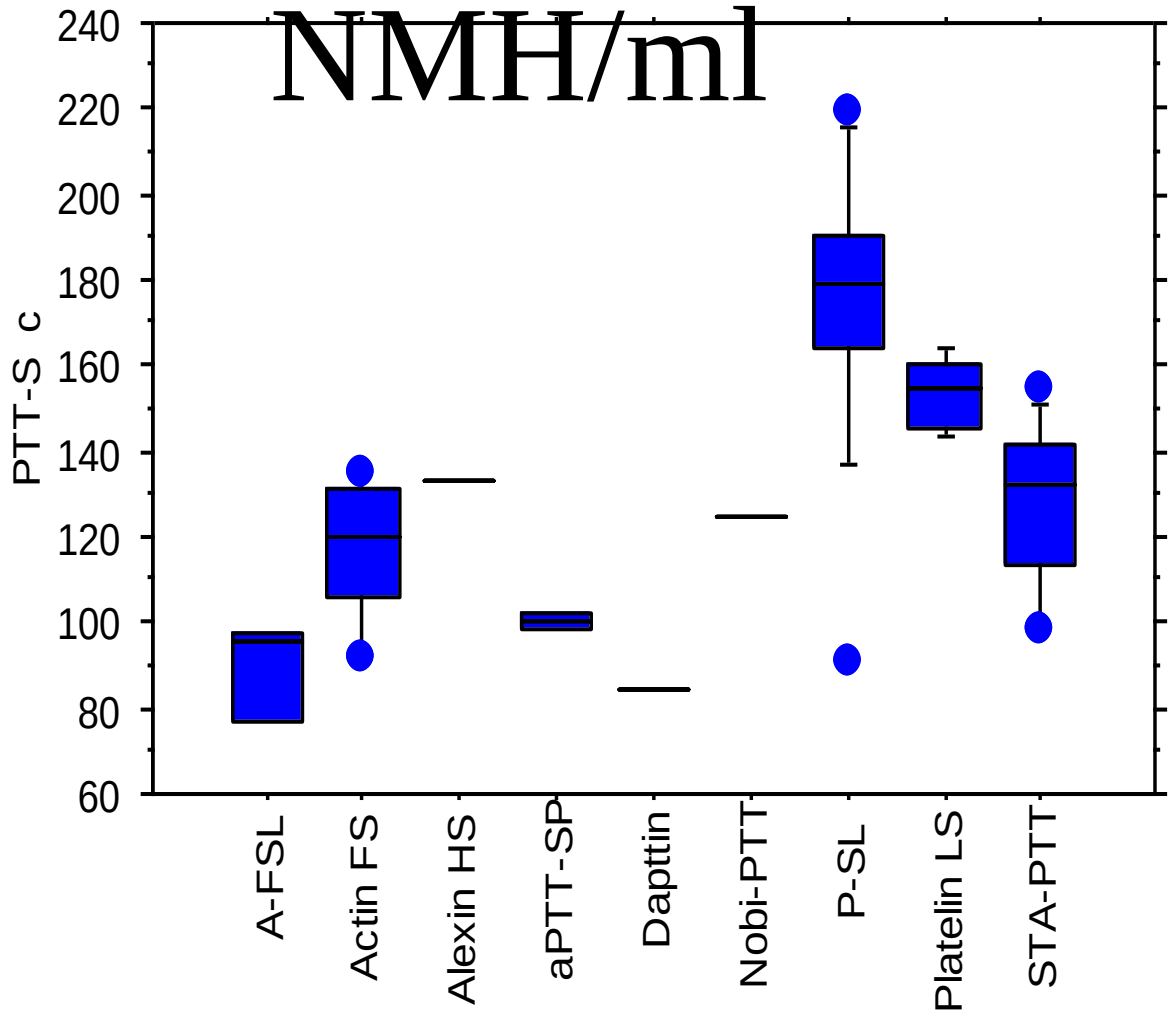
LMWH - MW - Anti-Xa / IIa Ratio

Substance	Preparation	MW(Da)	Anti-Xa/-IIa-Ratio
*Bemiparin-Natrium**	*Alkalische Depolymerisation*	*3.000-4.200*	*6-9*
Enoxaparin-Natrium	Benzylierung und alkalische Depolymerisation	3.500-5.500	3,6
Reviparin-Natrium	Depolymerisation mit salpetriger Säure	3.550-4.650	3,2
Nadroparin-Kalzium	Depolymerisation mit salpetriger Säure	4.200-4.800	3,2
Dalteparin-Natrium	Depolymerisation mit salpetriger Säure	5.000-5.950	2,5
*Parnaparin-Natrium**	*Peroxidative Depolymerisation*	*4.000-5.000*	*2,4*
*Ardeparin-Natrium**	*Peroxidative Depolymerisation*	*5.500-6.500*	*2,0*
Certoparin-Natrium	Aufspaltung durch Isoamylnitrat	6.000	2,0
Tinzaparin-Natrium	Digestion durch Heparinase	5.800-6.750	1,9

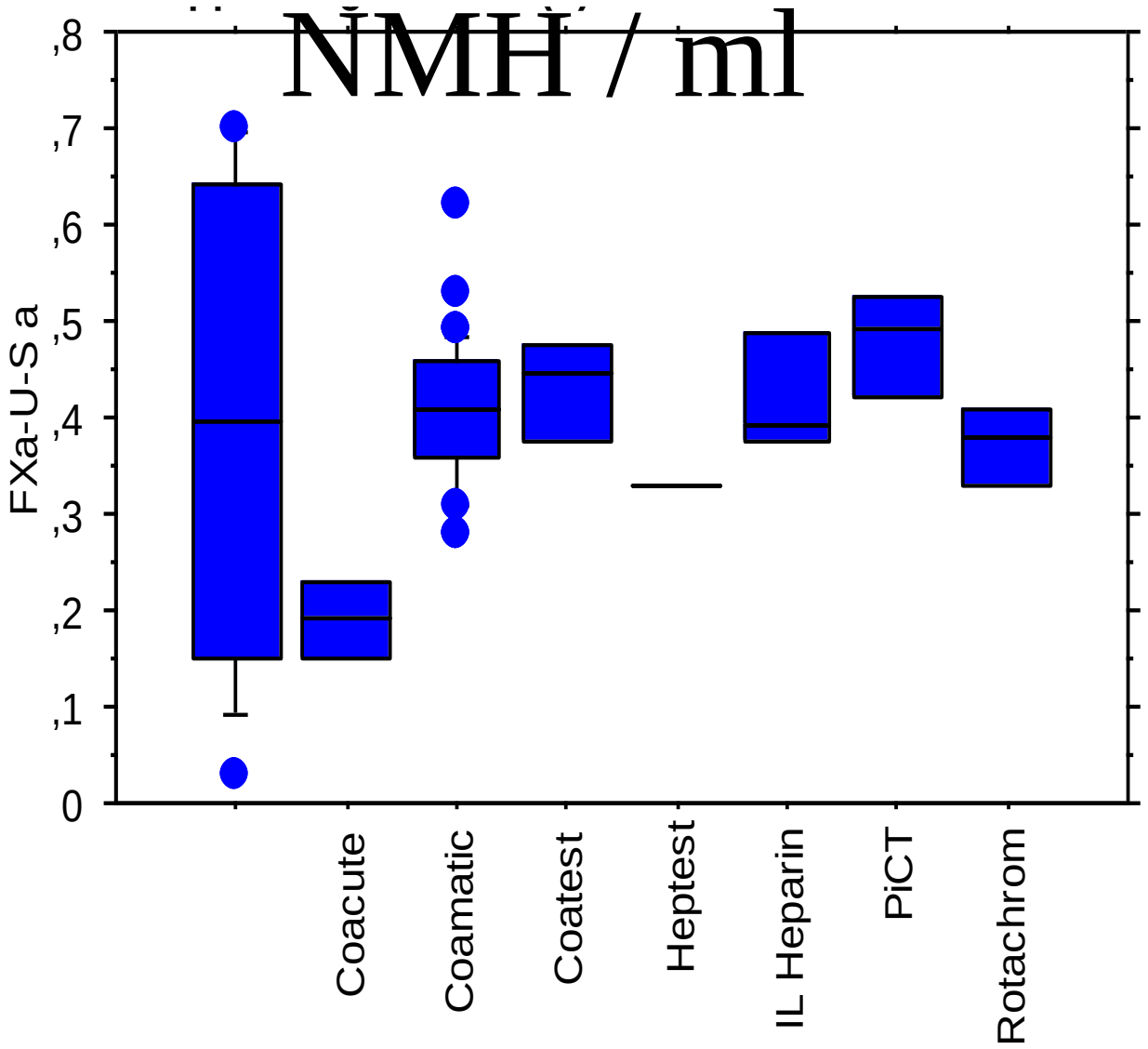
Heptest-Messungen unter Therapie von TVT mit fixer Dosierung



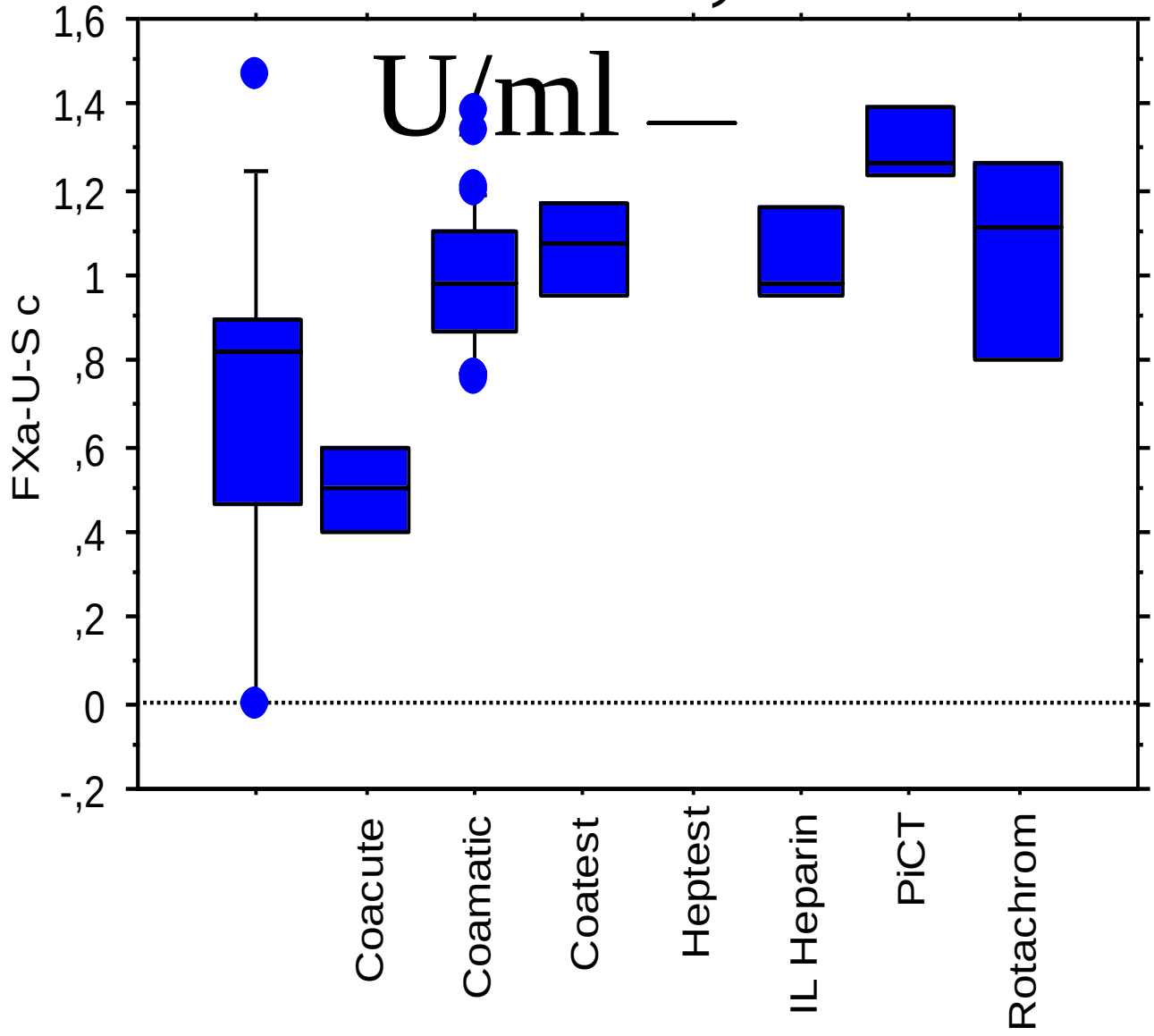
PTT 1,15 U NMH/ml



Anti Xa 0,45 U NMH / ml



Anti Xa 1,12 NMH



Streubreite der aPTT bei nicht-adjustierter Heparin-Dosierung