Standardization of blood coagulation tests. What has been achieved?

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Leiden: mills, canals, bicycles

Curriculum vitae

Year	
1979	Dutch Reference Laboratory for anticoagulant monitoring
1979	Certification of International Standards for Thromboplastins
1981	Working Group Dutch EQA Scheme for coagulation tests
1986	Subcommittee on Control of Anticoagulation (ISTH)
1997	Rapporteur WHO/ISTH Consultation on Thromboplastins and Plasmas to control Oral Anticogulant Therapy
2001	CLSI Subcommittee on Prothrombin Time Calibration
2004	Scientific and Standardization Committee of ISTH
2015	Consultant for “Coagulation Reference Laboratory” (Head: Prof. Dr. Christa Cobbaert)

Background

- Coagulation factors have a complex structure and function.
- Coagulation factors have a low concentration in blood plasma.
- Physico-chemical estimation is virtually impossible.
- Estimation relies on comparative bioassay, relative to a reference standard containing a known amount of analyte.

International Unit

- For most clotting factors in plasma, the unit of activity was first defined as the amount in “average normal plasma”.
- Once the first International Standard has been calibrated, it is assigned a value in International Units.
- Subsequent batches of International Standards are calibrated in International Units against the previous Standard.

International Standards (IS) held by NIBSC

Name	Plasma	Concentrate	Purified
Factor II	3rd IS	3rd IS	
Factor V	1st IS		
Factor VII	3rd IS	1st IS	
Factor VIIa			2nd IS
Factor VIII	6th IS	8th IS	
Factor IX	3rd IS	4th IS	
Factor IXa		1st IS	
Factor X	3rd IS	3rd IS	
Thrombin			2nd IS
Fibrinogen	2nd IS	1st IS	
Antithrombin	2nd IS	3rd IS	

From: Raut & Hubbard. Biologicals 2010;38:423-429

International Standards (IS) held by NIBSC

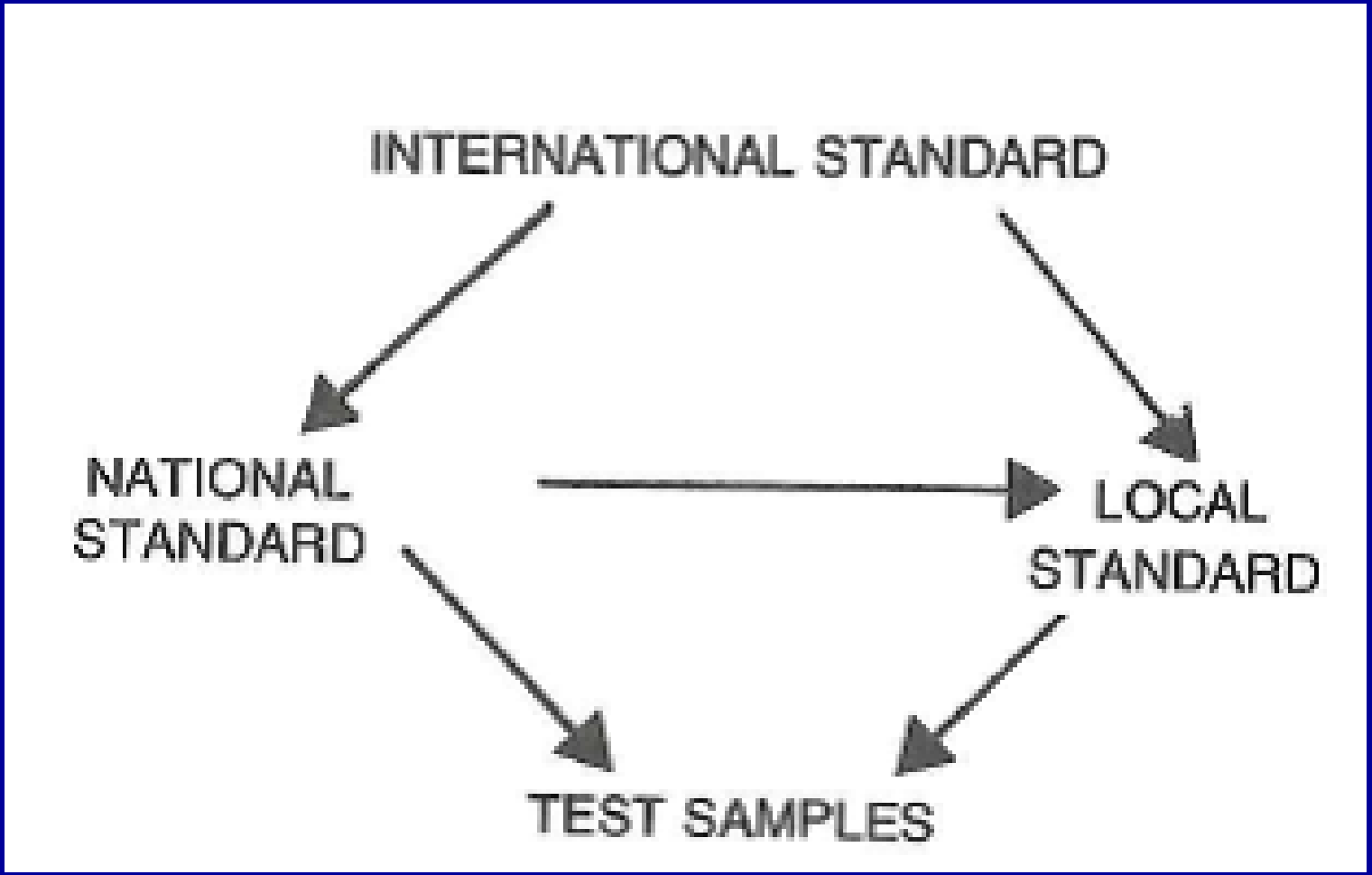
Name	Plasma	Concentrate	Purified
Factor XI	1st IS		
Protein C	2nd IS	1st IS	
Protein S	1st IS		
Von Willebrand Factor	6th IS	1st IS	
Factor XIII	1st IS		

Raut & Hubbard. Biologicals 2010;38:423-429

International Standards for Thromboplastins

Type	Code		Custodian
Rabbit brain Tissue Factor	RBT/05	4th IS	NIBSC
Recombinant human Tissue Factor	rTF/09	4th IS	NIBSC

Hierarchy of Quantitation



Barrowcliffe, 1981; In: Standardization of Coagulation Assays

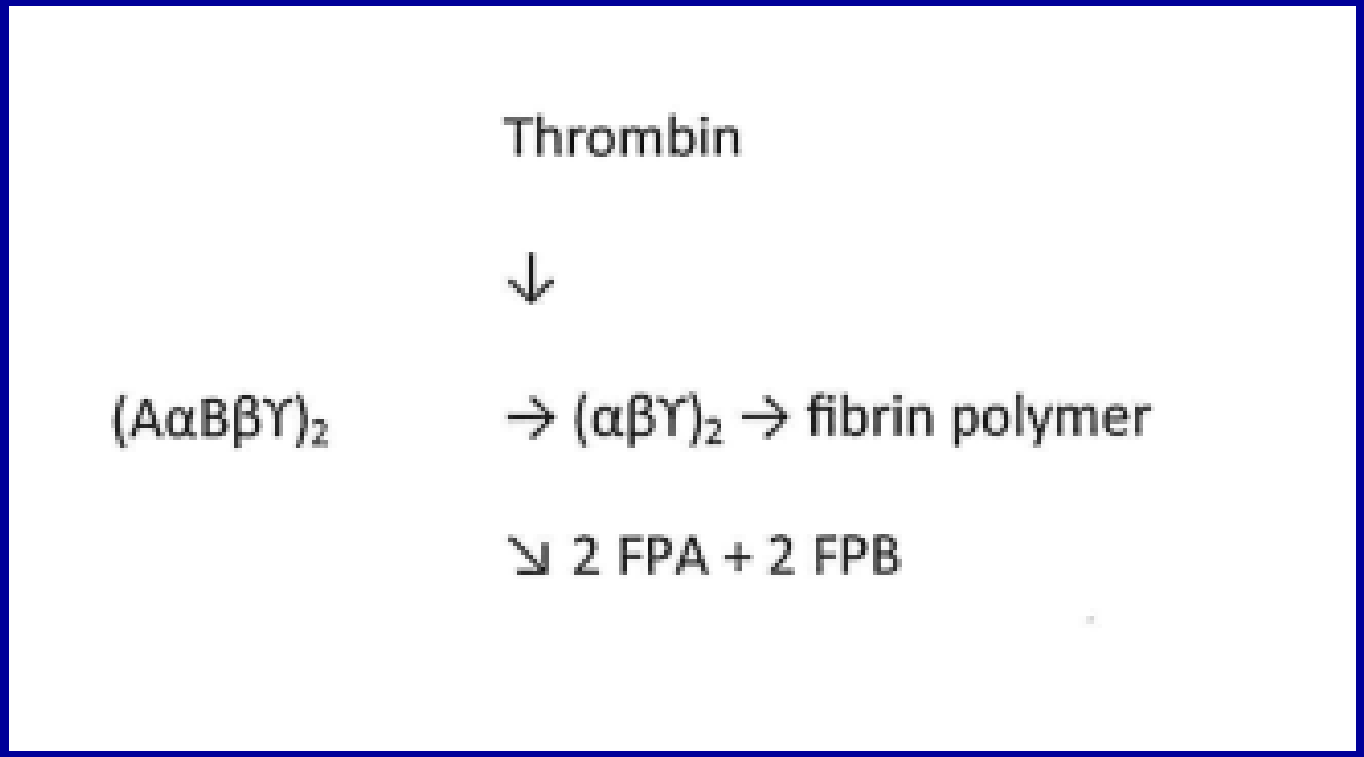
Fibrinogen

- Dimeric macromolecule
- Each half consists of three different polypeptide chains, Aα, Bβ and γ, linked together by disulphide bridges
- Three forms: HMW (340 kD), LMW (305 kD) and LMW’ (270 KD)
- Reference range: 1.7 – 4.0 g/L

Reasons for measuring fibrinogen

- Disseminated intravascular coagulation
- Liver dysfunction
- Dysfibrinogenaemia
- Thrombolytic therapy
- Arvin therapy
- Risk assessment profiling for arterial disease

Fibrinogen is cleaved by thrombin



Methods for fibrinogen estimation

Thrombin clottable protein	Clot opacity	Ellis *et al*, 1961
	Clot weight	Bang, 1957
	Clot harvest & dissolution	
	Colorimetric	Ratnoff *et al*, 1951
	Ultraviolet	Blombäck, 1958
	Thrombin time, modified	Clauss, 1957
Physicochemical methods	Heat precipitation	Millar *et al*, 1971
	Glycine precipitation	Kazal *et al*, 1964
	Salt precipitation	Parfentjev *et al*, 1953
Immunologic methods	Radial immunodiffusion	Brittin *et al*, 1972
	Nephelometric	Exner *et al,* 1979
	Enzyme immunoassay	Hoegee *et al*, 1988

Modified from: J.A. Koepke (1980)

First International Standard for plasma Fibrinogen

- Lyophilized pooled plasma in ampoules
- Recommended method: Clot harvest and dissolution, measurement of Optical Density at 280 nm and 315 nm
- 22 Laboratories in 9 different countries
- Mean value: 2.4 g/L, geometric CV: 9.18%

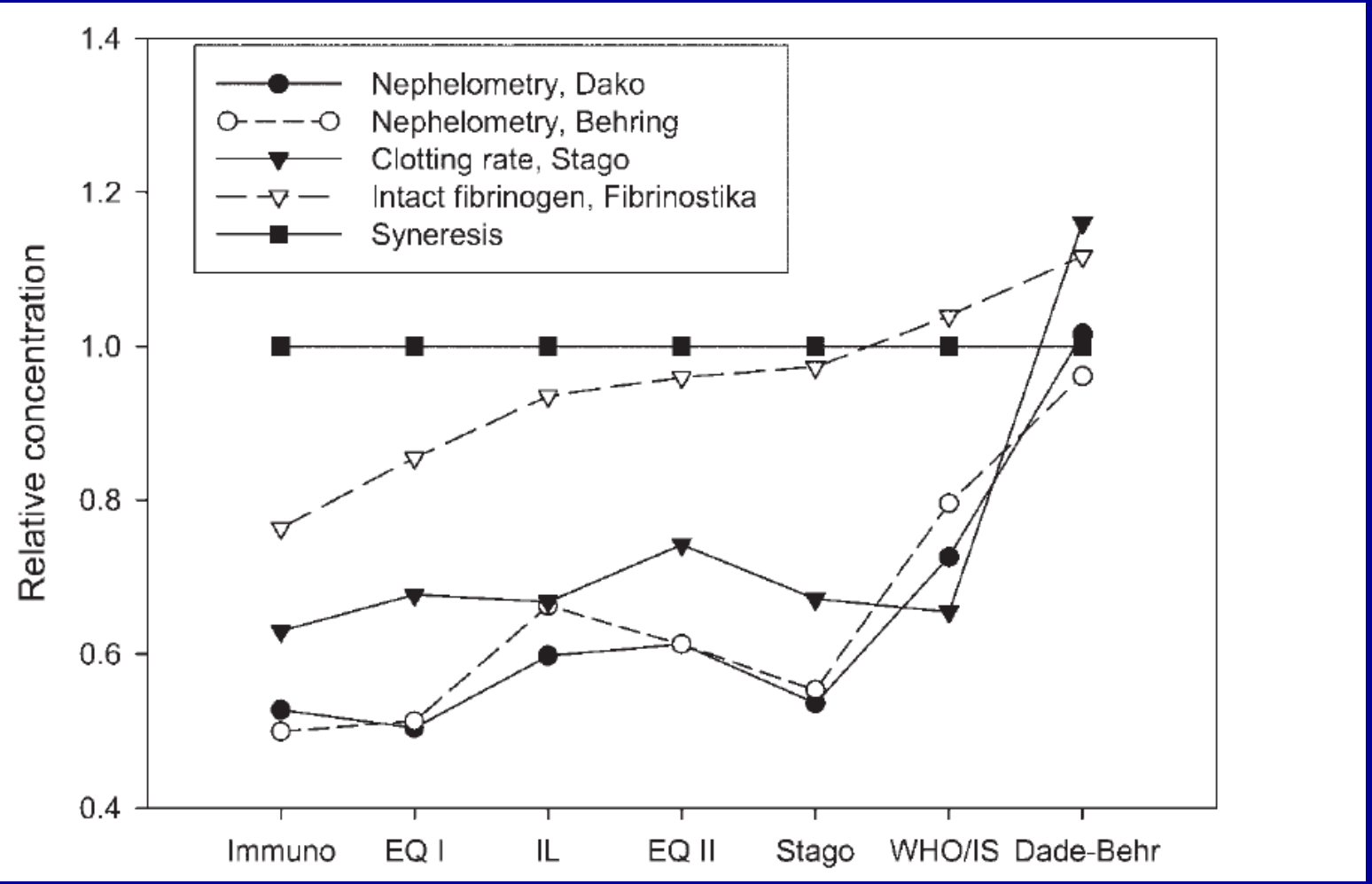
From: Gaffney and Wong (Thromb Haemost1992;68:428)

Second International Standard for plasma fibrinogen

- Lyophilized solvent/detergent treated plasma in ampoules
- Requested method: either automated “Clauss” assay (thrombin time) and/or Clot harvest assay
- Automated Clauss (n=11): 2.19 g/L
- Clot harvest assay (n=3): 1.93 g/L
- Potency of established 2nd IS: 2.2 g/L

From: Whitton *et al*, Thromb Haemost 2000;84:258

Commutability of fibrinogen reference materials



Kallner *et al*, 2003, Clin Chem Lab Med

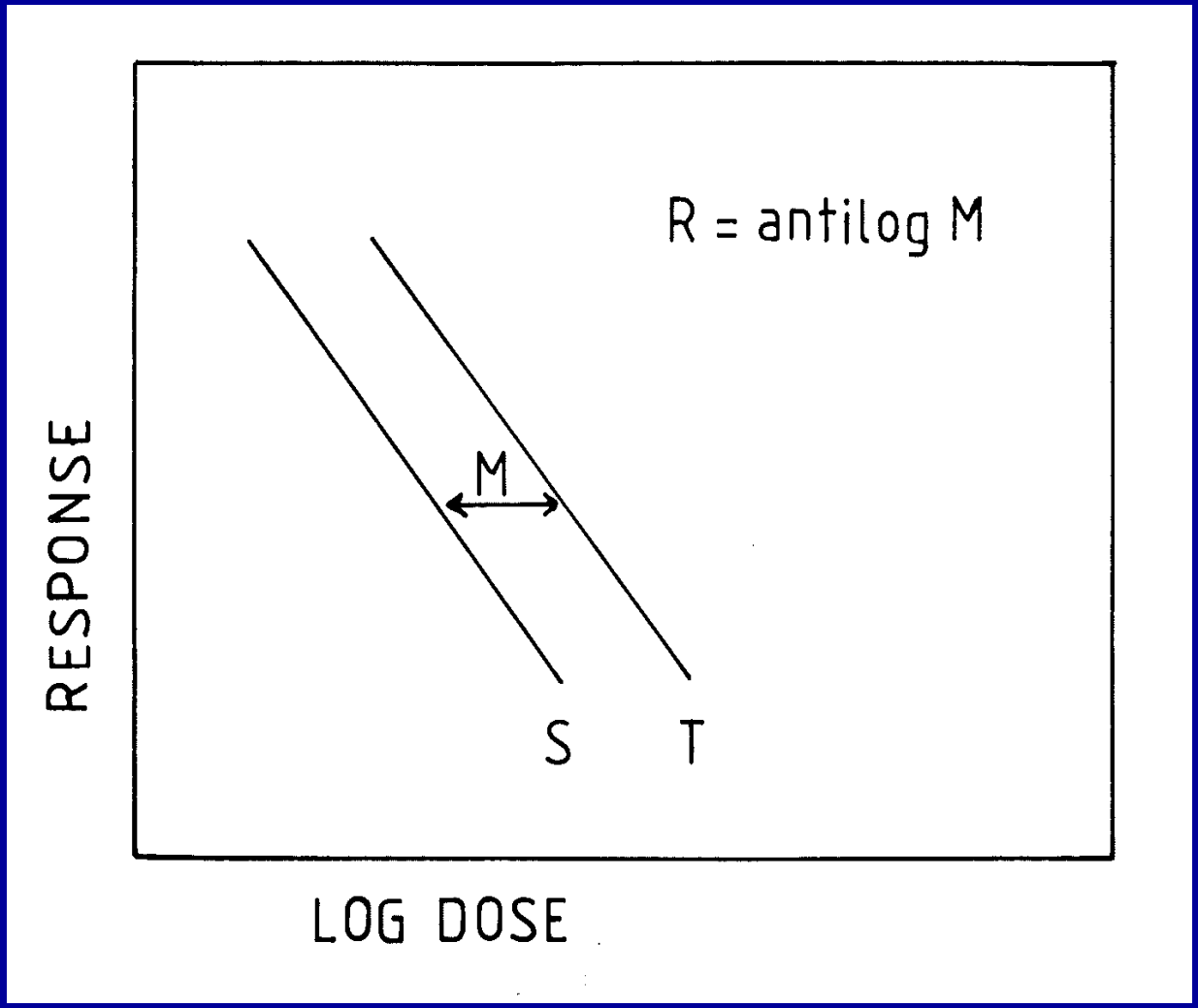
Reasons for measuring Factor VIII

- Potency of concentrates (by manufacturer)
- Analysis of patients' post-infusion samples, to monitor their haemostatic status, and to assess the pharmacokinetics of concentrates.
- Diagnosis and assessment of the severity of the haemophilic defect.

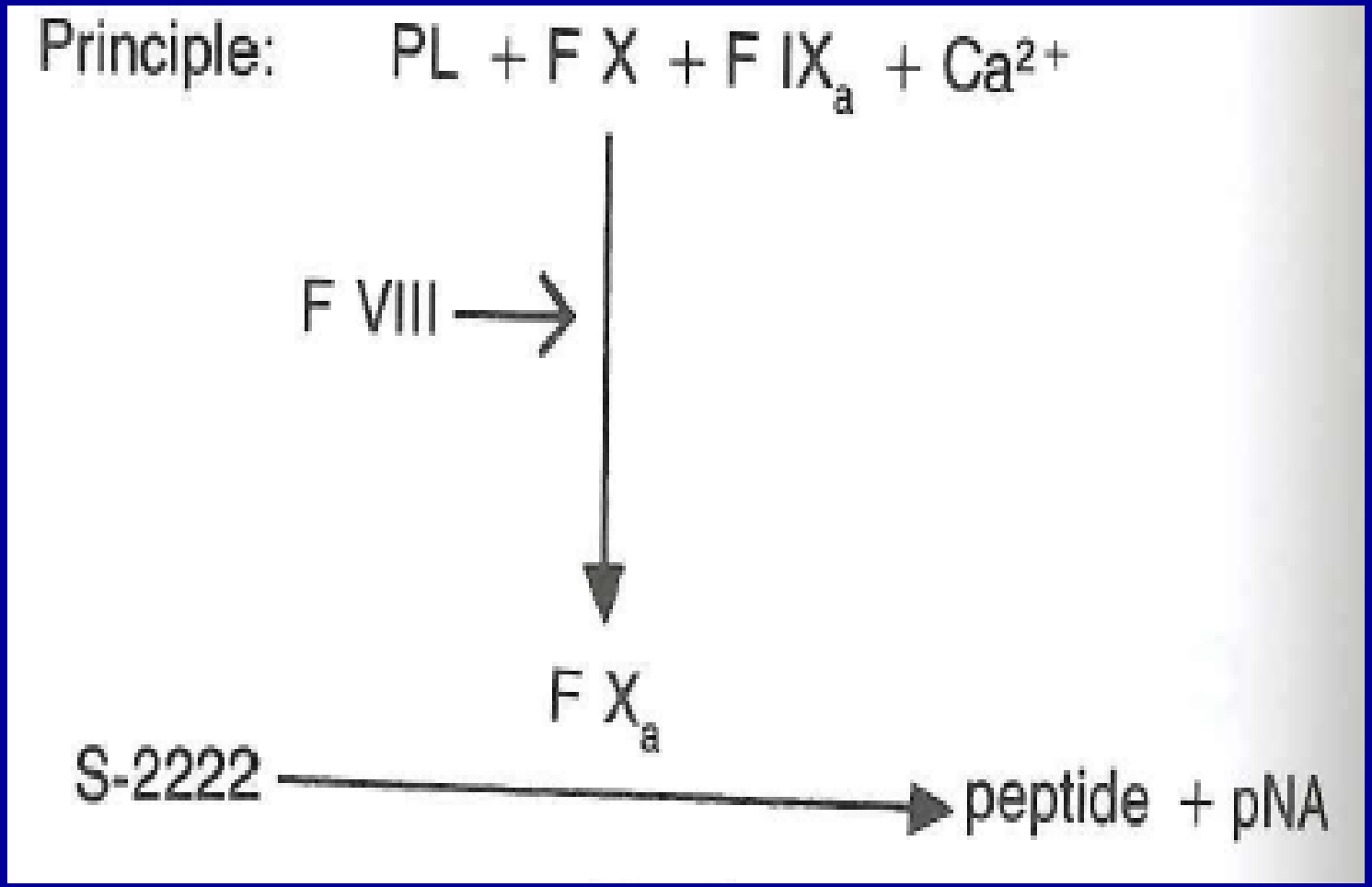
Methods for Factor VIII measurement

	Endpoint	Reference
One-stage clotting time test	Manual or automated	Langdell *et al*, 1953
Two-stage clotting time test	Manual or automated	Biggs *et al,* 1955
Factor Xa Generation	Chromogenic substrate	Rosén *et al*, 1981
Thrombin Generation	Clotting time	McIntosh *et al*, 2003
Thrombin Generation	Chromogenic substrates	Hemker *et al*,
Waveform analysis	Optical density, automated	Shima *et al*, 2002

Parallel Line Assay

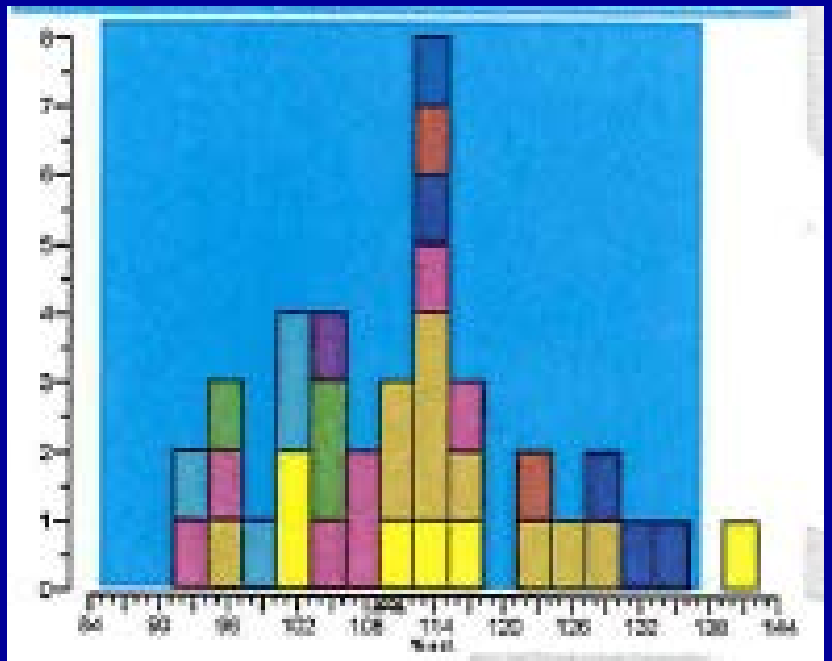


Factor Xa Generation



S. Rosén *et al*. 1981, In: Standardization of Coagulation Assays

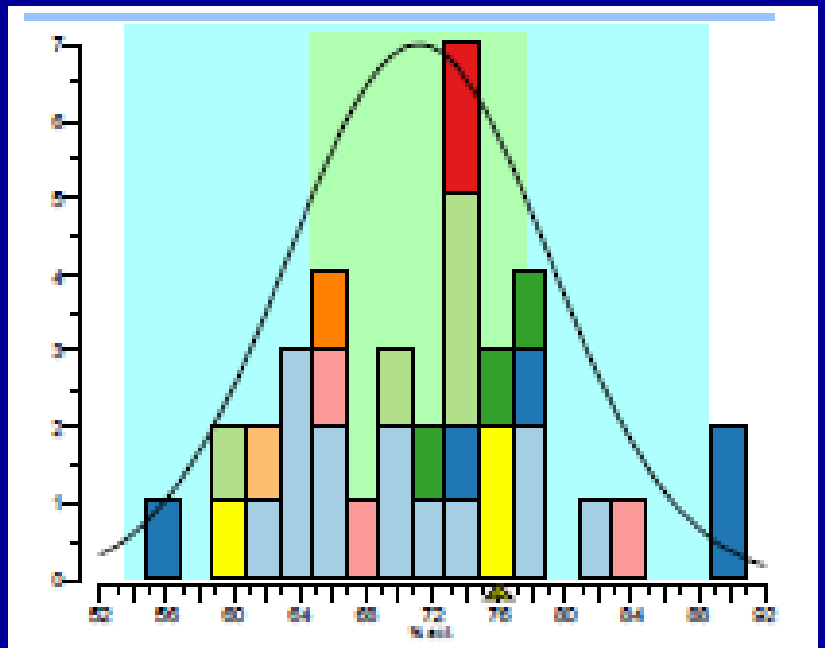
External Quality Assessment of Factor VIII



	cons.	meth.	ALTM	lab
gem.	111	110	111	110
SD	12	7	12	
n	38	6	38	

Source: Foundation Quality Assessment Medical Laboratory Diagnostics (SKML)

External Quality Assessment of Factor VIII



	cons.	meth.	ALTM	lab
gem.	71	70	71	76
SD	8		8	
n	36	3	36	

Source: SKML (2014)

Sources of variability in Factor VIII assays

Assay Method	One-stage, Two-stage, etc.
Reagents	Standard reference plasma
	Deficient substrate plasma
	Buffer
	Partial thromboplastin (phospholipids)
Equipment	Coagulometer, Photometer, tubes, pipettes
Technique	Preanalytical variables
	Dilutions
	Sequence of reagent addition
	Incubation times
	Calculation of assay results
	Analysis of assay quality (Linearity, parallelism, drift)

Prothrombin Time

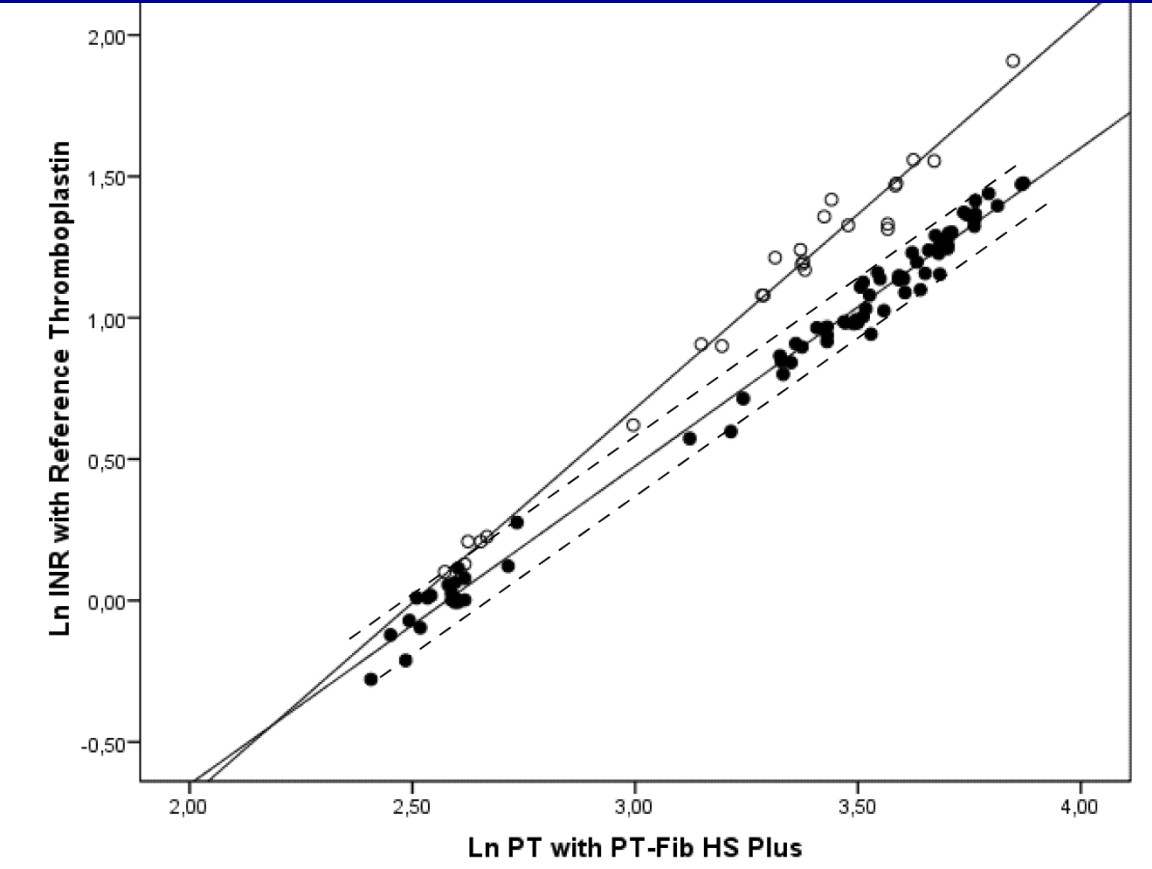
- PT is the most popular test for monitoring vitamin K antagonists (VKA).
- PT is not very specific because it is influenced by many factors.
- PT is determined with many different reagents (thromboplastin) and instruments.
- PT reference method: International Standard for thromboplastin + manual method (Tilt-tube).

Prothrombin Time

- PT for monitoring of VKA is expressed as International Normalized Ratio (INR), using a simple calibration model.
- Local calibration of PT methods may be performed with deep-frozen or freeze-dried plasmas with certified (INR) values.
- Commutability of deep-frozen or freeze-dried plasmas should be verified.

Certified plasmas: different types

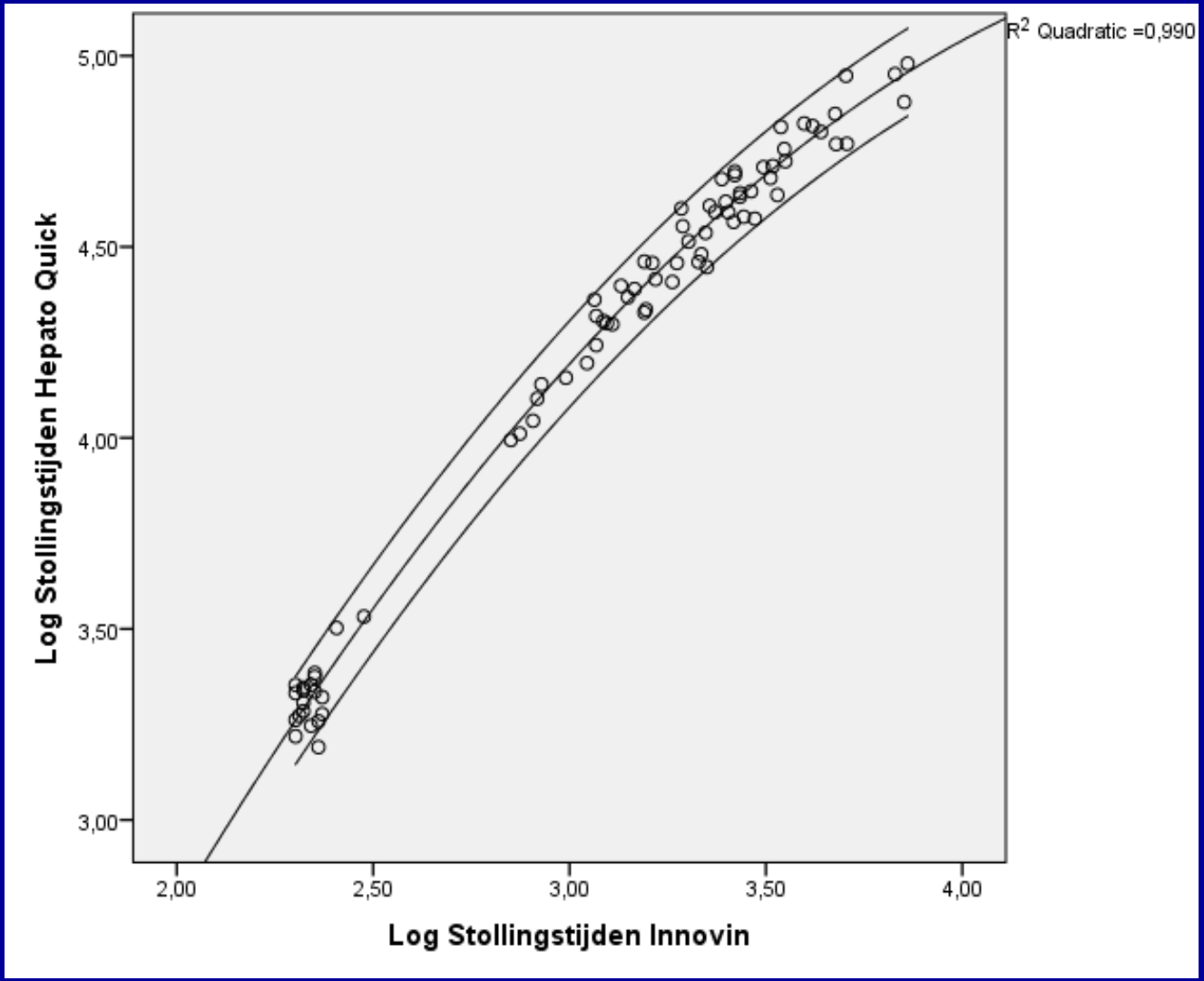
- Individual and pooled normal plasma
- Individual and pooled plasma from patients treated with VKA
- Individual and pooled normal plasma adsorbed with barium sulphate (artificially depleted plasma)
- Deep-frozen or freeze-dried



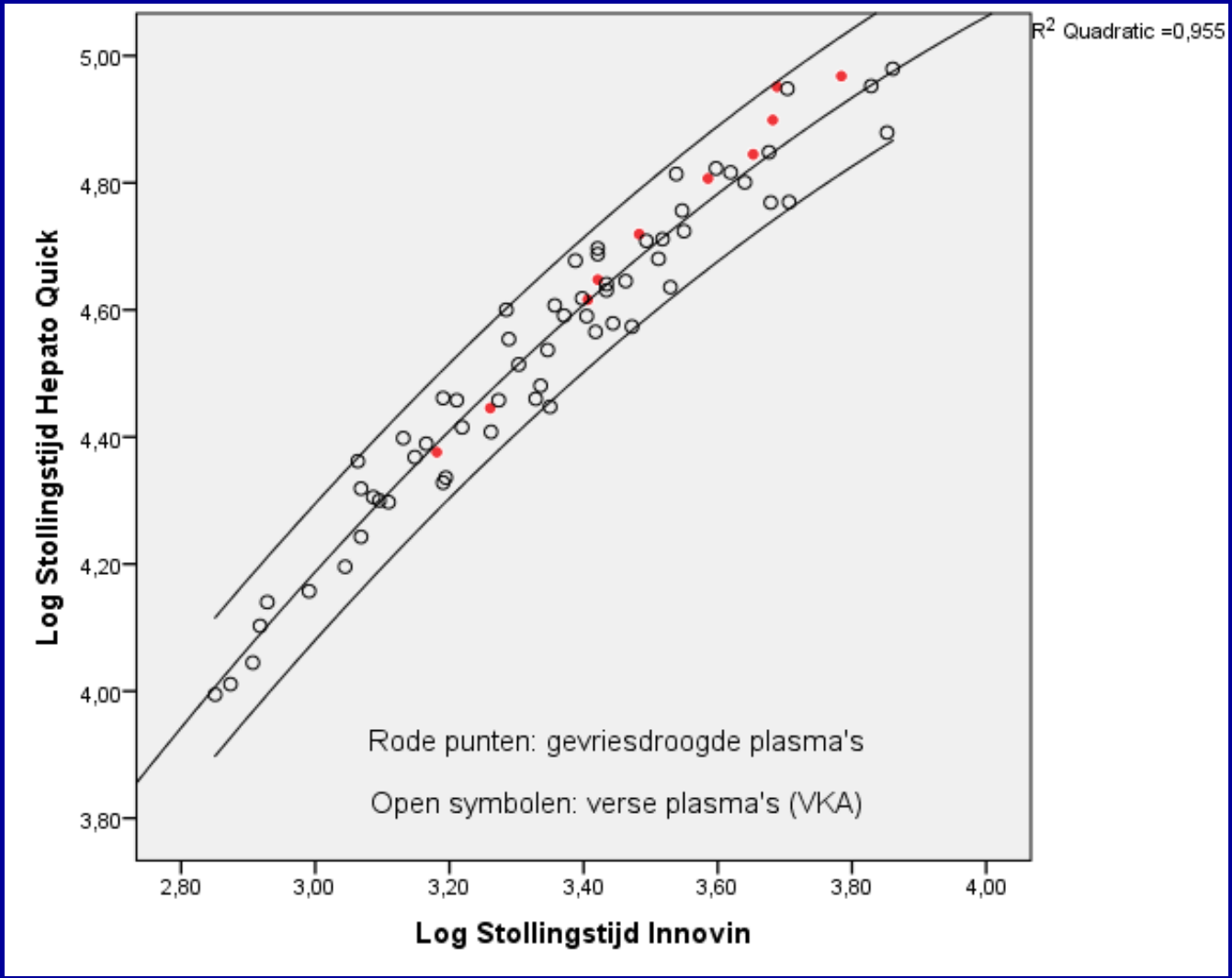
Commutability of Freeze-dried Artificially depleted Plasmas (J Thromb Haemost 2012;10:303)

Filled symbols: fresh native plasma samples of 20 normal and 60 VKA patients. Open symbols: 7 freeze-dried normal samples and 20 freeze-dried artificially depleted plasmas. Dotted lines: 95% prediction interval.

PT clotting times for fresh plasma samples from normal subjects and VKA patients



PT clotting times for fresh plasmas and for freeze-dried pooled VKA plasmas



Summary

- For most of the coagulation factors, international standard preparations are available.
- International standard for fibrinogen: g/L.
- Other standards: arbitrary units.
- There are no established reference methods for coagulation factor assays
- For PT/INR determination: manual tilt tube method is reference method.
- Commutability of processed plasmas can be problematic.