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Confidence in Biological Medicines

Have International Standards in haemostasis improved comparability of laboratory results?

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Measurement Systems-
Improving comparability of laboratory results

Metrological

- Primary reference method
- Defined analyte
- Quantify in SI
- Traceability to previous standard, with defined uncertainty
- ISO guidelines

Biological

- No established primary method
- Analyte often undefined – relative to effect/response
- Quantify in arbitrary units – International Unit
- A “physical” standard ie ampoule content defines the unit of measurement
- WHO guidelines

In an ideal Metrological World.....

ISO 17511:

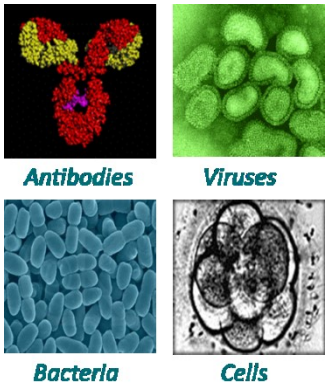
Definition of a Reference Method

"..... Having the highest metrological qualities, whose operation can be completely described and understood, for which a complete uncertainty statement can be written down in terms of SI units, and where results are therefore accepted without reference to a measurement standard of the quantity being examined."

- However, where reference method does not exist, harmonisation of measurement relies on a higher order reference material – measurement of biological analytes/medicines fall under this category

What are biological medicines?

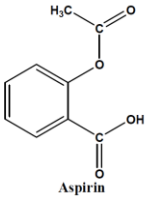
- Produced from biological systems
  - Cells, tissues, blood, organs
  - Include therapeutics manufactured by recombinant technology
- Highly complex in structure
  - Different to simple chemically synthesised drugs e.g. aspirin
  - Cannot be fully characterised and measured by physicochemical methods e.g. coagulation factors and inhibitors



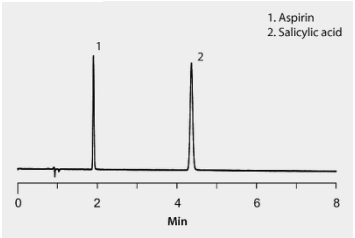
HUMAN COAGULATION FACTOR IX (rDNA) CONCENTRATED SOLUTION

Factoris IX coagulationis humani (ADNr) solutio concentrata

YNSGKLEEFV	QGNLERECME	EKCSFEEARE	VFENTERTTE	40
FWKQYVDGDQ	CESNPCLNGG	SCKDDINSYE	CWCPFGFEGK	80
NCELDVTCNI	KNGRCEQFCK	NSADNKVVCS	CTEGYRLAEN	120
QKSCEPAVPF	PCGRVSVSQT	SKLTRAEAVF	PDVDYVNSTE	160
AETILDNITQ	STQSFNDFTR	VVGGEDAKPG	QFPWQVVLNG	200
KVDAFCGGSI	VNEKWIVTAA	HCVETGVKIT	VVAGEHNIEE	240
TEHTEQKRNV	IRIIPHHNYN	AAINKYNHDI	ALLELDEPLV	280
LNSYVTPICI	ADKEYTNIFL	KFGSGYVSGW	GRVFHKGRSA	320
LVLQYLRVPL	VDRATCLRST	KFTIYNNMFC	AGFHEGGRDS	360
CQGDSGGPHV	TEVEGTSFLT	GIISWGEECA	MKGKYGIYTK	400
VSRYVNWIKE	KTKLT			415



Aspirin



How do we measure haemostasis related analytes?

- Biological activity are measured using bioassays
- Bioassays determine the amount of biologically active substance present in complex mixtures
- Measurement can be estimated:
  - In absolute quantities i.e. independent of an external reference standard e.g by mass balance – synthetic direct thrombin and Xa inhibitors
  - In comparative quantities i.e. activity expressed relative to a defined reference standard e.g. in International Unit against International Standard – FVIII and FIX

Advantages of International Unit (IU)

- 1 IU = activity found in 1 ml of normal plasma (Exceptions: activated factors e.g. thrombin, FVIIa)

1 IU/ml = 100% normal

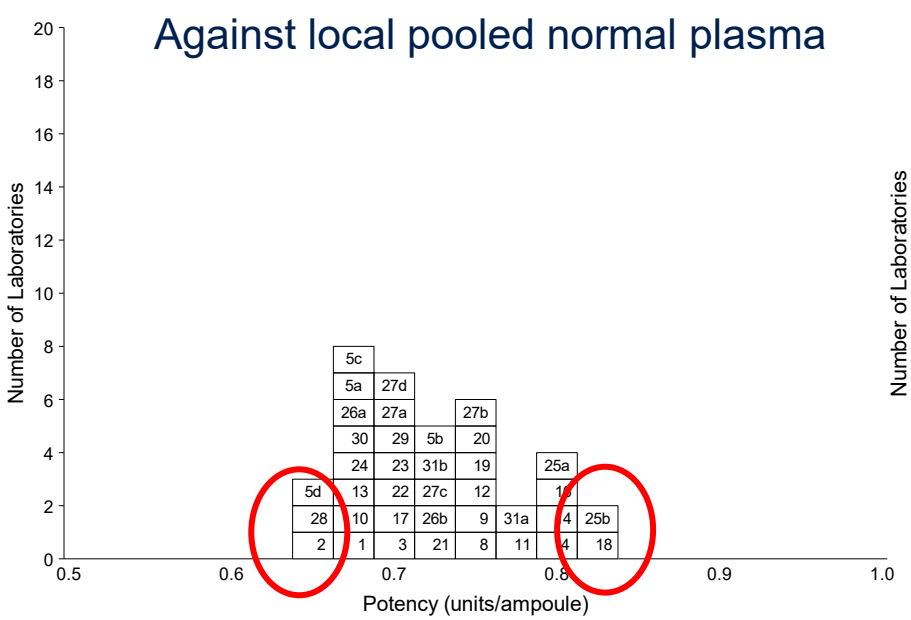
- While activity of local normal pool can change and that normal pool from different labs are “not the same”, once the IU is defined for the first standard then it is fixed for subsequent replacement preparations
- Local pools should be calibrated against the International Standard (IS) or other reference preparations traceable to the IS

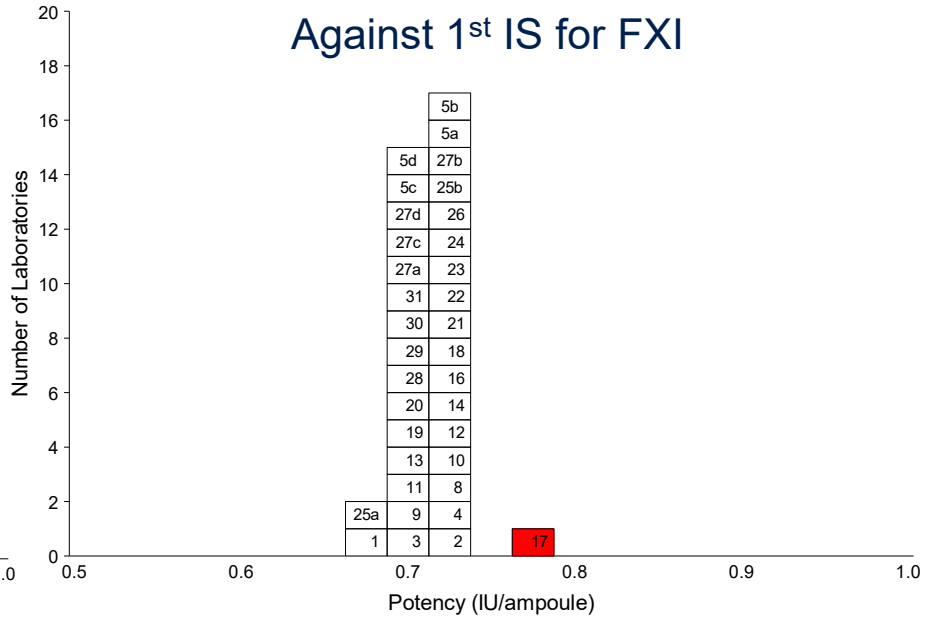
Facilitate agreement of level of “activity” between labs

- Labelled potency of products in IU are linked to the Plasma IU

Enable the understanding of normal and deficient levels and aids the calculation of target levels for therapy.

Value assignment of 2nd International Standard for Blood Coagulation Factor XI, Plasma





Overall geometric mean: 0.72 u/ampoule
GCV: 7.2%, n = 29

Overall geometric mean: 0.71 IU/ampoule
GCV: 1.8%, n = 29

Assay against a common standard improves inter-laboratory agreement

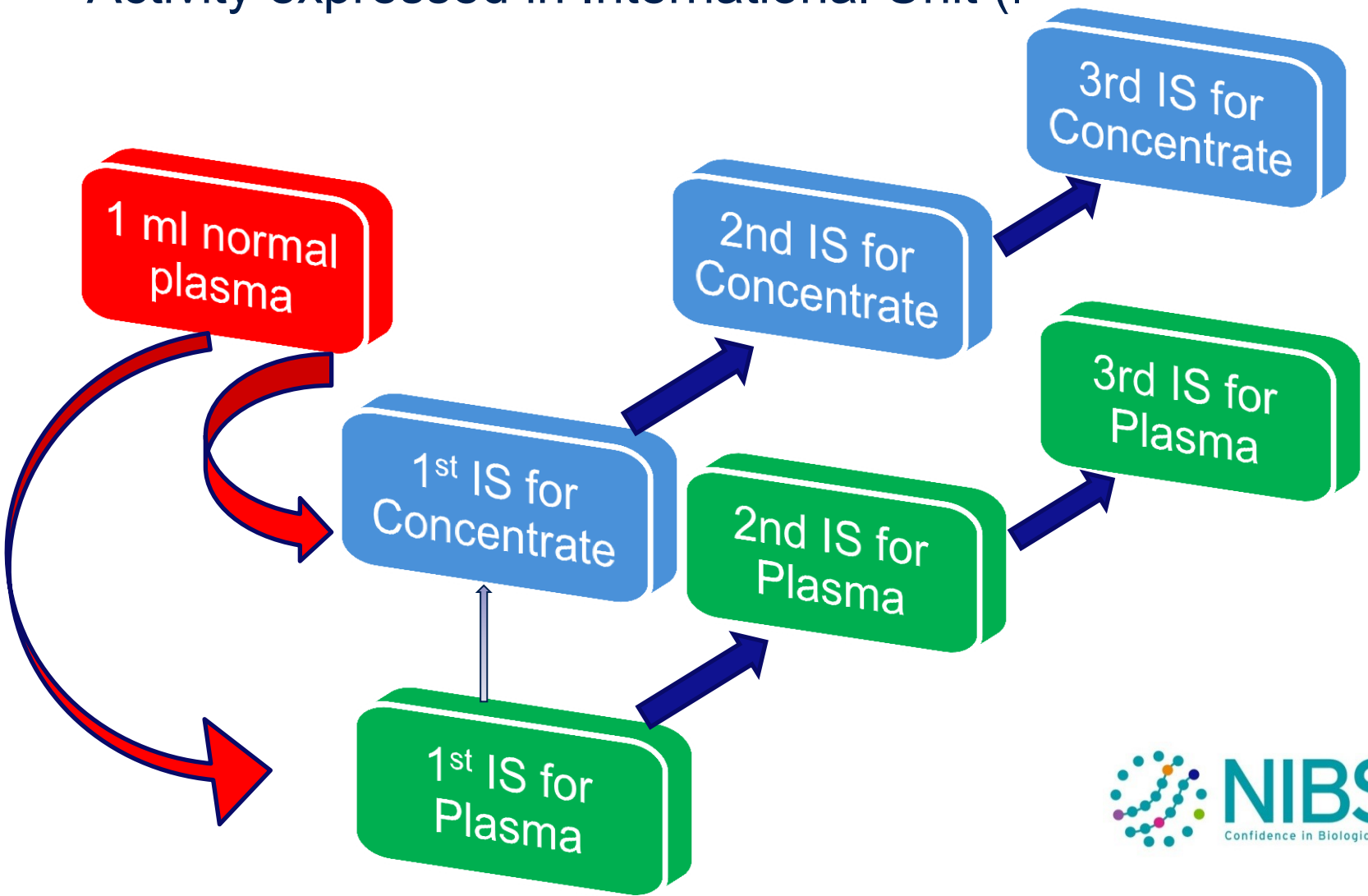
Comparison of potency estimates relative to the 3rd IS and the local pooled plasmas (assuming 1 u/ml)

	Vs sample S, 3rd IS		Vs sample N, normal pooled plasma		Difference between potencies	t-test, *p* value
	Potency estimates (95% CL) IU/ampoule	Inter-lab GCV	Potency estimates (95% CL) IU/ampoule	Inter-lab GCV		
FII	0.892 (0.888 – 0.897)	1.2 %	0.880 (0.862 – 0.897)	5.1%	1.4%	0.316
FVII	0.987 (0.977 – 0.998)	2.9%	0.933 (0.896 – 0.971)	11.2%	5.5%	0.007
FIX	0.863 (0.854 – 0.873)	2.8%	0.812 (0.788 – 0.837)	8.0%	6.0%	<0.001
FX	0.887 (0.882 – 0.893)	1.5%	0.847 (0.821 – 0.874)	8.0%	4.5%	0.004

No of normal plasma donor ≥ 747, from 29 laboratories, 14 countries

Unit of activity for clotting factors

Based on biological activity of the coagulation factor
Activity expressed in International Unit (IU)



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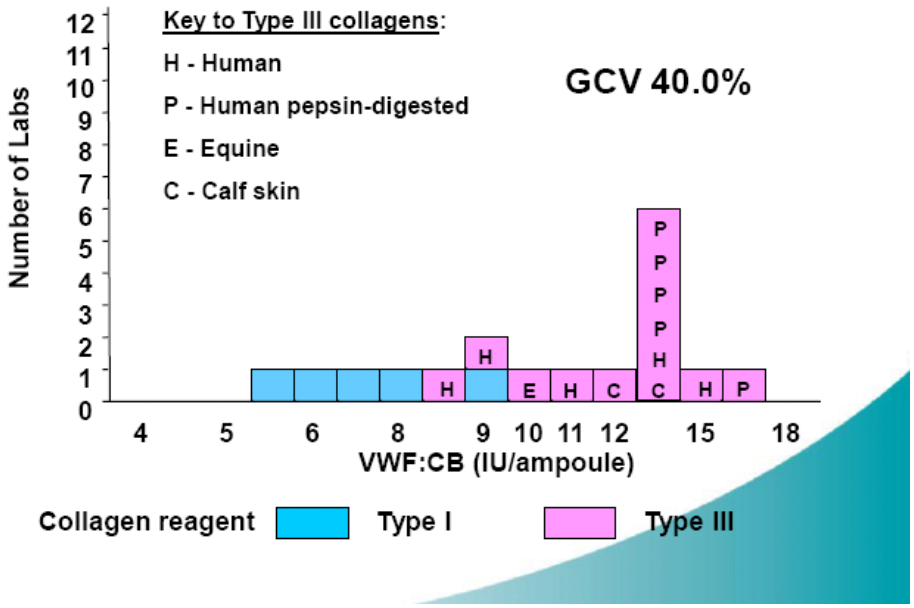
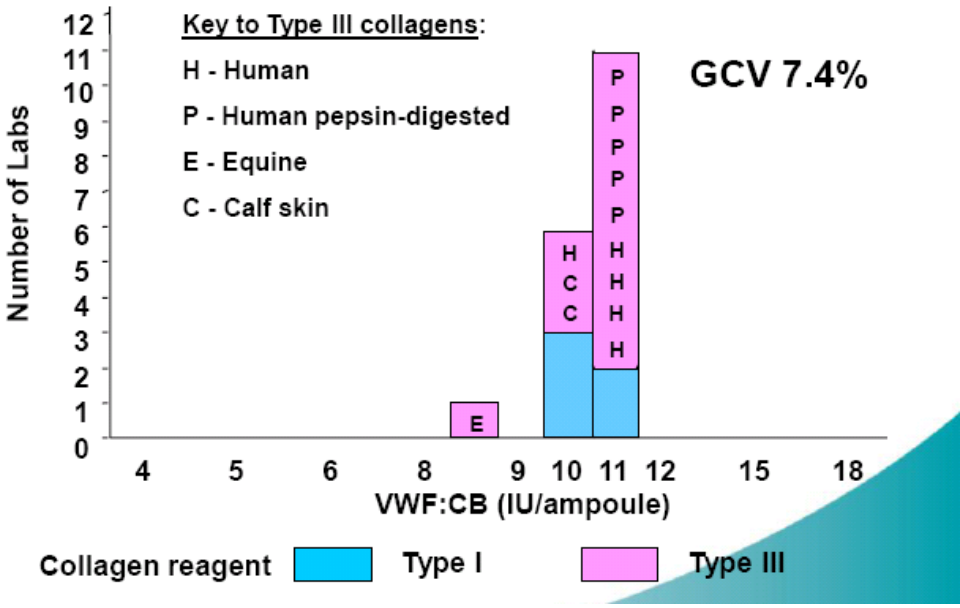
Like against Like: Principle of Biological Standardisation

Test sample should behave as dilutions of reference standard

von Willebrand Factor Concentrate- Collagen Binding Activity





Against the 4th IS for vWF, Plasma

Against the 1st IS for vWF, Concentrate

Assay like against like (concentrate against concentrate) improves inter-laboratory agreement

Traceability of International Unit
Example: Unfractionated Heparin

- The “Howell”/”Toronto” unit (1923): One unit of heparin is an amount required to keep 1 ml of cat's blood fluid for 24 hours at 0 °C.
- International Unit (IU) is the activity contained in a one ampoule of the current International Standard for Unfractionated Heparin
  - Derived by consensus mean of all assay methods used by different participating laboratories in a collaborative study against the previous standard
  - Continuity of unit from the 1st International Reference Preparation, established in 1941 by The League of Nation to the current 6th IS

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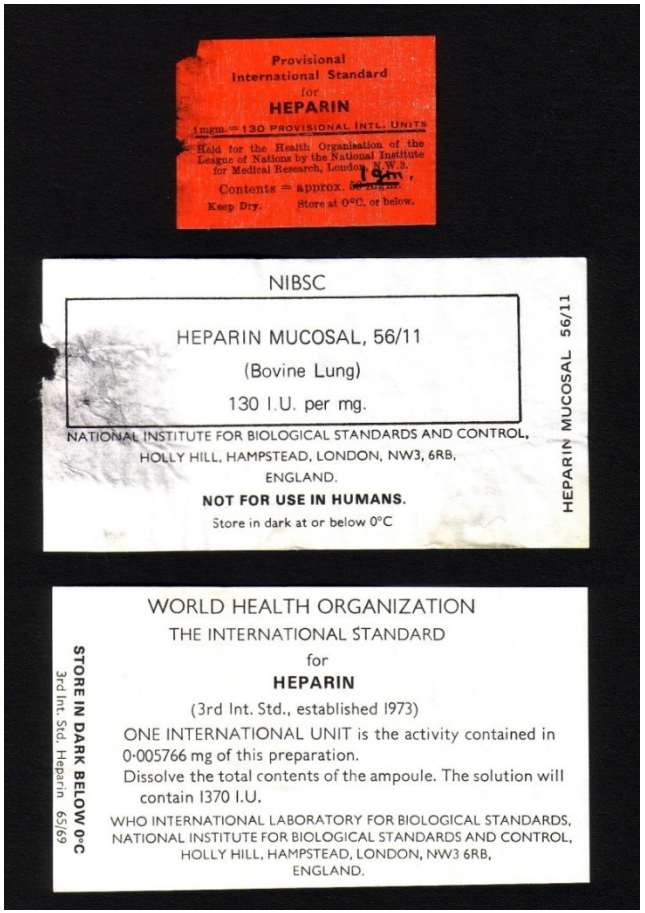
International Standards for Unfractionated Heparin

Samples	Labelled IU/mg	Specific Activity IU/mg (95% confidence limits)		
		Anti-Xa	Anti-IIa	APTT
1st IS Bovine lung	130	136 128 - 144	145 133 - 157	140 131 - 149
2nd IS Bovine Lung	130	135 127 - 144	136 125 -146	139 131 - 147
3rd IS Porcine mucosa	173	176 161 - 191	178 161 - 195	173 160 - 186



Against the 5th IS for Unfractionated Heparin

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WHO International Standard: Material of Higher Order

WHO Technical Report Series, No. 932, 2006

Annex 2

Recommendations for the preparation, characterization and establishment of international and other biological reference standards (revised 2004)

The process whereby international biological reference standards are established, and the technical specifications with which they comply, are set out in this guidance document, which is intended to be scientific and advisory in nature.
The parts of each section printed in large type are definitive requirements for international biological reference standards. The parts of each section printed in smaller type are comments for additional guidance and are intended to provide further explanation of the text in large print.

Is it possible to measure haemostasis related analytes in SI?

- Activated coagulation factors and fibrinolytic proteins are enzymes
- Activity can be measured in derived SI unit e.g.
  - 1µmol in 1 min
  - katal
- Requires a reference method

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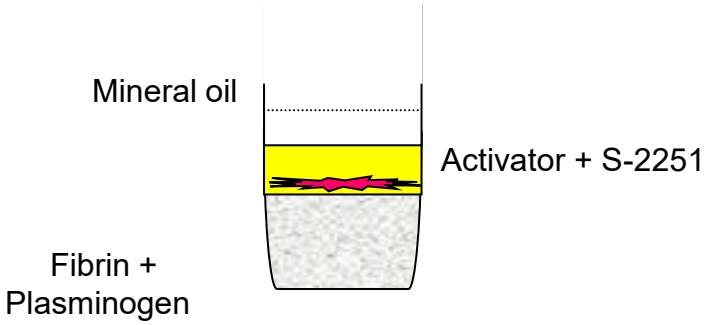
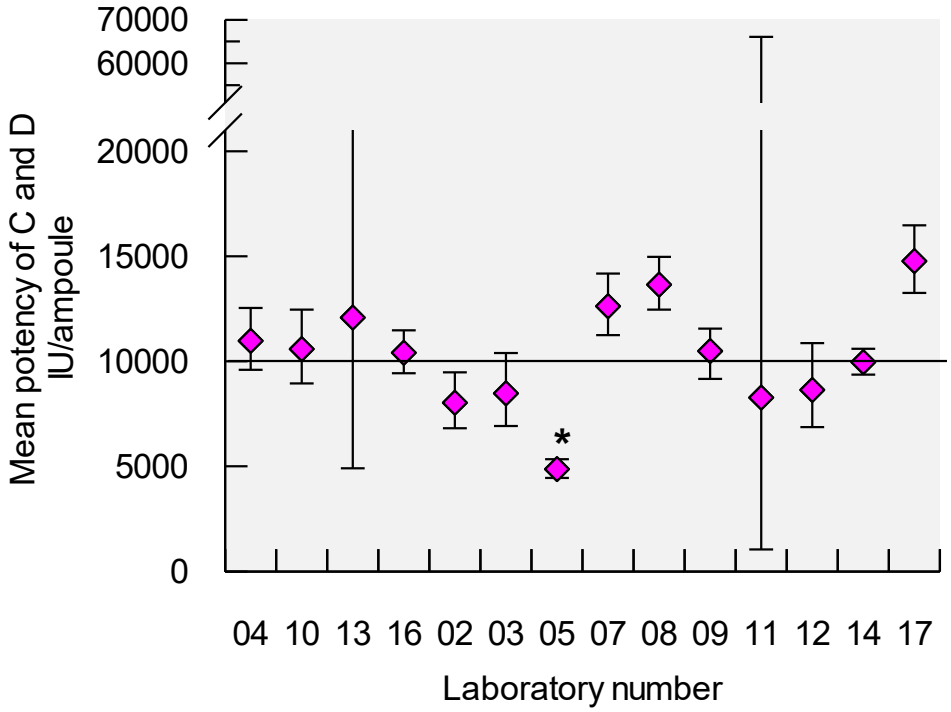
SSC (Colin Longstaff): Can we measure fibrinolysis proteins in absolute units?

([Plasmin]/s in a defined method)





- *From Sands, Whitton, Merton and Longstaff Thromb Haemost 2002, 88: 294*

A reference method for all plasminogen activators?

Journal of Thrombosis and Haemostasis, 2: 1416–1421

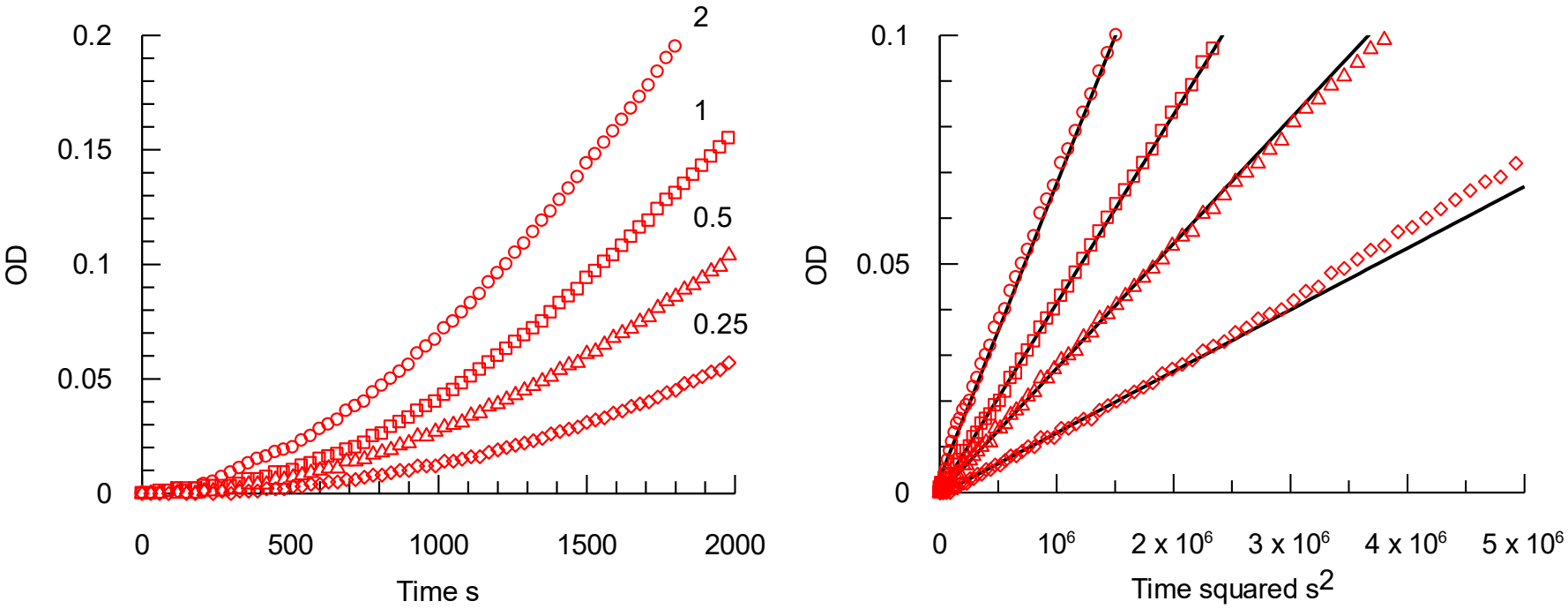
ORIGINAL ARTICLE

A proposed reference method for plasminogen activators that enables calculation of enzyme activities in SI units

C. LONGSTAFF and C. M. WHITTON
Division of Haematology, National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, UK

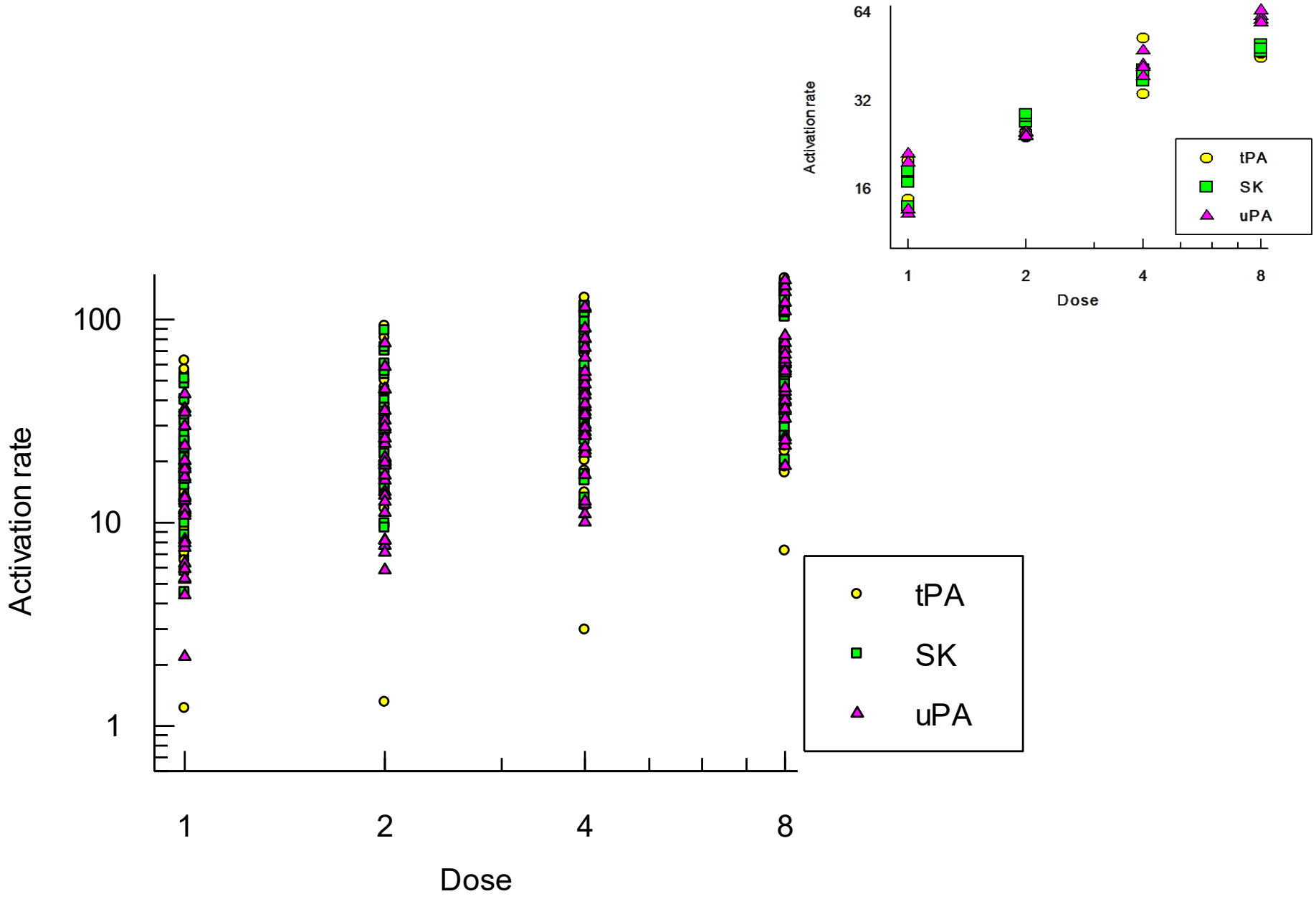
- A method was developed that could be used to measure all plasminogen activators
- The activities could be expressed in SI units
- In theory this would avoid the need for multiple standards for engineered variants

Raw and transformed data from fibrin overlay method with streptokinase



Materials and Methods

Reagent	Code.	Number provided	[6]Reconstituion volume ml	[7]Dilution	[8]Recommended volumes	Working conc
[1]Fgn	98/614	3	1.0	5	0.8->4.8 ml	2.5mg/ml
[2]Plgn	97/536	3	0.5	-	-	-
[3]Thrombin	01/578	1	1.0	25	80µl + 2.0ml	4IU/ml
tPA	98/714	3	1.0	78.125	80µl->0.5ml 80µl->1.0ml	128IU/ml
SK	00/464	3	1.0	312.5	40µl->0.5ml 40µl->1.0ml	3.3IU/ml
uPA	87/594	3	1.0	40	25µl->1.0ml	107.5IU/ml
[4]CS-41(03)	n/a	1	15.0	4	0.75->3.0ml	0.8mM
[5]Plasmin	97/536	1	1.0	4	0.1ml->0.4ml	1.3IU/ml


Activation rate
100
10
1
1
2
4
8
Dose
tPA
SK
uPA
64
32
16
Activation rate
1
2
4
8
Dose
tPA
SK
uPA

The reference method performed poorly in the field

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OFFICIAL COMMUNICATION OF THE SSC

An international collaborative study to investigate a proposed reference method for the determination of potency measurements of fibrinolytics in absolute units

C. LONGSTAFF,* C. WHITTON,* C. THELWELL* and D. BELGRAVE†, ON BEHALF OF THE FIBRINOLYSIS SUBCOMMITTEE OF THE SSC OF THE ISTH
**Haemostasis Section; and †Biostatistics Section, National Institute for Biological Standards and Control, South Mimms, Herts, UK*

- **Absolute methods are difficult - more difficult than relative methods to apply in practice**

Conclusions

- Haemostasis related analytes are complex biologicals
- Current technology and physio-chemical assay methods cannot fully characterise and quantify these analytes
- Robust primary reference methods are currently not available for measurement in SI
- International Standards, defining International Units have served well to improve agreement within and between laboratories

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Acknowledgments

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Helen Wilmot

Tony Hubbard

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Thank you for your attention

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