

# WHO International Standards

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# Overview

- Background WHO, NIBSC, International Standards and International Units
- How to make an International Standard
- Worked examples from Haemostasis/Blood products
  - C1-inhibitor, Alpha-1-Antitrypsin
- Interactions and joint standards with other organisations
- Engineered proteins-standardisation challenges
- Stability and continuity considerations for lyophilised standards
- Summary

# The National Institute for Biological Standards and Control



*Assuring the Quality of Biological Medicines*

# History of International Standards

- 1921 the Health Committee of the League of Nations identified biological standards as high priority
- Responsibility later passed on to the UN and WHO.
- The Expert Committee on Biological Standards (ECBS) of the WHO began to establish standards in 1947 by the process that is familiar today.
- IS receive the final approval of the Director General of WHO following recommendation from the ECBS and member nations of the UN agree to implement these units.

# WHO Biological Reference Standards I

## Key points

WHO Constitution (article 2) “ to promote international standardisation with respect to food biological, pharmaceutical and similar products”

- A Biological Substance
  - “a substance which cannot be completely characterised by physico-chemical means alone and which therefore requires some form of bioassay”
- Standard
  - “Like versus like” principle
  - May be poorly characterised, heterogeneous, complex, macromolecular
  - Labelled in International Units (arbitrary)
  - Potency agreed as consensus value following a collaborative study
- Methods
  - Comparative not absolute
  - Standards should be independent of methods used
  - Over-rigid definition of methods is to be avoided
  - Possible to define minimum requirements (avoid matrix effects)

# WHO Biological Reference Materials II

## SI Units

- Where appropriate a WHO biological reference standard may be calibrated in SI units
- The choice of IU or SI unit should be made on a case-by-case basis reflecting the biological, medical and physicochemical information available.
- SI units should be derived from, and traceable to, physicochemical procedures.
- More likely to necessitate the existence and use of defined methods and an assignment of uncertainty, derived from calibration data.

*See ISO 17511 and WHO TRS 932*

# Standards & Reference Materials

- **Central to NIBSC's function**

- Originating from insulin work in 1920s

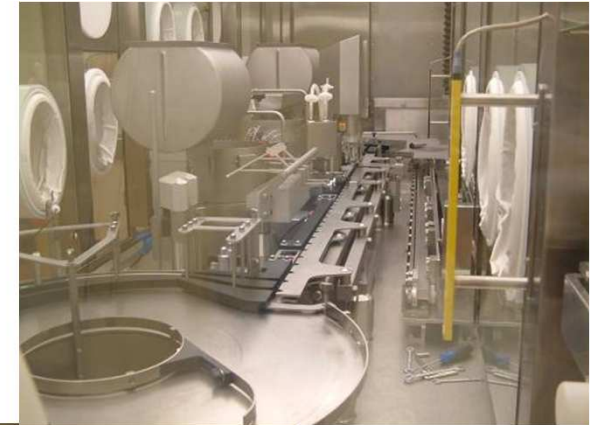


- **NIBSC produces >90% of World's International Standards for biological medicines**

- WHO International Laboratory for Standards

- **Centre for Biological Reference Materials**

- Catalogue list of ~600 items (>300 IS)
- Distributed ~100,000 items in 5000 shipments
- Inventory ~ 2m ampoules and vials



- **Centre for AIDS Reagents**

- **CJD Resource Centre**

- **UK Stem Cell Bank**



# League table of standards shipped (non-influenza) 2010/11

1-10		11-20		21-30		31-40		41-50	
Coagulation Factor	<b>IS</b>	Coagulation Factor	<b>IS</b>	Inhibitor	<b>IS</b>	Inhibitor	<b>IS</b>	Coagulation Factor	<b>IS</b>
	WS		<b>IS</b>		<b>CE</b>		SD	Coagulation Factor	<b>IS</b>
Coagulation Factor	<b>IS</b>		<b>IS</b>		<b>IS</b>	Coagulation Factor	<b>IS</b>	Coagulation Factor	<b>IS</b>
	<b>CE</b>	Coagulation Factor	<b>IS</b>		<b>IS</b>		<b>IS</b>		SD
Plasma	WS		<b>CE</b>		<b>IS</b>		<b>IS</b>		SD
	WS		<b>CE</b>		SD		SD		SD
	WS	Coagulation Factors	<b>IS</b>		<b>IS</b>	Coagulation Factor	<b>IS</b>		<b>IS</b>
	<b>IS</b>		<b>IS</b>		<b>CE</b>		<b>CE</b>		<b>IS</b>
Inhibitor	<b>IS</b>		<b>IS</b>	Coagulation Factor	<b>IS</b>	Coagulation Factor	WS		<b>IS</b>
Inhibitor	<b>IS</b>	Inhibitor	<b>IS</b>		<b>IS</b>	Coagulation Factor	<b>IS</b>		<b>IS</b>

19/50

Haemostasis  
Blood products

Blood virology

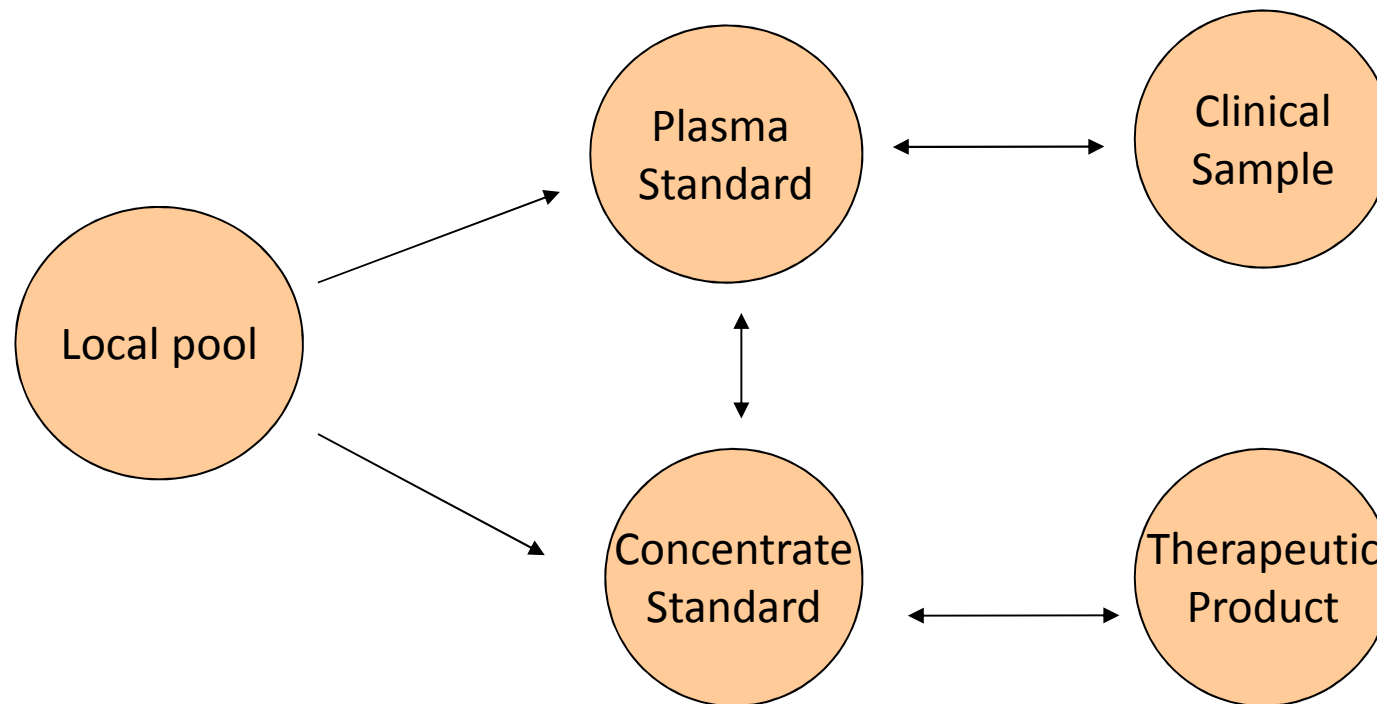
Hormones  
Cytokines  
Vaccine etc

# How to make an International Standard



# International Units-Haemostasis

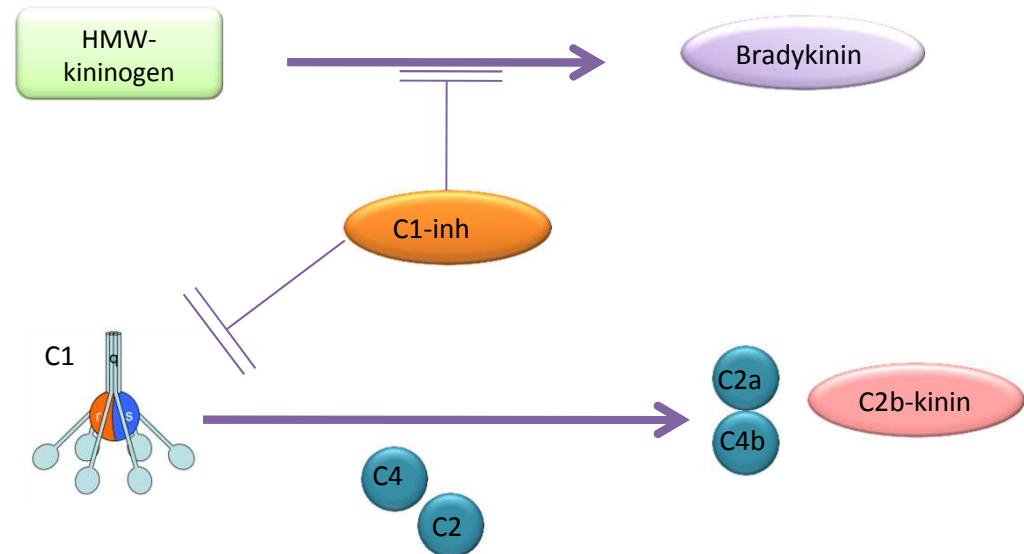
- Clotting factors-the traditional approach
  - The IU is linked to plasma concentration
  - “the activity in fresh normal plasma”



# C1-inhibitor IS for plasma and concentrate

- **Disease association**
  - C1-inh deficiency is most commonly associated with hereditary angioedema (HAE)
- **Diagnosis**
  - HAE diagnosis determined by functional assay:
- **Treatment**
  - Purified C1-inh used in replacement therapy for treatment of HAE:
    - Plasma derived:
      - Berinert P (CSL Behring)
      - Ceter (Sanquin)
      - Cinryze (Lev)
    - Recombinant:
      - Rhucin (Pharming) – [Phase III]

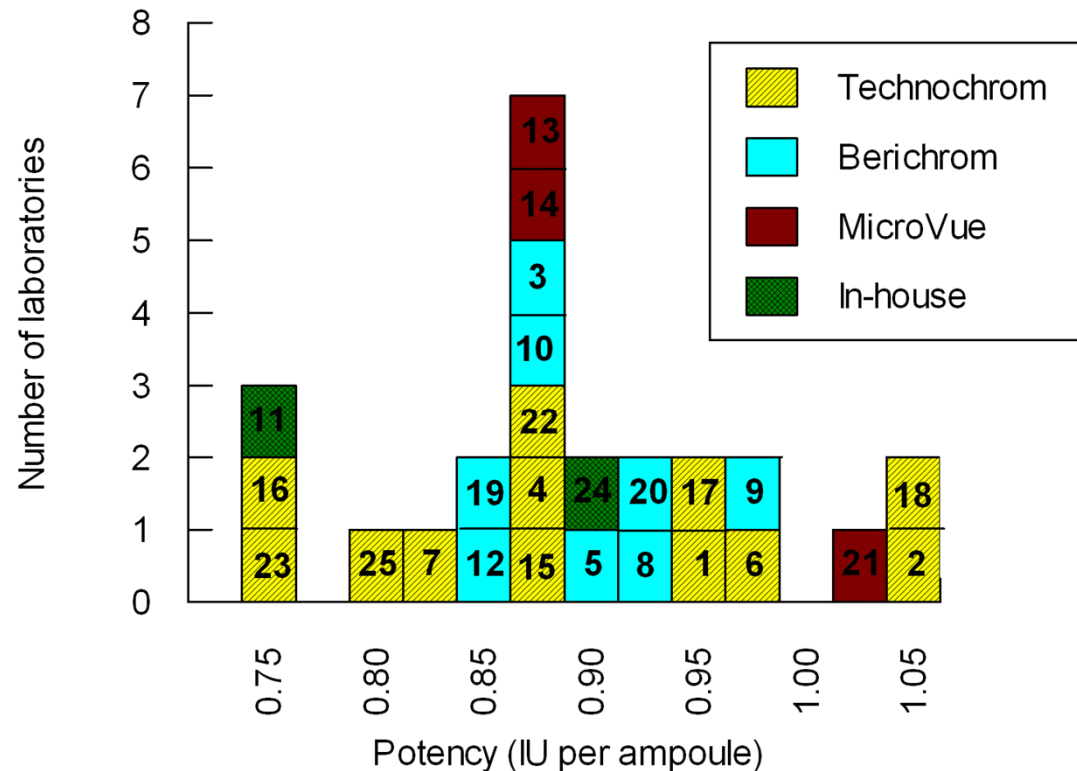
C1-inhibitor, inhibits C-1 esterase and proteases of the fibrinolytic, clotting and kinin pathways



# The WHO 1<sup>st</sup> IS for C1-inhibitor, plasma (08/262)

Diagnostic standard

Freeze dried candidate plasma pool is measured against local pools (I IU/ml)



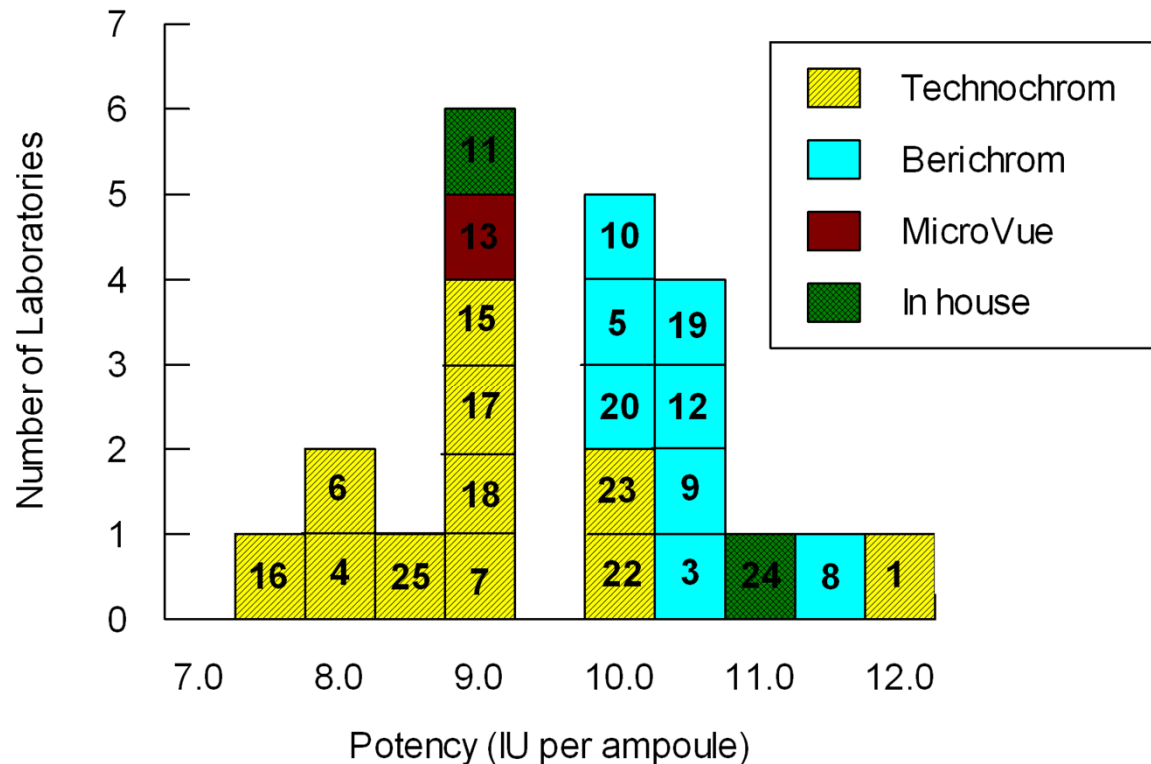
**Geometric mean 0.89 IU**

GCV = 9.9 %

*Thelwell et al, J Thromb  
Haemost, 2011, in press*

# The WHO 1<sup>st</sup> IS for C1-inhibitor concentrate (08/256)

- The concentrate is measured against the local pools
- There is a discrepancy associated with method used
- The agreed potency of the new concentrate IS will help harmonise results from different manufacturers using different methods



**Geometric mean 9.6 IU**

GCV = 12.8 %

*Thelwell et al, J Thromb Haemost, 2011, in press*

# Background: Alpha-1

## ■ Clinical background

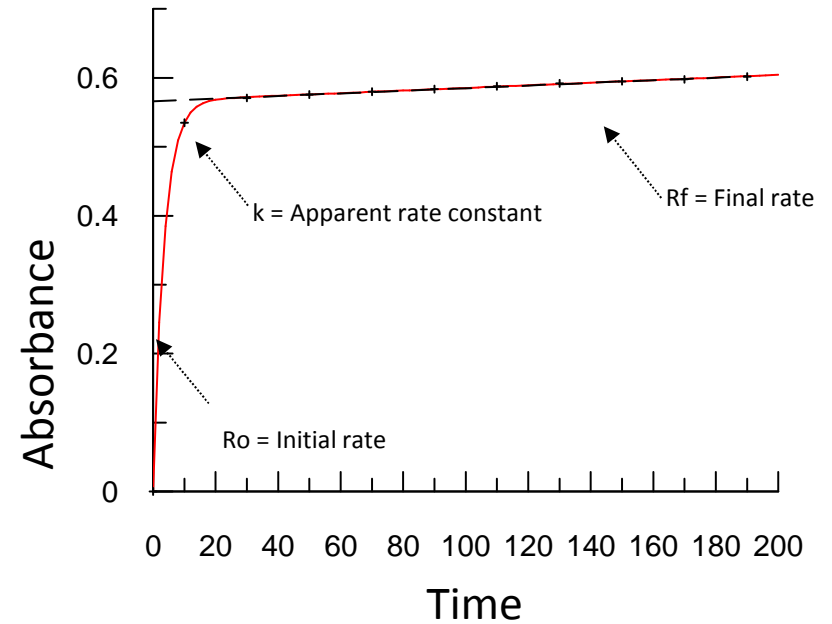
- Alpha-1 has been identified in virtually all populations. It is estimated that as many as 1 in every 2,500 Americans have Alpha-1, for example.
- Alpha-1 can lead to lung destruction and is often misdiagnosed as asthma or smoking-related **Chronic Obstructive Pulmonary Disease (COPD)**.
- Treatment can be augmentation therapy with plasma-derived Alpha-1-Antitrypsin

## ■ Goals of the study

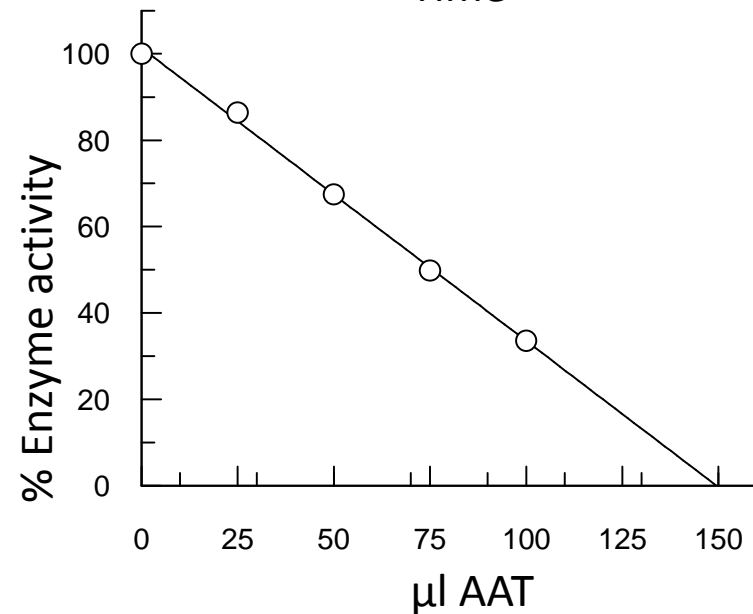
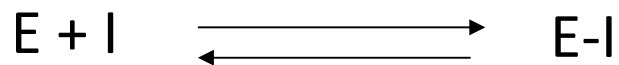
- New International standard for AAT to standardise dosing for replacement therapy
- Units of mg/ampoule (SI units)
- New Eur Ph monograph method for potency testing
- Collaboration between NIBSC, FDA/CBER, Regulators, Industry, Academia, Eur Ph Group 6B, Alpha-1 Foundation

# Calibrating Trypsin and AAT

- Stage 1
  - Active site titration of trypsin (E)
  - % activity of stock trypsin



- Stage 2
  - Titration of AAT against trypsin



# The WHO 1<sup>st</sup> IS for AAT (05/162)

	A (05/150)	B (05/152)	<b>C (05/162)</b>	D (05172)
Ampoules	9674	9481	<b>9792</b>	708
Filling weight (cv)	1.0058 (0.19)	1.0061 (0.21)	<b>1.0057 (0.15)</b>	1.0057 (0.21)
SEC HPLC % Monomer	95.4	77.3	<b>91.4</b>	98.0
nmoles AAT (mg)	205 (10.6)	218 (11.1)	<b>243 (12.4)</b>	197 (8.8)
Total protein mg	10.4	12.0	<b>12.4</b>	nd
Antigen mg	10.6	10.6	<b>12.4</b>	nd

- Choose the “best” candidate from the study (good agreement with all products)
- The molar concentration is converted to mg-the units familiar to clinicians
- All candidates satisfactory, recombinant products can be standardised using the appropriate Mwt
- Subsequent studies assigned total protein and antigen values to the IS

# Examples of collaborations and joint standards

## An international collaborative study to establish the WHO 1st international standard for alpha-1-antitrypsin

C. Thelwell,<sup>1</sup> E. Marszal,<sup>2</sup> P. Rigsby<sup>3</sup> & C. Longstaff<sup>1</sup>

<sup>1</sup>Biotherapeutics Group, Haemostasis Section, National Institute for Biological Standards and Control, South Mimms, Herts, UK

<sup>2</sup>Division of Hematology, FDA/CBER, Lincoln Drive, Bethesda, MD, USA

<sup>3</sup>Biostatistics Group, National Institute for Biological Standards and Control, South Mimms, Herts, UK

*WHO IS with FDA/CBER, new Eur Ph monograph with Group 6B*

## A reunification of the US (“NIH”) and International Unit into a single standard for Thrombin

Colin Whitton<sup>1</sup>, Dawn Sands<sup>2</sup>, Timothy Lee<sup>3</sup>, Andrew Chang<sup>3</sup>, Colin Longstaff<sup>1</sup>

<sup>1</sup>Division of Haematology, and <sup>2</sup>Informatics Division, National Institute for Biological Standards and Control, Herts, UK

<sup>3</sup>Center for Biologics Evaluation and Research, U.S. Food and Drug Administration, Rockville, Maryland, USA

*Unification of “NIH” unit and IU for Thrombin in a joint WHO and US standard*

## An international collaborative study to replace the 1st international standard for prekallikrein activator

C. Longstaff,<sup>1</sup> M.-E. Behr-Gross,<sup>2</sup> A. Daas<sup>2</sup> & F. Lackner<sup>3</sup>

<sup>1</sup>Division of Haematology, National Institute for Biological Standards and Control, South Mimms, Herts, UK

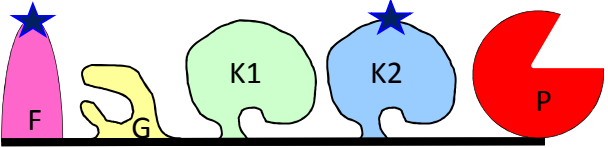
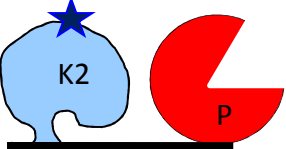
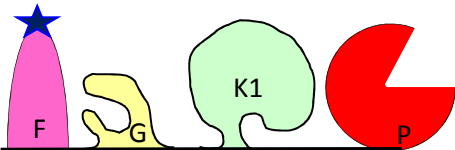
<sup>2</sup>Division IV, European Directorate for the Quality of Medicines, Council of Europe, Strasbourg, France

<sup>3</sup>Federal Institute for Medicines, Vienna, Austria

*New WHO IS for PKA and working standard with EDQM*

# Standardisation of engineered proteins

- Tissue plasminogen activator (Genentech) was a blockbuster clotbuster, recombinant drug from the 80's.
- Variants were developed which posed problems for standardisation
- Very different binding properties, which regulate biological activity
- Are separate standards necessary for each variant?

Standard	Domain structure ★ = Important fibrin-binding domains	Commercial Product
<b>3<sup>rd</sup> IS for tPA, recombinant</b>		<b>Alteplase</b>
<b>In house standard for rPA</b>		<b>Reteplase</b>
<b>In house standard for Desmoteplase</b>		<b>Desmoteplase (Phase III)</b>

# Units of enzyme Activity

- Units (1 $\mu$ mol in 1 min; katal)-Absolute
  - Measure product or substrate
  - Conditions carefully defined
- WHO International Units (IU)-Relative
  - Specific for each enzyme
  - Reference preparations defines the unit
  - Method not defined

# 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

# Enzyme activities in the reference method

Activity units	PA	
	tPA (98/714)	Streptokinase (00/464)
IU/ampoule (from collaborative study)	10 000	1030
<sup>1</sup> nM plasmin per s	1.50	7.69
<sup>1</sup> pmoles plasmin per s	0.06	0.31
<sup>1</sup> Specific Activity μmoles plasmin per s for 1 mole PA	0.20	1.45

<sup>1</sup> Determined using proposed reference method

# 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**

Reference system	Goals
Reference Material	Traceability to defined reference method
Reference Method	Specified uncertainties
Reference Laboratories	Commutability with clinical values

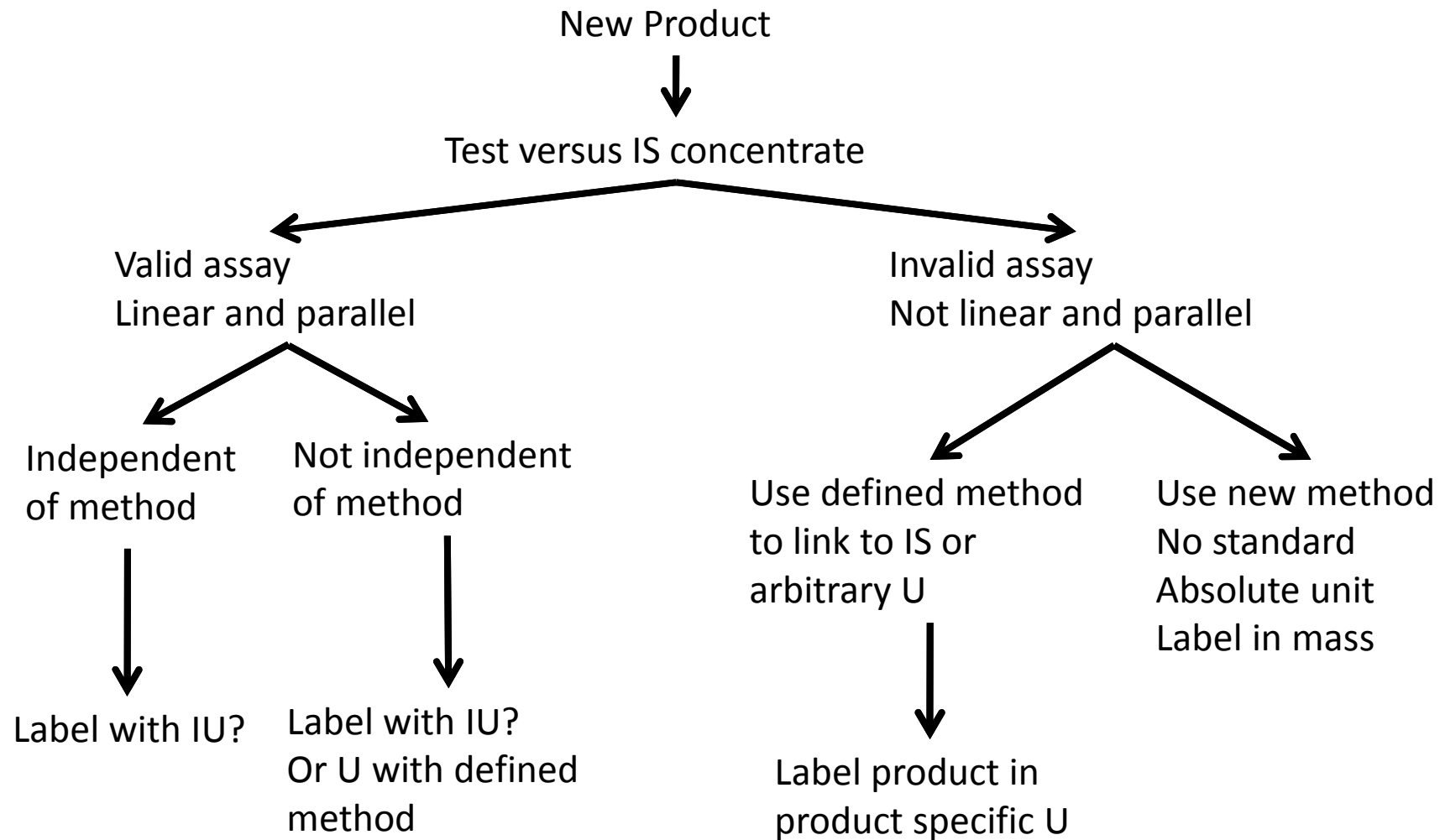
# A single IU for all products – for how much longer?

- novel bioengineering developments – Factor VIII:
  - improve expression (yield) of recombinant products:
    - truncated molecules (B-domain-deleted FVIII)
  - increase stability of activated FVIII
    - disulphide bond stabilised FVIIIa
  - extend plasma half-life:
    - chemical modification (pegylation, sialylation)
    - formulation with pegylated-liposomes
    - FVIII-Fc fusion
  - resistance to FVIII inhibitory antibodies:
    - recombinant porcine B-DD FVIII
    - human/porcine FVIII hybrids

# Harmonised approach to potency labelling of new products

- The diversity of new products requires a case by case approach
- Need agreement between licensing authorities on the principles which will be applied in deciding the route towards potency estimation
- Discussion and guidance should be available to manufacturers during product development
- Possible approaches to standards and methods development should be investigated by industry, academic and standardisation societies, scientists and regulatory bodies (licensing authorities, pharmacopoeias etc)

# Options for modified products

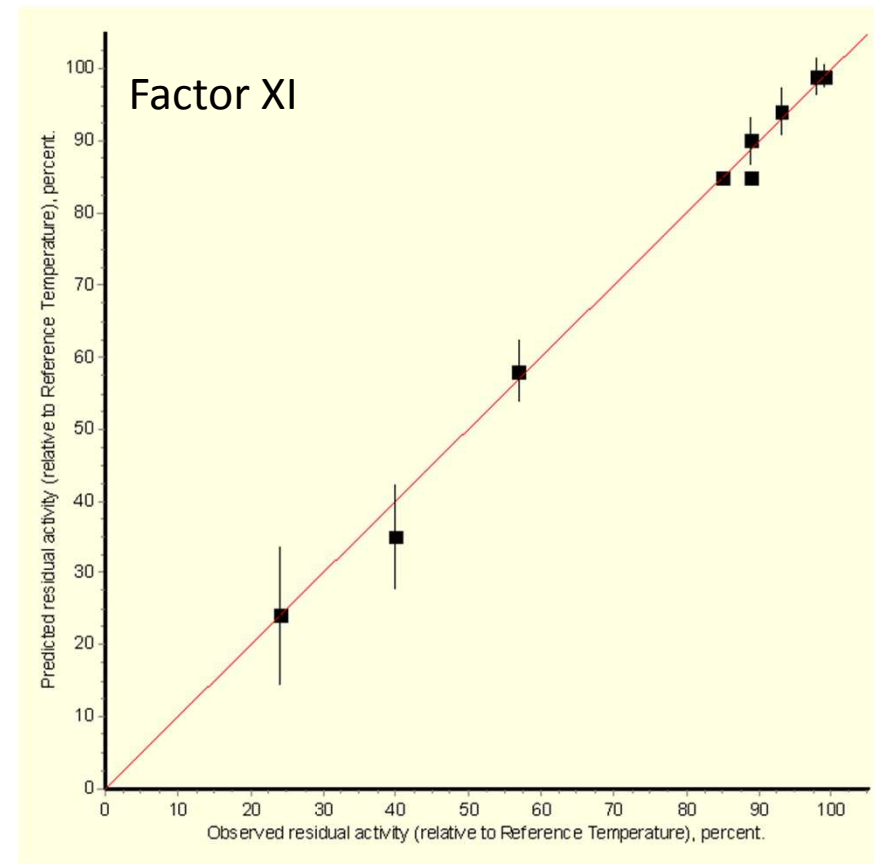
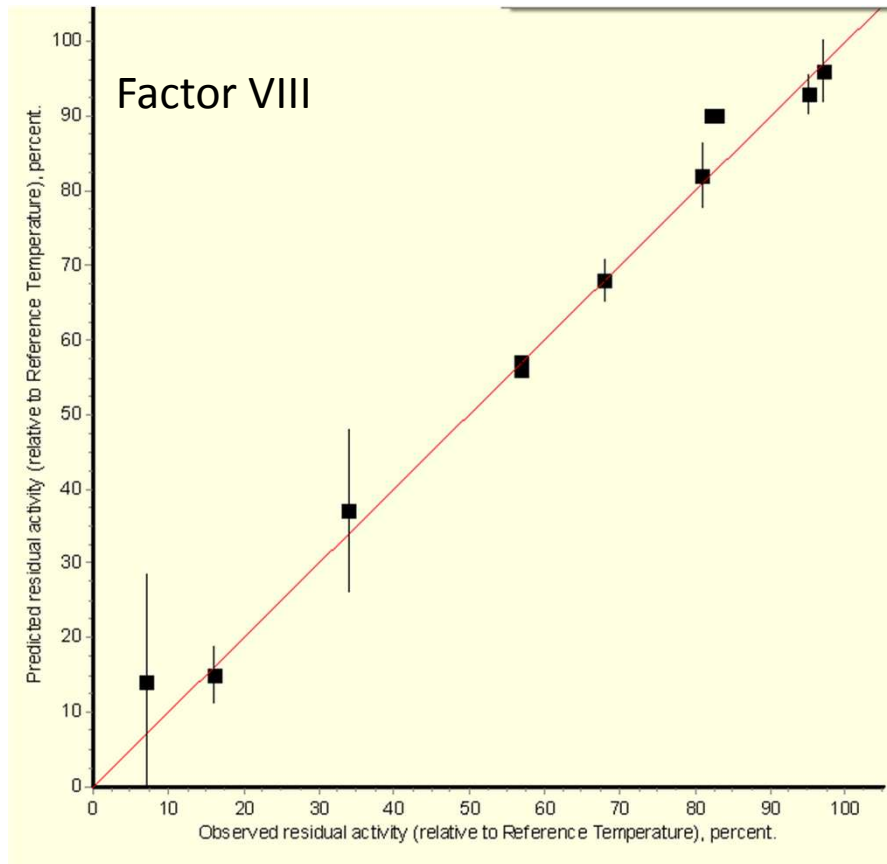


# Stability and IU continuity

- Standards need to be demonstrably stable
- The IU should be conserved between IS replacements
- The formulation of IS may be adjusted to enhance stability
- Accelerated degradation studies are performed to investigate long term stability (Arrhenius model)
- Ongoing real time stability studies are also carried out
- Example data from lyophilised plasma WS (ISTH/SSC Coagulation Secondary Standard)

# ISTH/SSC Lot #3 lyophilised plasma Accelerated degradation study

## Observed vs Predicted residual activity 6 years of data



# SSC Lot #3

## Accelerated degradation study

- predicted % loss per year at various storage temperatures

Storage temp (°C)	<u>Factor V</u>	Factor VII	<u>Factor VIII</u>	Factor XI
-20	0.034	0.015	0.098	0.004
+4	1.08	0.40	1.76	0.24
+20	7.74	2.66	8.95	2.55
+37	41.60	15.10	36.65	21.79

# SSC Lot #3

## Real-time stability

- potency estimates for vials stored at -70 °C relative to vials stored at -20 °C 6.2 years

Assay	Factor V		Factor VII		Factor VIII		Factor XI	
	NIBSC	RHH	NIBSC	RHH	NIBSC	RHH	NIBSC	RHH
<b>Mean</b>	<b>97%</b>	<b>95%</b>	<b>98%</b>	<b>100%</b>	<b>96%</b>	<b>96%</b>	<b>100%</b>	<b>102%</b>

# SSC Lot #3

## Predicted stability and shelf-life (-20°C)

- combined data, 6 years

<b>Predicted loss and shelf-life</b>	<b>Factor V</b>	<b>Factor VII</b>	<b>Factor VIII</b>	<b>Factor XI</b>
Mean % loss per year at -20 °C	0.034	0.015	0.098	0.004
Upper 95% confidence limit of loss	0.056	0.039	0.207	0.012
†Shelf-life (years)	91	131	25	444

† - time period during which relative potency drops < 5% based on upper (95%) confidence limit of degradation rate

# Summary

- WHO IS are generated for diagnostic, quality, safety and potency roles
- WHO IS are established as primary reference materials to assist in the calibration of secondary or working standards
- WHO IS are prepared, characterised and established by a well recognized, rigorous, formalised system of approval
- The long history of IS and investigations into stability supports the continuity of IU over time
- The selection of IS and unit is robust, collaborative and flexible
- Over many years IS have met the needs of clinicians, manufacturers, regulators, NGOs and academia
- IS are valued worldwide and are able to respond rapidly to emerging needs