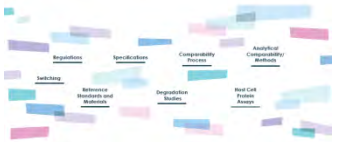


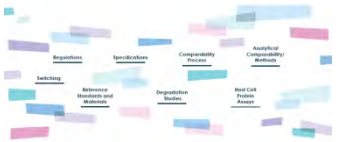
Elaine Gray, PhD, UK

- Principal Scientist, National Institute for Biological Standards and Control, UK

GaBI Scientific Meetings

Second Colombian Scientific Meeting on Quality Assessment of **BIOSIMILARS/SIMILAR BIOTHERAPEUTIC PRODUCTS**

15 August 2017, Hiilton Bogotá, Colombia



Value assignment of International Standards: challenges for potency labelling of biotechnology/ biosimilar products

Elaine Gray, PhD

15 August 2017

GaBi
GENERICS AND BIOSIMILARS INITIATIVE
Building trust in cost-effective treatments

invima
Instituto Nacional de Vigilancia de Medicamentos y Alimentos

PPCI
Pro Pharma Communications International

Biotechnology Products are biologicals

World Health Organization (WHO) definition of a biological:

"Substances and complex materials, whether of biological, biotechnological or synthetic origin, that cannot be characterized fully by chemical and/or physical means alone.....and which therefore requires the use of some form of a bioassay**."**

Naturally derived	Biotechnology produced
Plasma derived clotting factors	Monoclonal antibodies
Heparin, low molecular weight heparin	Recombinant DNA vaccines
Thrombolytics	Recombinant DNA hormones
Vaccines	Recombinant clotting factors
Anti-venoms	Transgenic clotting inhibitors

Measurement of Bioactivity - Bioassays

Exact nature and mechanisms of action of biologicals not always known – most will cause ‘multiple’ effects (or relevant ‘end’ effect may result from ‘cascade’ of events)

A biological measurand does not necessarily refer to a defined analyte. It also can refer to groups of components, antigen epitopes, catalytic activity, immunoreactivity or infectivity

Bioassays are usually complex systems, direct measurement of analyte/measurand using primary measurement methods not always possible

Underlying principle of such assays is that they depend on the comparison of the response of the test substance with that of a reference standard

WHO Role

"To define an internationally agreed unit to allow comparison of biological measurements worldwide"

WHO International Standards

International standards for biologicals are materials of higher order, endorsed and established by the World Health Organisation.

Serve as primary reference materials -

- the standard should be calibrated in arbitrary rather than absolute units
- the unit is directly traceable to a standard with a physical existence
- the calibration of the standard, and therefore the unit, is unrelated to a specific method of determination

World Health Organization

Attributes of Primary Reference Materials

Definition of unit - Arbitrary unit, relates to effect/response of the biological

Continuity of unit - Similarity to current standard

Multiple method assignment - Minimise assay bias

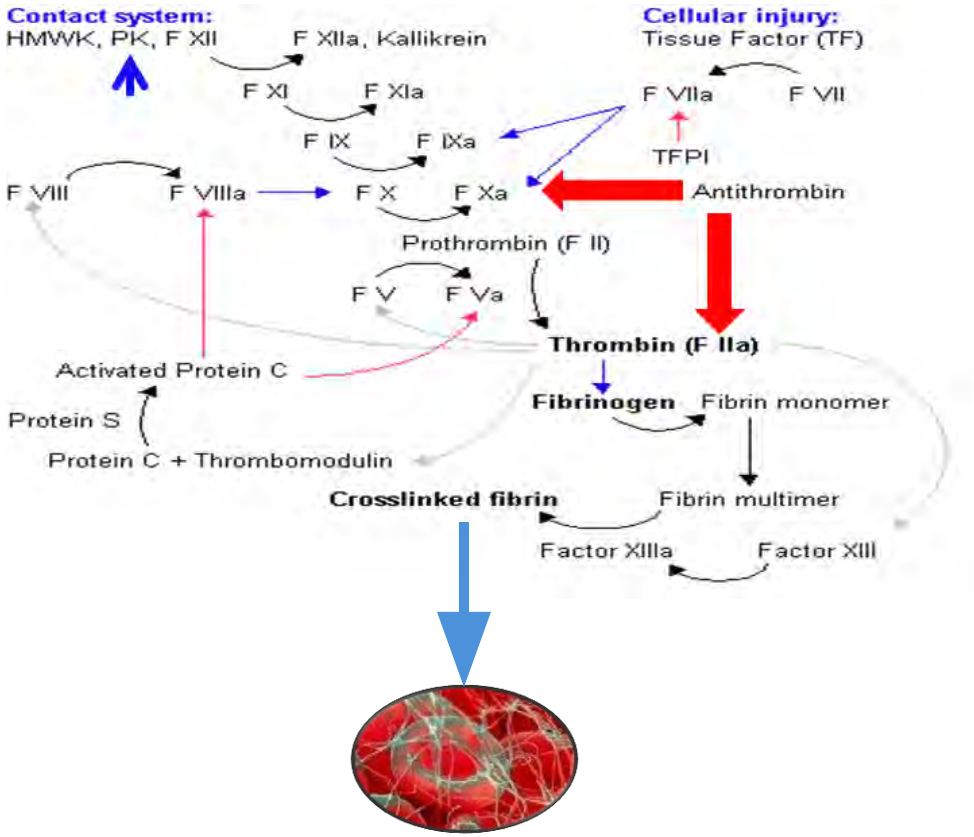
Like against like - Similarity to test samples

Case study – Clotting factors and inhibitors

Haemostasis – a balanced interaction of blood cells, vasculature and coagulation factors and inhibitors –
no bleeding and no thrombosis

Deficiency of coagulation factor(s) – bleeding disorders e.g. FVIII deficiency – haemophilia A

Deficiency of coagulation inhibitor(s) – Thrombosis e.g. antithrombin deficiency - thrombophila



Measurement of clotting factors and inhibitors

- Important for diagnosis of congenital and acquired deficiencies
- Important for potency labelling of replacement therapeutics
- Important for correlation of circulating levels of therapeutics and clinical outcome

Bioactivity assays of target coagulation factors and inhibitors
- No direct measurement
- Endpoint readout usually several steps from target factor in the cascade
- Require reference standard to give relative potency/activity

Attributes of Primary Reference Materials

Definition of unit - Arbitrary unit, relates to effect/response of the biological

Continuity of unit - Similarity to current standard

Multiple method assignment - Minimise assay bias

Like against like - Similarity to test samples

Units of Coagulation Factors and Inhibitors

1 International Unit =

Amount or activity in 1mL of

“average fresh normal plasma”

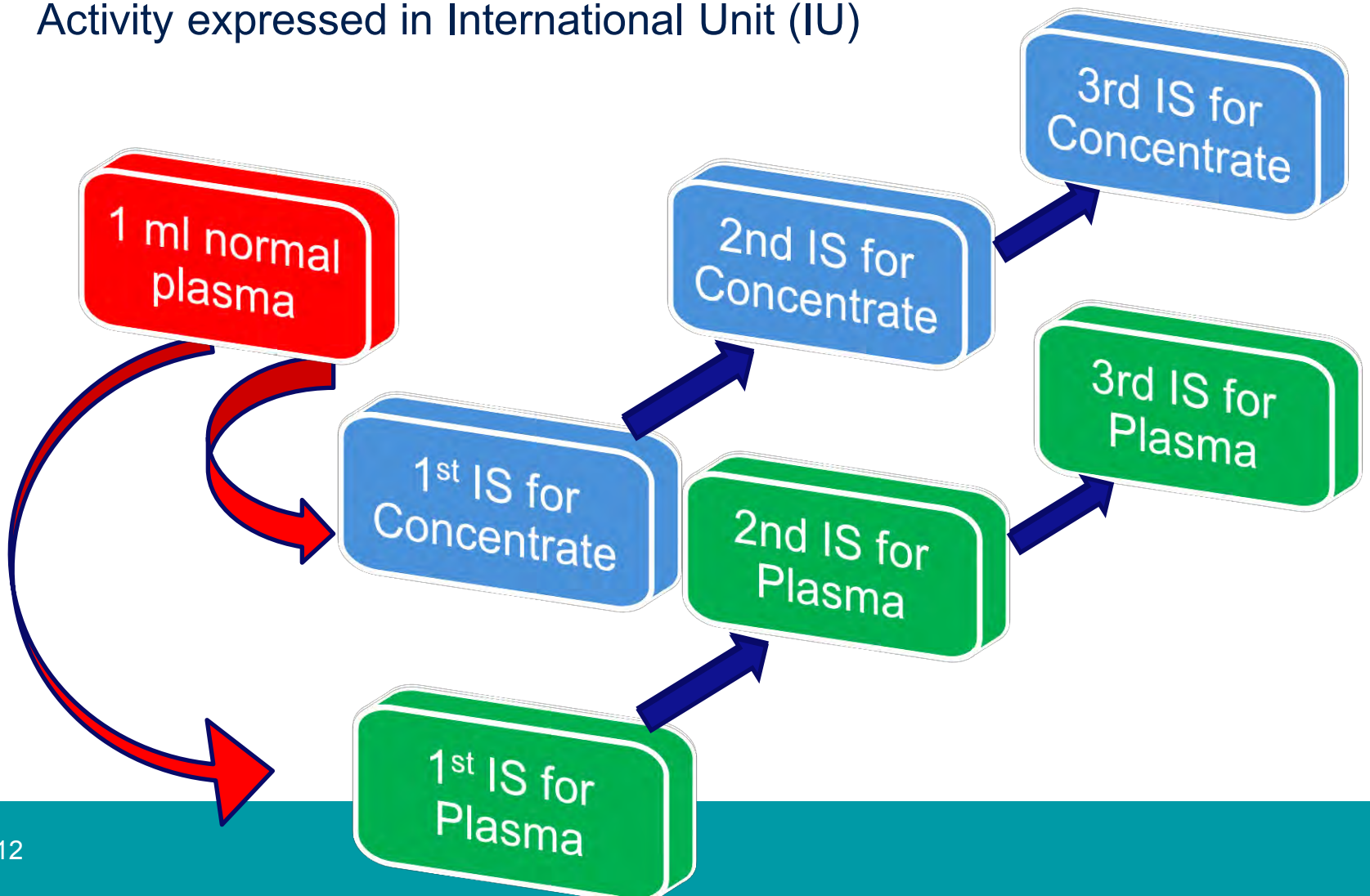
As defined by pools of fresh plasma collected from normal donors in labs participating in international collaborative study, according to a defined protocol (normally > 300 donors – diverse ethic background, gender, age)

Other reference standards – unit can be totally arbitrary

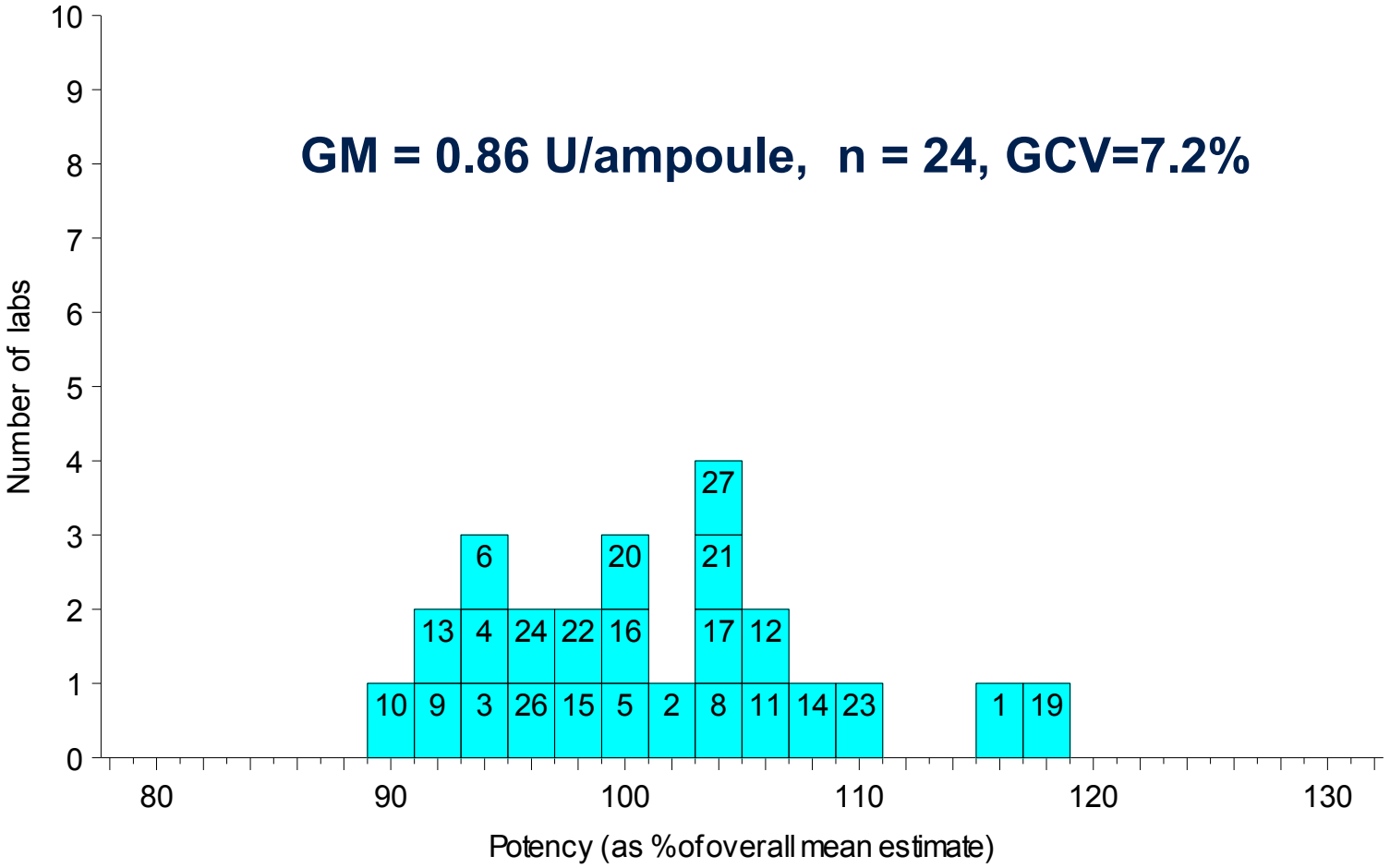
Unit of activity for clotting factors and inhibitors

Based on biological activity of the coagulation factor

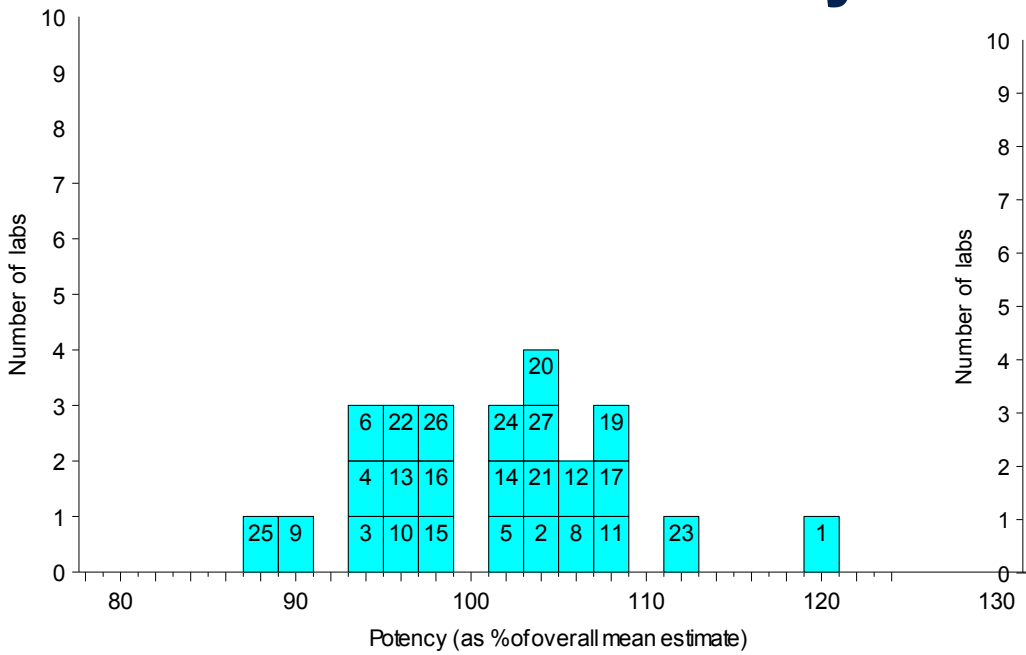
Activity expressed in International Unit (IU)

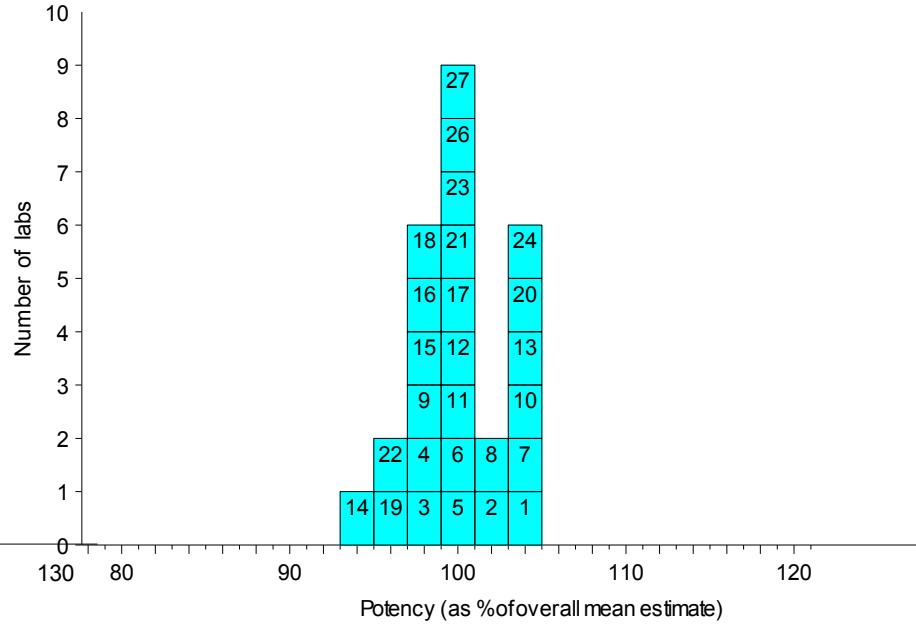


1st IS for FXI, plasma –Value assigned against >300 donors plasma



SSC Lot #3 Secondary Plasma Standard





Against local pooled normal plasma
GM = 0.87 U/vial
GCV = 7.7%

Against 1st IS for FXI, Plasma
GM = 0.88 IU/vial
GCV = 3.1%

Attributes of Primary Reference Materials

Definition of unit - Arbitrary unit, relates to effect/response of the biological

Continuity of unit - Similarity to current standard

Multiple method assignment - Minimise assay bias

Like against like - Similarity to test samples

Continuity of Unit

Long-term use of same batch of standard?

- Dependent on batch size and demand
- Assuming no significant degradation

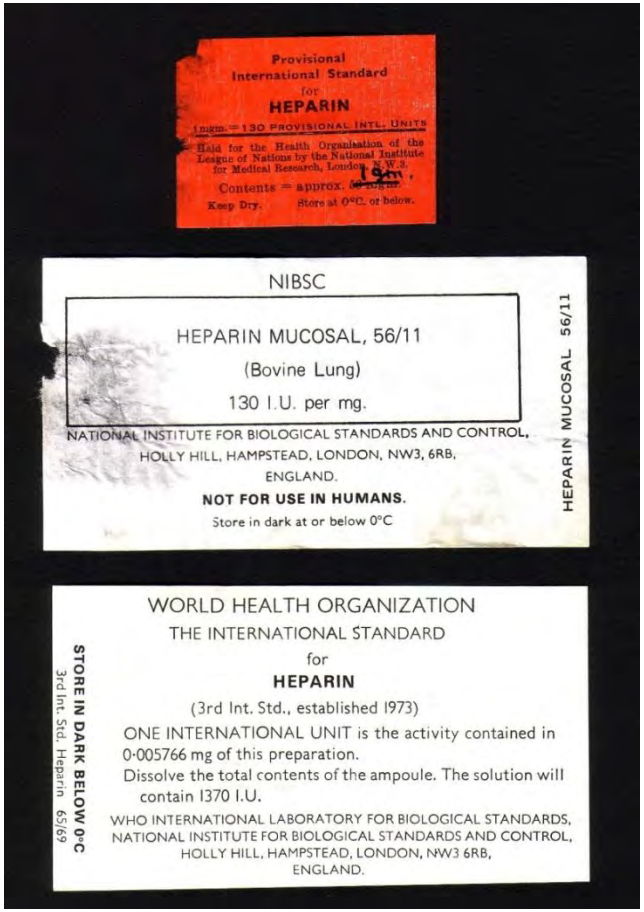
Replacement by similar material?

- Not always possible, products changes over time
- Same lab, same methods? – not always possible

Calibrate against preceding standard

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 Est. 1941	130	136 128 - 144	145 133 - 157	140 131 - 149
2nd IS Bovine Lung Est. 1956	130	135 127 - 144	136 125 -146	139 131 - 147
3rd IS Porcine mucosa Est 1973	173	176 161 - 191	178 161 - 195	173 160 - 186



Against the 5th IS for Unfractionated Heparin in 2008

Attributes of Primary Reference Materials

Definition of unit - Arbitrary unit, relates to effect/response of the biological

Continuity of unit - Similarity to current standard

Multiple method assignment - Minimise assay bias

Like against like - Similarity to test samples

“All Methods” Approach

- “Reference methods” not easily definable for biologicals such as coagulation factors/inhibitors

- WHO Standards should be suitable for use with all current methods

Deviation from the mean of any assay result is composed of two elements: assay imprecision and method bias

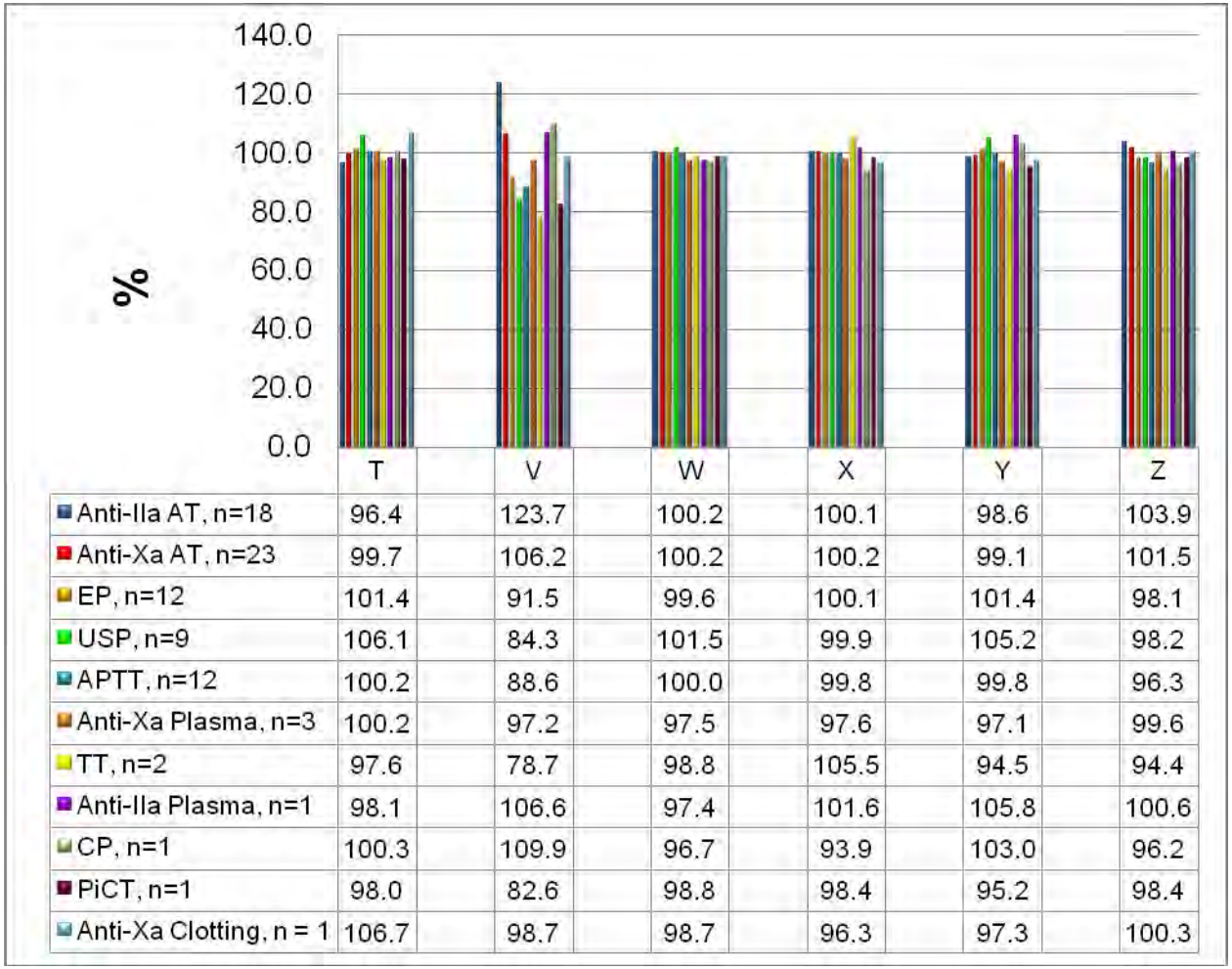
- **The WHO multi-method approach, by including all assays, seeks to average out, and therefore minimise the bias effect.**

- **The WHO approach will provide an estimate which is “accurate” but not “precise”**

- **The reference-method (SI) approach will provide an estimate which may be “precise”, but not “accurate”**

6th IS for Unfractionated heparin collaborative study

Potency Estimates by Each Assay Method Expressed as the % of the Overall Mean Potency Estimates for Each Candidate



	T	V	W	X	Y	Z
Anti-IIa AT, n=18	96.4	123.7	100.2	100.1	98.6	103.9
Anti-Xa AT, n=23	99.7	106.2	100.2	100.2	99.1	101.5
EP, n=12	101.4	91.5	99.6	100.1	101.4	98.1
USP, n=9	106.1	84.3	101.5	99.9	105.2	98.2
APTT, n=12	100.2	88.6	100.0	99.8	99.8	96.3
Anti-Xa Plasma, n=3	100.2	97.2	97.5	97.6	97.1	99.6
TT, n=2	97.6	78.7	98.8	105.5	94.5	94.4
Anti-IIa Plasma, n=1	98.1	106.6	97.4	101.6	105.8	100.6
CP, n=1	100.3	109.9	96.7	93.9	103.0	96.2
PiCT, n=1	98.0	82.6	98.8	98.4	95.2	98.4
Anti-Xa Clotting, n = 1	106.7	98.7	98.7	96.3	97.3	100.3

Exceptions to combined potency by all methods

Antigen and biological activity – always separate

Different biological activities if related to differences in mechanism of action, for example:

- ristocetin co-factor and collagen binding activities of Von Willebrand Factor
- anti-Xa and anti-IIa activities of Low Molecular Weight Heparin
- TNF-α neutralising activity and ligand binding of infliximab

Attributes of Primary Reference Materials

Definition of unit - Arbitrary unit, relates to effect/response of the biological

Continuity of unit - Similarity to current standard

Multiple method assignment - Minimise assay bias

Like against like - Similarity to test samples

Importance of “Like vs Like”

Biological assays or bioassays are based on the principle of assaying like against like

Reference standard and test sample should have “similar” characteristics and the test sample dilution should behave as if it is a dilution of the standard

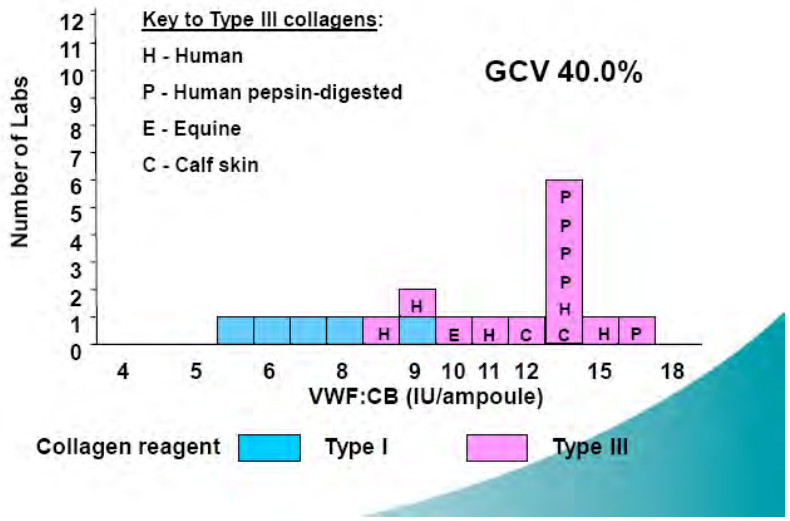
- To minimise “matrix” effects
- Concentrate standards for assay of concentrate products
- Plasma standards for assay of patients’ plasma

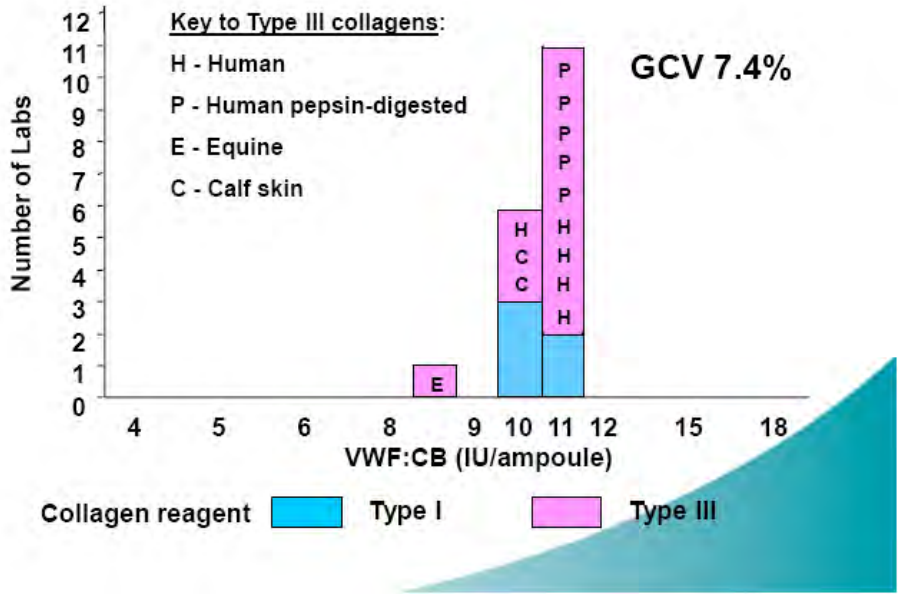
Choice of RS should be based on how well a candidate compare with all products that it needs to cover

Challenge:

Primary standard needs to cover a product type with wide diversity of characteristics

Importance of Like against Like: von Willebrand Factor (vWF) therapeutic concentrate - Collagen Binding Activity





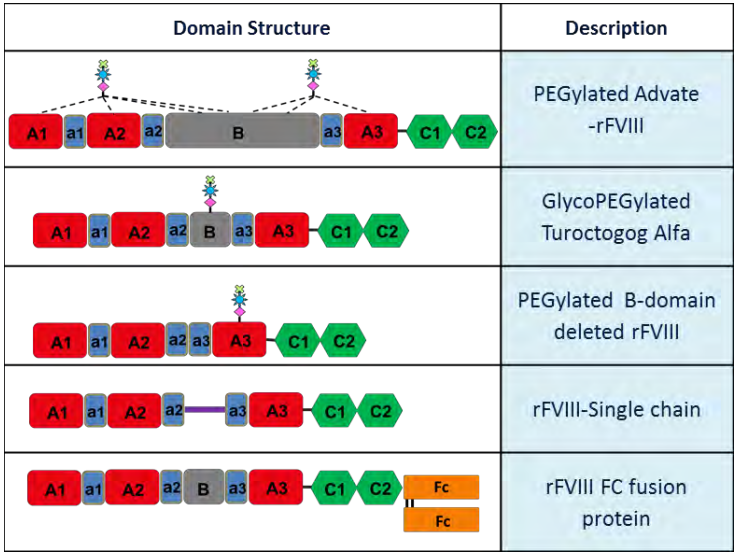
Against the 4th IS for vWF, Plasma

Against the 1st IS for vWF, Concentrate

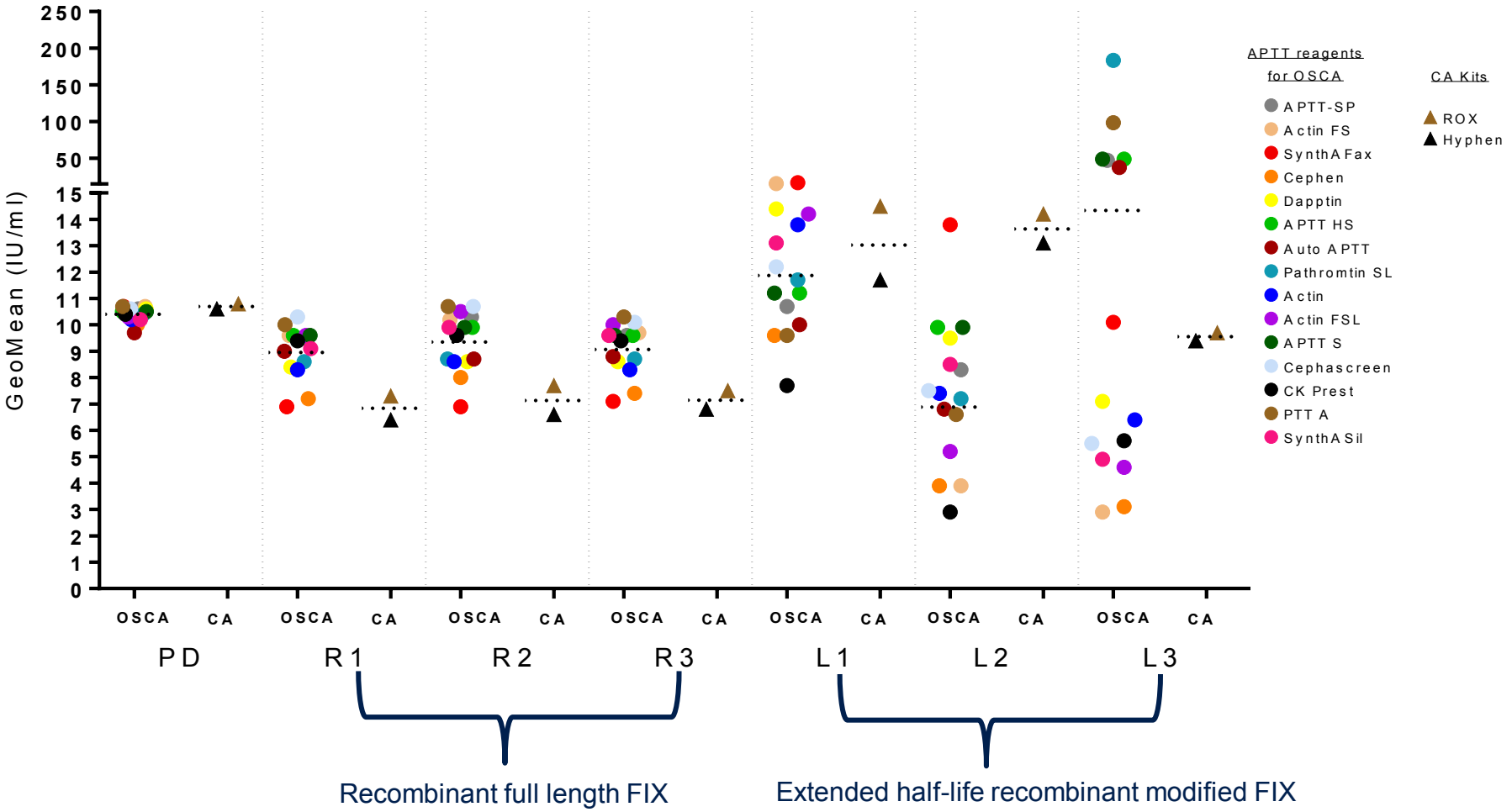
A single IU for all products – for how much longer?

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



Plasma derived (PD) International Standard as comparator for biotechnology products



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)

Journal of Thrombosis and Haemostasis, **11**: 988–989 DOI: 10.1111/jth.12167

OFFICIAL COMMUNICATION OF THE SSC

Recommendations on the potency labelling of factor VIII and factor IX concentrates

A. R. HUBBARD,* J. DODT,† T. LEE,‡ K. MERTENS,§ R. SEITZ,† A. SRIVASTAVA,¶ M. WEINSTEIN‡ and ON BEHALF OF THE FACTOR VIII AND FACTOR IX SUBCOMMITTEE OF THE SCIENTIFIC AND STANDARDISATION COMMITTEE OF THE INTERNATIONAL SOCIETY ON THROMBOSIS AND HAEMOSTASIS

**National Institute for Biological Standards and Control, Potters Bar, UK; †Paul-Ehrlich-Institut, Langen, Germany; ‡Center for Biologics Evaluation and Research/Food and Drug Administration, Rockville, MD, USA; §Sanquin Blood Supply Foundation, Amsterdam, the Netherlands; and ¶Christian Medical College, Vellore, India*

Workshop on "Characterisation of new clotting factor concentrates (FVIII, FIX) with respect to potency assays used for labelling and testing of post infusion samples" ***28-29 November 2013***

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

edqm
European Directorate for the Quality of Medicines & HealthCare | Direction européenne de la qualité du médicament & soins de santé

COUNCIL OF EUROPE
CONSEIL DE L'EUROPE

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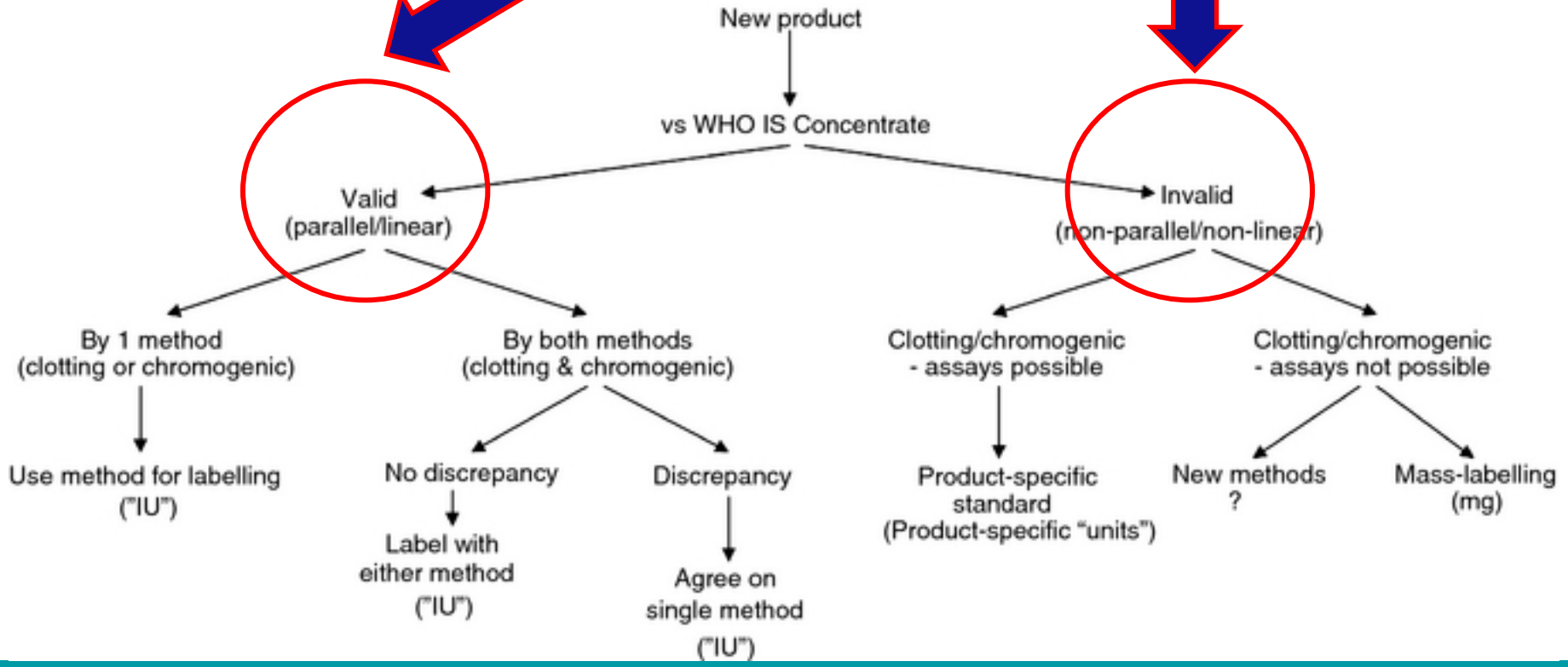
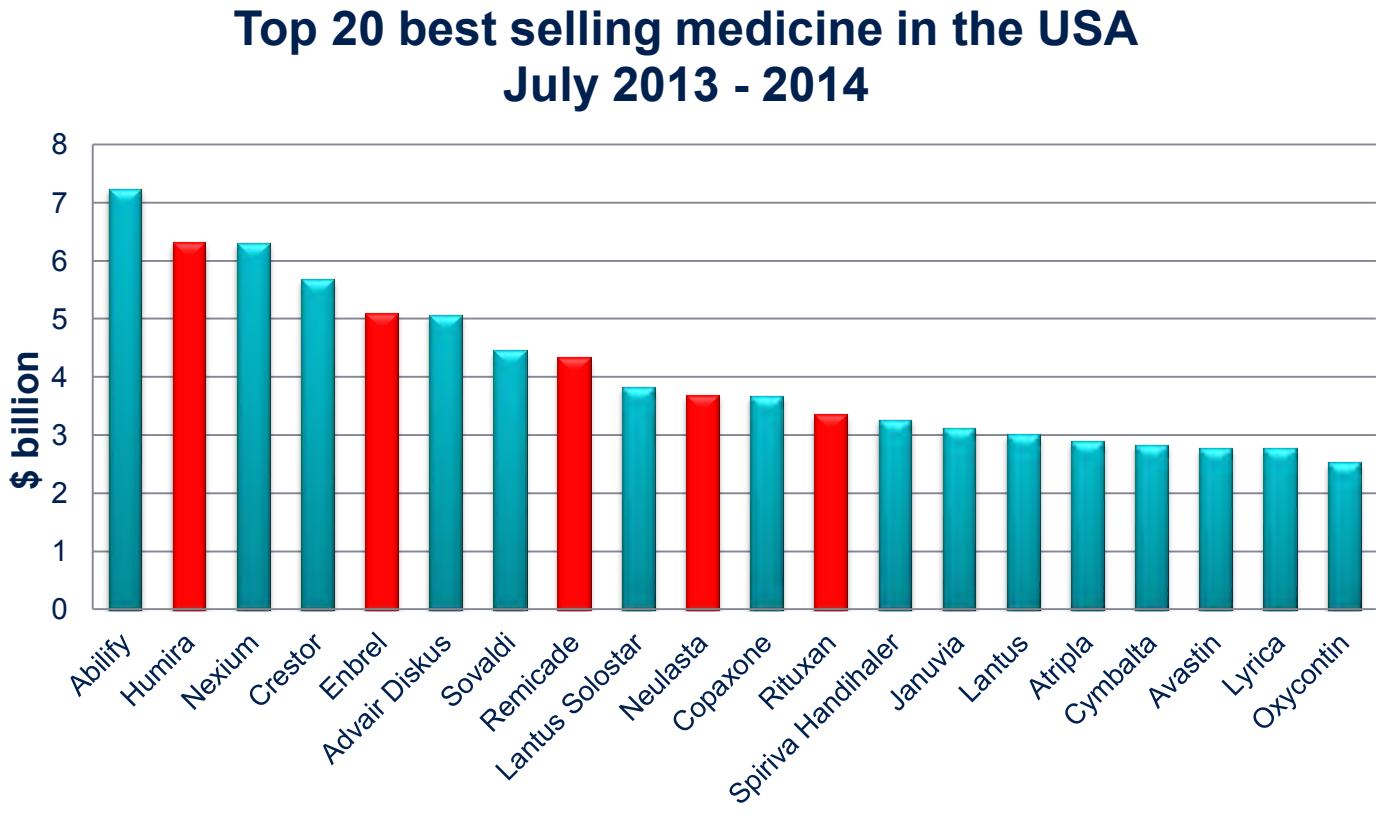
Statistical Assessment



5 out of 20 top selling therapeutics are biologicals produced by biotechnology: all potency labelled in mass unit



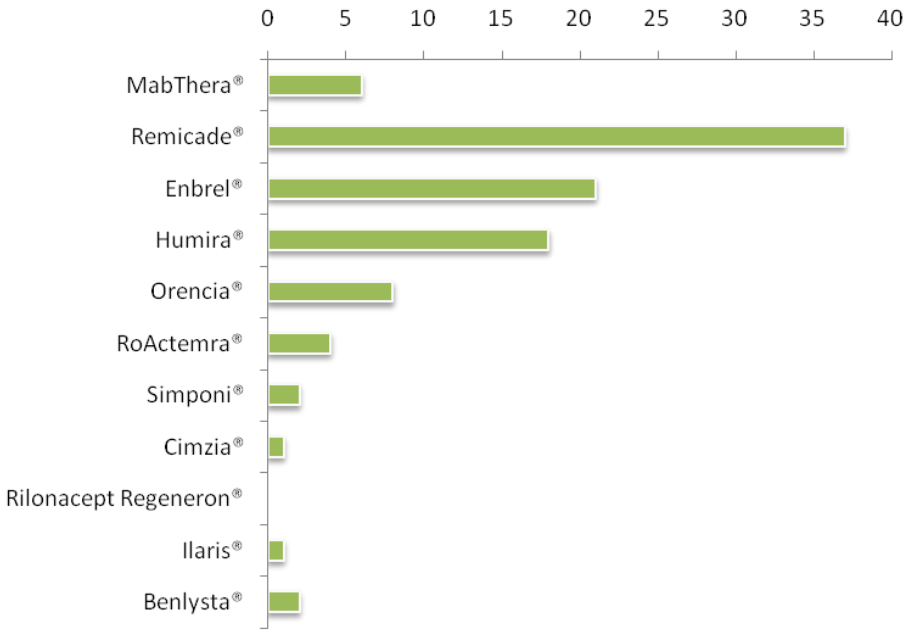
Source - Medscape.com August 2014(http://www.medscape.com/viewarticle/829246#vp_1)

The role of bioactivity standards for engineered biotherapeutics like mAbs

- Therapeutic protein where potency is assigned in milligram/ strength is defined as concentration (mg/ml)
- This could be a biosimilar, or a "post-change" new version with an optimized manufacturing process.
- Request to regulatory authorities:

"In order to match the strength of <originator> <pre-change product>, the <biosimilar> <post-change product> needs to have the same deliverable volume and the same total protein concentration. Therefore, the concentration should be at xx mg/ml, the same as the <originator> <pre-change product> total protein concentration. However, the <originator> <pre-change product> on average contains approximately yy % misfolded species that are biologically inactive, whereas the <biosimilar> <post-change product> contains no misfolded species. Therefore, So <biosimilar> <post-change product> at xx mg/ml actually contains yy % higher amounts of active protein compared to the <originator> <pre-change product>."

Changes in the manufacturing process of originator biologicals



Schneider CK. Ann Rheum Dis. 2013 Mar;72(3):315-8.
(Data source: EPARs on EMA website)

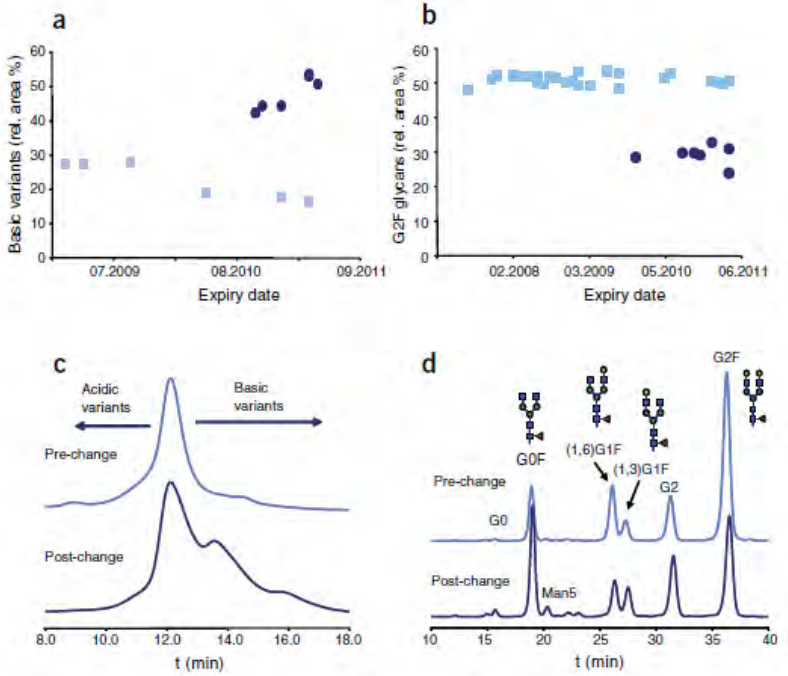


Figure 3 Comparison of the different pre- and post-change batches of Enbrel. (**a**) Relative amounts of basic variants of the pre-change ($n = 6$) and the post-change ($n = 6$) batches as measured by CEX. (**b**) Relative amount of the G2F glycan of the pre-change ($n = 25$) and the post-change ($n = 9$) batches. (**c**) Exemplary CEX chromatograms. (**d**) Exemplary glycan mapping chromatograms.

Schiestl M et al. Nat Biotechnol. 2011 Apr;29(4):310-2.

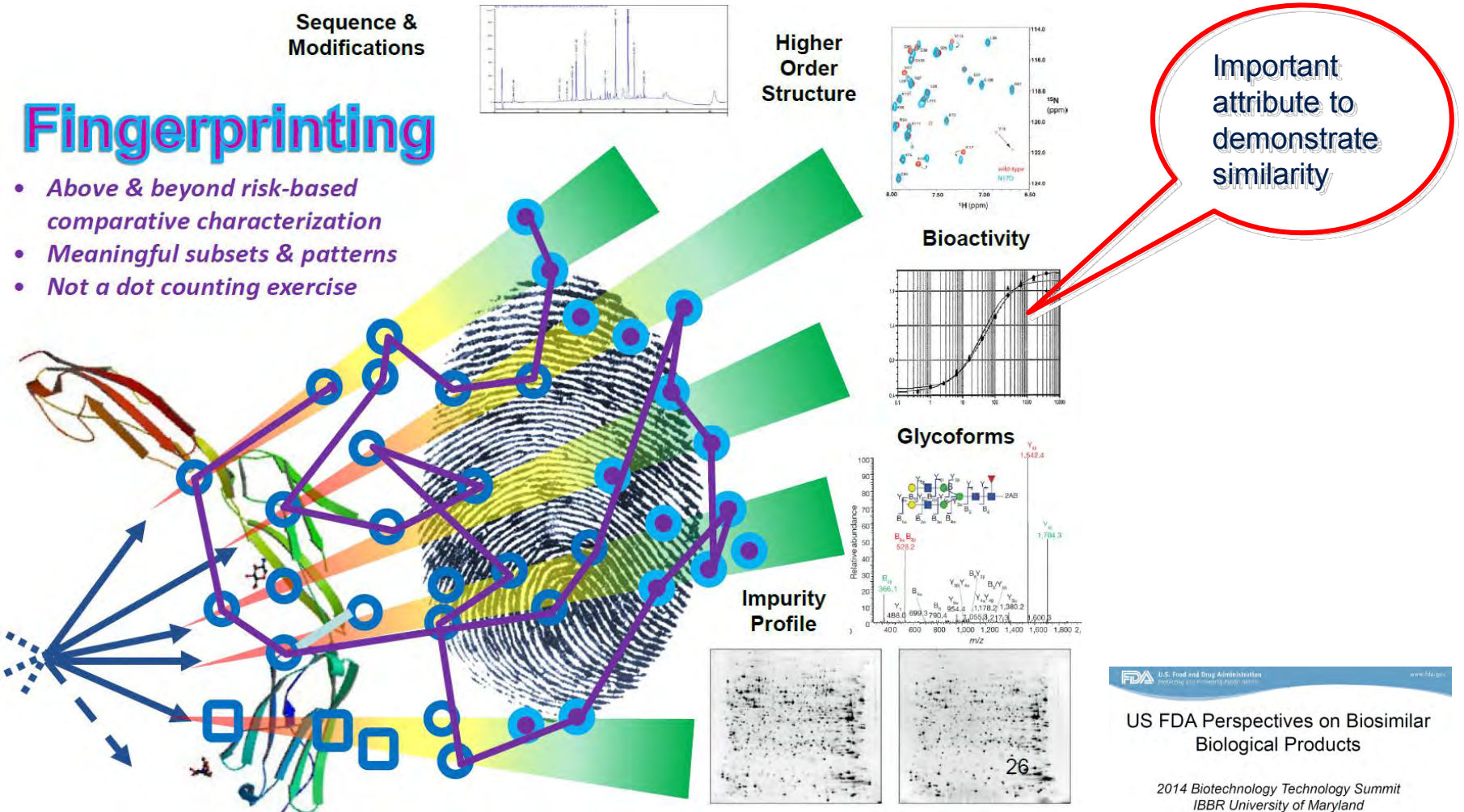
Current status of infliximab biosimilar authorisation worldwide

Data compiled from GaBI Online - Generics and Biosimilars Initiative. Available at: http://www.gabionline.net/.

Product name	Manufacturer	Authorisation	Date Approved	Therapeutic Indications of reference product[1]
Flixabi/Renflexis	Samsung Bioepis/Merk	Europe	26 May 2016	All
		Korea	4 Dec 2015	All
		Australia	28 Nov 2016	All
		USA	21 Apr 2017	All
Remsima/Inflectra	Celltrion/Hospira	Europe	10 Sep 2013	All
		USA	5 Apr 2016	All
		Korea	23 Jul 2012	All
		Japan	4 Jul 2014	Crohn's disease Rheumatoid arthritis Ulcerative colitis
		Australia	19 Aug 2015	All
		Russia (Marked as Flemingas)	13 Jul 2015	All
		Columbia	16 Dec 2013	All
		Brazil	27 Apr 2015	All
		Venezuela	29 Apr 2015	All
Inflectra	Hospira	Canada	15 Jan 2014	Ankylosing spondylitis Psoriatic arthritis Psoriasis Rheumatoid arthritis
			14 Jun 2016	Crohn's disease Ulcerative colitis
Remsima	Celltrion	Canada	15 Jan 2014	Ankylosing spondylitis Psoriatic arthritis Psoriasis Rheumatoid arthritis

[1] All therapeutic indications are; ankylosing spondylitis, Crohn's disease, psoriatic arthritis, psoriasis, rheumatoid arthritis and ulcerative colitis.

Demonstration of similarity



US FDA Perspectives on Biosimilar Biological Products

2014 Biotechnology Technology Summit
IBBR University of Maryland
Rockville, MD

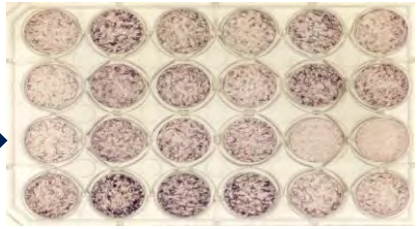
June 13th, 2014

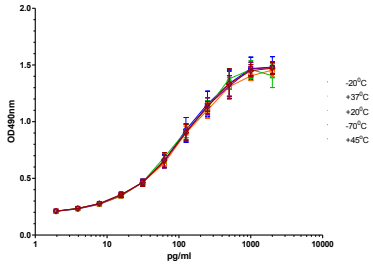
Steven Kozlowski, M.D.
Director, Office of Biotechnology Products
OPS/CDER / U.S. FDA

Bioassays and their critical role

- **Bioassays** are pivotal for assessing bioactivity throughout development (i.e. characterisation, batch release)
- **In house qualified reference standards** are used (i.e. process change, lot release, stability, system suitability …) to define activity.
- Product development often in parallel with bioassay qualification and development of reference standards

ICH guideline Q6B:
"The results of biological assay should be expressed in units of activity calibrated against **an international or national reference standard. Where no such reference standard exists, a characterised in-house reference material should be established and assay results reported as in-house units."**





Making the case for international measurement standards

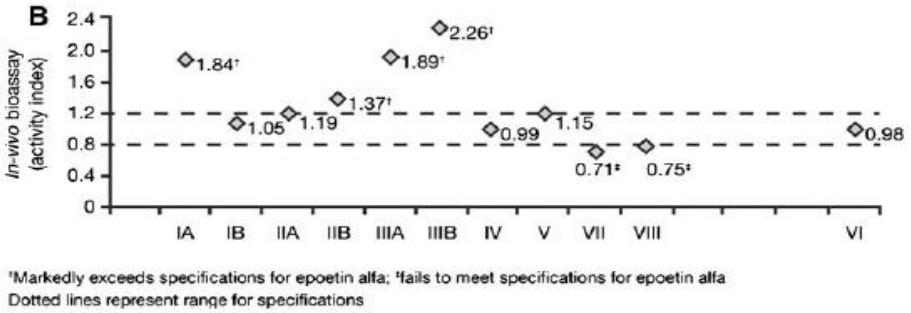
Non-innovator erythropoietins“

Medicines & Healthcare products Regulatory Agency

NIBSC
Confidence in Biological Medicines

WHO International Standard
3rd WHO International Standard for Erythropoietin, recombinant, for bioassay
NIBSC code: 11/170
Instructions for use
(Version 1.0, Dated 30/10/2012)

Care should be taken to avoid cuts and projectile glass fragments that might enter the eyes, for example, by the use of suitable gloves and an eye shield. Take care that no material is lost from the ampoule and no glass falls into the ampoule. Within the ampoule is dry nitrogen gas at slightly less than atmospheric pressure. A new disposable ampoule breaker is provided with each DIN ampoule.



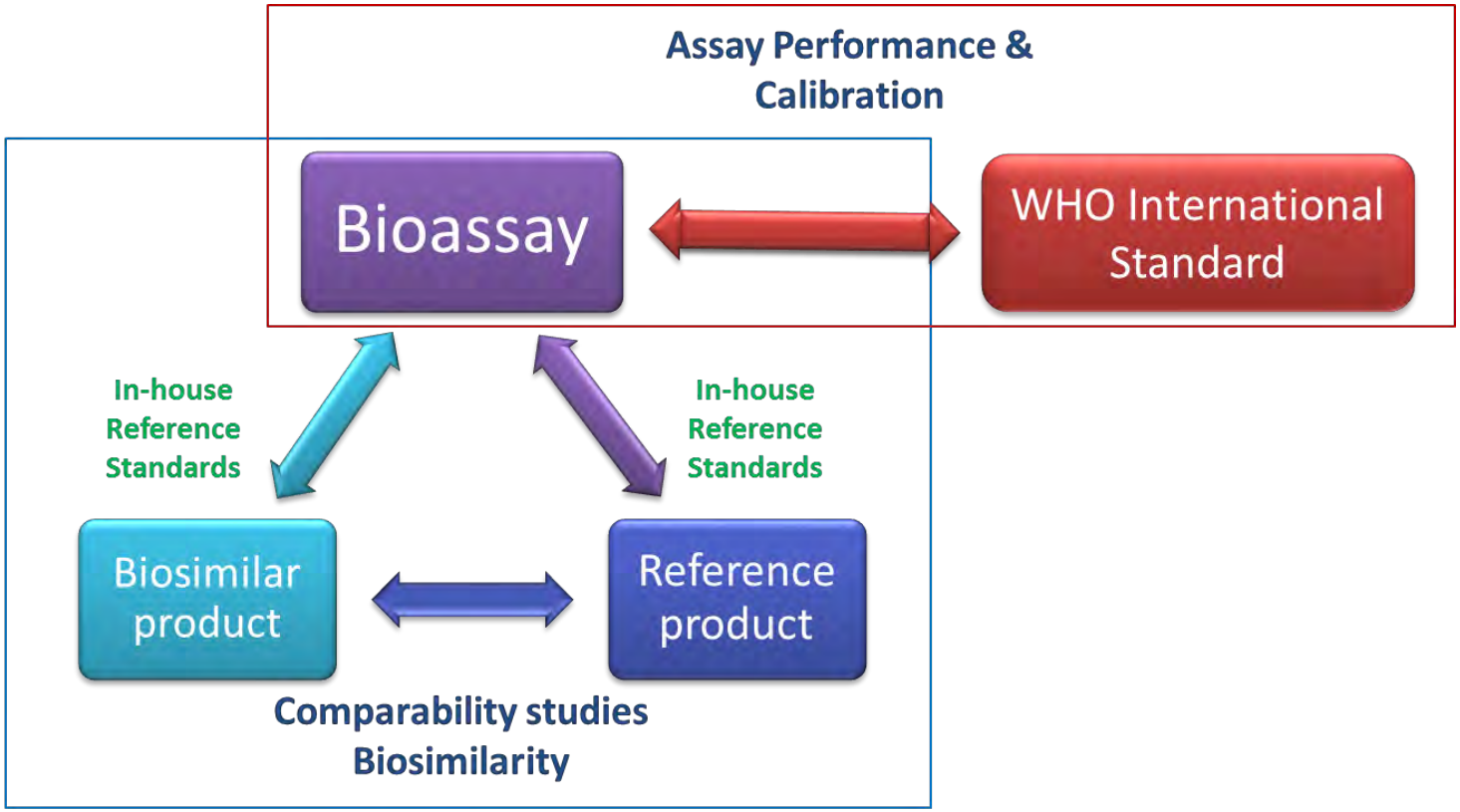
†Markedly exceeds specifications for epoetin alfa; ‡fails to meet specifications for epoetin alfa
Dotted lines represent range for specifications

VI	4000	India
VII	10 000	China
VIII	6000	China

Schellekens H, NDT Plus (2009)

- Working with USP, EDQM, WHO, NIFDC, MFDS to make sure the importance of standards is understood

Evolving Role of IS



Reference product and reference standard are DISTINCT
Reference product is NOT reference standard

WHO International Standards

Availability of International Standards (IS) for bioactivity -

- would provide assurance on method performance
- facilitate early development and support multiple products through their life-cycle
- would help to control ‘drift’ during process changes post-approval of products incl biosimilars (impact on efficacy and safety)
- should not impact the biosimilarity paradigm (requires reference medicinal product)
- should not impact the dosing and product labelling (‘mg’ to continue)

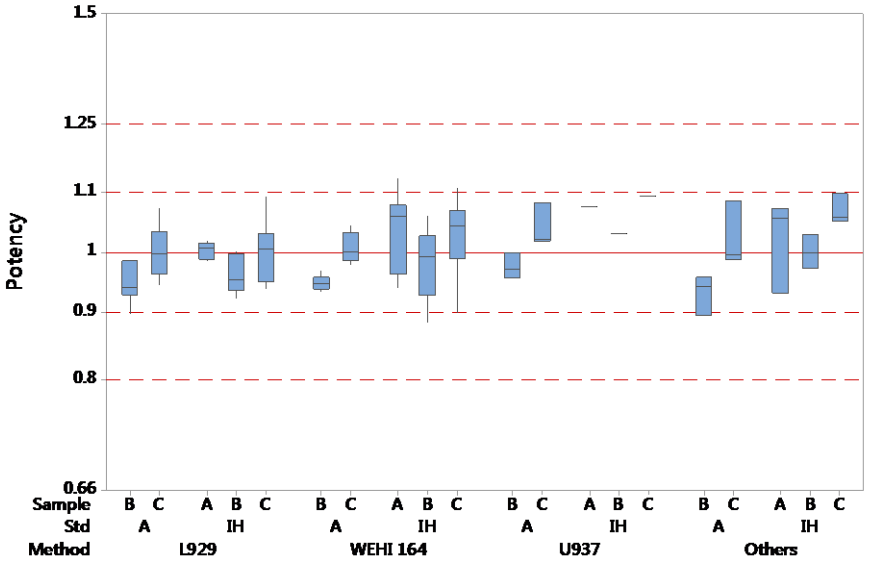
Seokkyun Kim et al (2017) Drifts in ADCC-related quality attributes of Herceptin®: Impact on development of a trastuzumab biosimilar, mAbs, 9:4, 704-714, DOI: 10.1080/19420862.2017.1305530

ECBS report- Proposed 1st IS for Infliximab (2017)

Clive Metcalfe, Chris Bird, Thomas Dougall, Peter Rigsby and Meenu Wadhwa

Sample A- Candidate 16/170; Sample B – Comparator infliximab; Sample C – coded duplicate of sample A

TNF-α neutralisation assays



	Relative potency Ranges of Geometric mean (ranges of Intra-lab GCV)	
	Vs A	Vs in-house standard
A		0.60 – 2.86 (0.8% – 16.8%)
B	0.90 – 1.00 (1.1% - 13.4%)	0.79 – 2.59 (1.6% – 28.9%)
C	0.94 -1.09 (0.9% - 18.1%)	0.90-2.67 (0.8% - 20.3%)

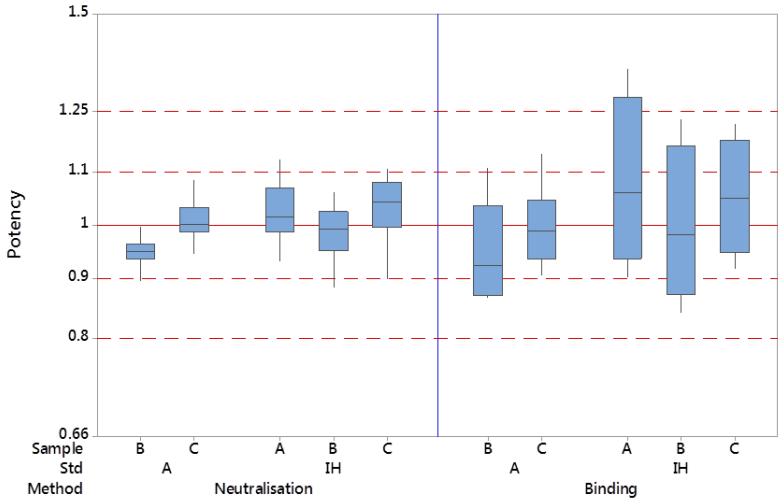
- Candidate A, a stable homogeneous reference preparation decreased day to day variability of assay for some laboratories
- Availability of an International Standard would improve laboratory performance of bioactivity assay

ECBS report- Proposed 1st IS for Infliximab (2017)
Clive Metcalfe, Chris Bird, Thomas Dougall, Peter Rigsby and Meenu Wadhwa

Sample A- Candidate 16/170; Sample B – Comparator infliximab; Sample C – coded duplicate of sample A

TNF-α neutralisation and binding assays

- Assay against a common reference standard lowered inter-laboratory %GCV – improved inter-laboratory agreement



	Method	Relative potency Geometric mean (95% CL); Inter-lab GCV	
		Vs A	Vs in-house standard
A	Neutralisation		1.02 (0.99 – 1.06); 5.9%
	Binding		1.09 (0.83 – 1.43); 18.6%
B	Neutralisation	0.95 (0.94 – 0.96); 2.7%	0.98 (0.96 – 1.01); 5.1%
	Binding	0.95 (0.86 – 1.05); 10.4%	1.00 (0.78 – 1.29) 17.1%
C	Neutralisation	1.01 (1.00 – 1.03); 3.9%	1.03 (1.00 – 1.07); 6.5%
	Binding	1.00 (0.91 – 1.09); 8.6%	1.06 (0.88 – 1.28); 12.5%

ECBS report- Proposed 1st IS for Infliximab (2017)
Clive Metcalfe, Chris Bird, Thomas Dougall, Peter Rigsby and Meenu Wadhwa

Study showed:

- Statistically valid assays could be obtained when candidate preparation 16/170 was assayed against the comparator sample and laboratories' own in house standards
- Improvement of assay performance, both within and between laboratory (lower assay variability expressed as %GCV compared with in-house standards) when test samples were assayed against candidate preparation

ECBS report- Proposed 1st IS for Infliximab (2017)
Clive Metcalfe, Chris Bird, Thomas Dougall, Peter Rigsby and Meenu Wadhwa

Conclusion and Proposal

- Based on the results of the multi-centre collaborative study, both infliximab preparations 16/160 and 16/170 showed good activity in all *in vitro* TNF-α neutralization bioassays (cytotoxicity, apoptosis and reporter gene assays), ADCC, CDC and TNF-α binding assays (ELISA, SPR and FRET) that were performed.
- Therefore, the infliximab candidate preparation NIBSC code 16/170 is suitable to serve as the 1st WHO International Standard for the *in vitro* biological activities of infliximab.
- It is proposed to the WHO ECBS that the candidate preparation 16/170 be accepted as the WHO 1st IS for infliximab with assigned values per ampoule of 500 IU of TNF-α neutralising activity and 500 IU of binding activity.

This infliximab International Standard is intended to support *in vitro* bioassay calibration and validation by defining international units of bioactivity. The proposed unitages, however, are not intended to revise product labelling or dosing requirements, as any decisions regarding this relies solely with the regulatory authorities. Furthermore, the infliximab International Standard is not intended for defining specific activity of products, nor to serve any regulatory role in defining biosimilarity.

NOR SWITCH

Articles

Interpretation The NOR-SWITCH trial showed that switching from infliximab originator to CT-P13 was not inferior to continued treatment with infliximab originator according to a prespecified non-inferiority margin of 15%. The study was not powered to show non-inferiority in individual diseases.

Switching from originator infliximab to biosimilar CT-P13 compared with maintained treatment with originator infliximab (NOR-SWITCH): a 52-week, randomised, double-blind, non-inferiority trial

Kristin K Jørgensen, Inge C Olsen*, Guro L Goll*, Merete Lorentzen*, Nils Bolstad, Espen A Haavardsholm, Knut E A Lundin, Cato Mørk†, Jørgen Jahnsen†, Tore K Kvien†, on behalf of the NOR-SWITCH study group*

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This online publication has been corrected. The corrected version first appeared at thelancet.com on May 23, 2017

See Editorial page 2263

See Comment page 2266

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Summary

Background TNF inhibitors have improved treatment of Crohn's disease, ulcerative colitis, spondyloarthritis, rheumatoid arthritis, psoriatic arthritis, and chronic plaque psoriasis, but are expensive therapies. The aim of NOR-SWITCH was to examine switching from originator infliximab to the less expensive biosimilar CT-P13 regarding efficacy, safety, and immunogenicity.

Methods The study is a randomised, non-inferiority, double-blind, phase 4 trial with 52 weeks of follow-up. Adult patients on stable treatment with infliximab originator treated in a hospital setting for at least 6 months were eligible for participation. Patients with informed consent were randomised in a 1:1 ratio to either continued infliximab originator or to switch to CT-P13 treatment, with unchanged dosing regimen. Data were collected at infusion visits in 40 Norwegian study centres. Patients, assessors, and patient care providers were masked to treatment allocation. The primary endpoint was disease worsening during 52-week follow-up. 394 patients in the primary per-protocol set were needed to show a non-inferiority margin of 15%, assuming 30% disease worsening in each group. This trial is registered with ClinicalTrials.gov, number NCT02148640.

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Thank You for Your Attention

"Biological assay, as carried out by the majority of workers in the world, still remains a subject for amusement or despair, rather than for satisfaction or self-respect. We have cat units, rabbit units, mouse units, dog units, and latest addition of all, pigeon units. The field of tame laboratory animals having been nearly exhausted, it remains for the bolder spirits to discover methods in which a lion or elephant unit may be described."

Professor Joshua Harold Burn
The errors of biological assay. 1930:
Physiol. Rev. Burn 10 (1): 146