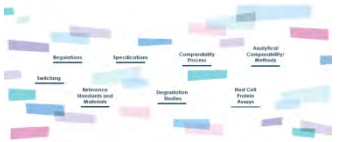


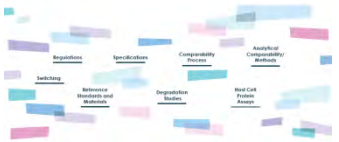
Paul Matejtschuk, BSc Biochemistry, PhD, CChem, UK

- Principal Scientist Standardisation Science, 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



Preparation and production of reference standards in support of biotechnology products

Paul Matejtschuk, BSc Biochemistry, PhD, CChem

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

NIBSC is a UK Government Laboratory

Medicines and Healthcare Products Regulatory Agency

- **>95% WHO global measurement standards developed by NIBSC**
  - Leading WHO International Laboratory for Biological Standardisation
  - Stock of >3.5m items
  - >150,000 items shipped to >80 countries p.a.
  - UK contribution to global public health
- **UK Official Medicines Control Laboratory (OMCL)**
  - Leading member of EU network
  - Broad range of medicines testing expertise
- **Global centre for Regulatory Science Research**
  - Strong externally and internally funded research prog
  - Extensive collaborative network
  - Influenza Resource Centre/UK Stem Cell Bank
  - CJD resource Centre
- **Since 2014 UK Designated Metrology Institute for bioactivity related to International Units**

CPRD NIBSC MHRA

Fundamental remit of assuring confidence in biological medicines - We do this by:

Establishing Reference materials (Standards) for biological medicines
- used in therapy, to ensure safe and effective drugs
- measured in diagnosis, to support reliable clinical interventions

Testing biological medicines
- within the European batch release scheme
- in response to adverse reactions or other incidents
- within market surveillance schemes

Underpinning research and development
-aimed at supporting and improving the activities above
-Over 340 staff, 70 post-doctoral

Supporting the Pharmaceutical industry through
- advice and interaction
- contractual arrangements
- within the context of conflict of interest policy

Outline of talk

1. WHO International Standards for Biologics
   - How they are made - key criteria – process
   - Role of biological standards in Biotech
2. Where potency is assigned or cross–referenced to IU though product is dosed in mg
3. Where no traceability to IU system exists (e.g. Mabs)
4. Role of IS in bioassay/bioactivity
5. System suitability standards
   - SEC HPLC
   - Others

The Biologics Revolution

- Huge explosion in importance: 10/20 top selling pharmaceuticals are now biologics

Share of total drug revenues over 20%. The global biosimilars market is expected to reach USD 10.90 Billion by 2021 from USD 3.39 Billion in 2016, http://www.marketsandmarkets.com/Market-Reports/biosimilars-40.html?gclid=EAIaIQobChMI9ZbRo-ST1QIVo7vtCh2bXgITEAAYASAAEgIz7PD_BwE

- Deep and diverse pipeline of novel biological products
- But measurement problem has not changed

  How can you measure what you don't fully understand?

  Sophisticated analytical technologies do not always provide solutions

  Biological standardisation needed now more than ever

WHO International Standards (IS)

Fulfill an essential role for Biological Medicines :

- Ensure consistency in product quality throughout manufacture
- Ensure consistency and harmonization in dosage to patients globally
- Enabling the safe development and use of multiple versions of the same product with the aim of reducing costs and increasing access to medicines

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)

Primary standards (‘gold standards’) intended for calibration of potency assays - **As ‘higher order’ standards**, IS are used for calibrating secondary standards e.g., pharmacopoeial standards (fewer labs, single method), regional standards (multiple labs& methods), manufacturers in-house standards (single method in-house, multiple sites)

WHO International Standards (IS)

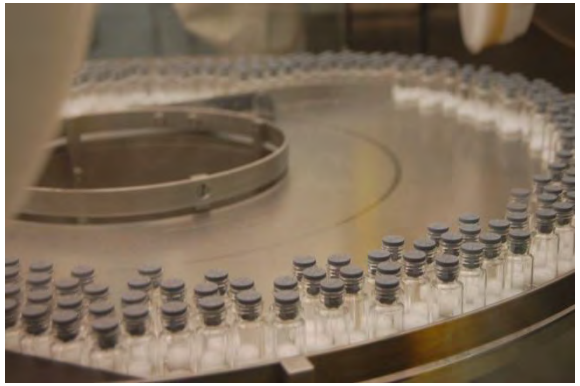
- Each project takes 2-3 years and significant resource
  - Capital investment, scientific input, significant management framework, quality assurance
- Stepwise process
  - Dependent on embedded practical and theoretical expertise
- Project Initiation
  - Industry, regulators, societies, WHO endorsement
- Sourcing of materials
  - Requires strong support from manufacturers,
  - ‘‘like vs like”, 1 or more candidates, clinical grade, replacement strategy, batch size, safety and ethical considerations with human-derived materials
- Process development (most WHO ISs are freeze dried)
  - Optimal for stability, homogeneity, ease of storage and transportation
  - Pilot fills to select formulation of lyophilised product - robust and biologically active; excipient fill
  - Definitive fill
- Collaborative Study (multi-centre, multi-method)
  - Essential not only for calibration purposes but also to demonstrate ‘fitness for purpose’
  - Study participants – end-users, expertise in assays
- Calibration and value assignment
  - Usually relative to an existing International Standard, unless the first IS (arbitrarily assigned)

Preparation Challenges

CBRM – Purpose-built facility (2003) - £10m

Gravimetric
- low CV of fill
- static
- solubility
- stability
- wide dry mass range

Biosafety
- potent
- microbial load
- infectivity



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Filling

- Flame sealed ampoules or stoppered vials
- 100% weighing checks with automated discard
- 3,000 containers per hour
- 25,000 lyo capacity
- Negative pressure isolator available

CS150 filling line

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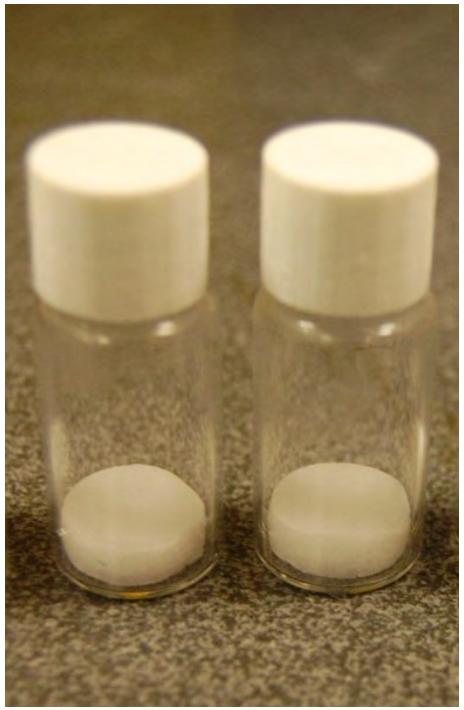
Lyophilised format

- Required for long term stability
- Homogeneous, robust cake
- More convenient to ship
- Readily reconstituted
- Ampoule
- Screw capped vial
- Amber glass option



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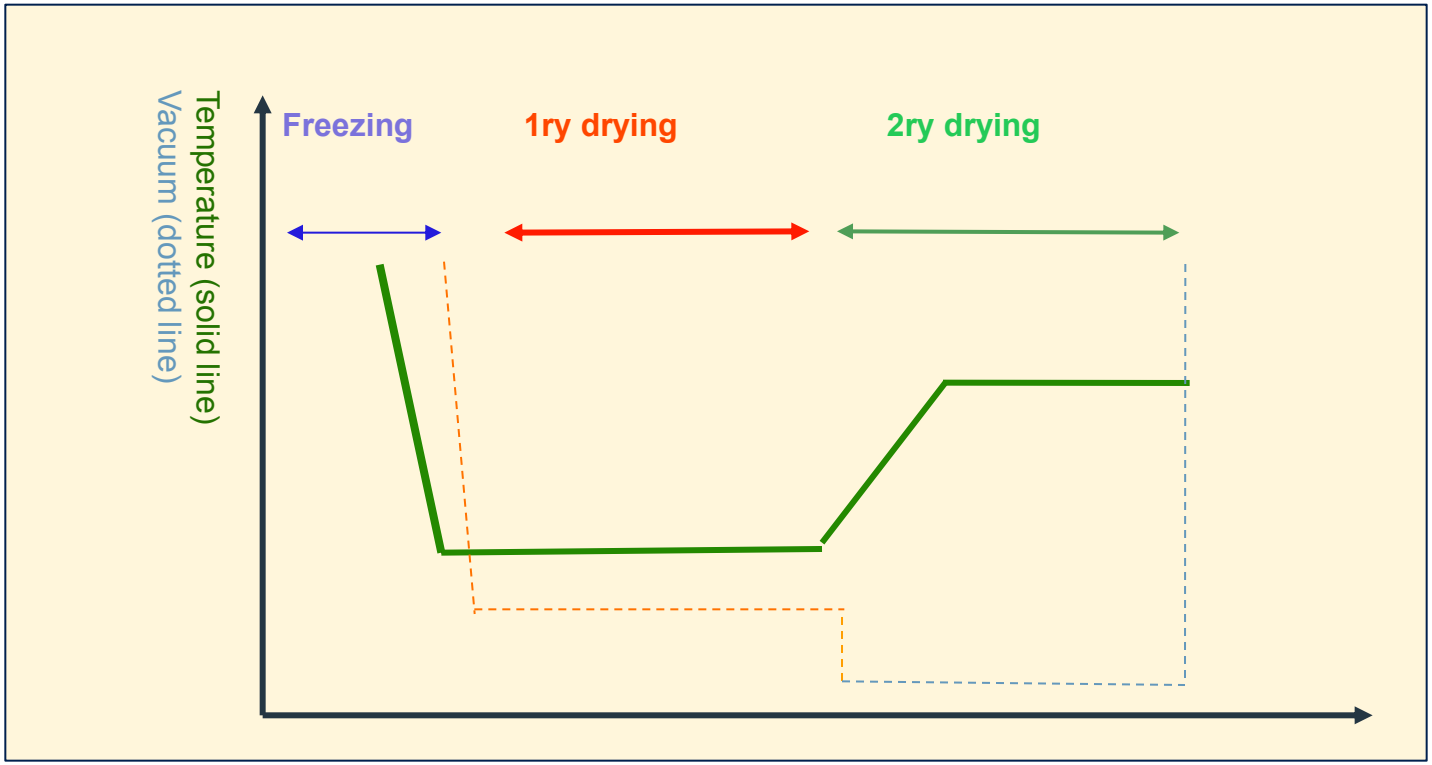
Process scale sealing of ampoules

Ampoules stoppered in dryer
Ampoule stoppers raised just before sealing
Ampoules flame sealed by heat fusion
Automated process

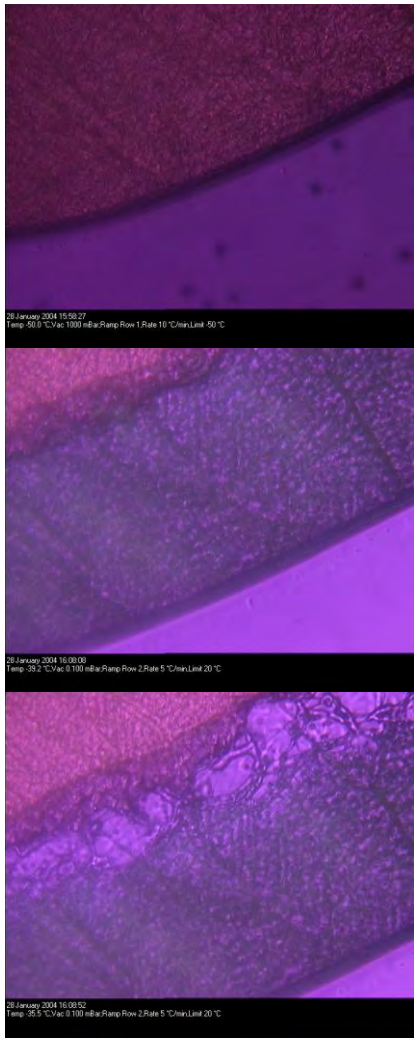
Freeze Drying



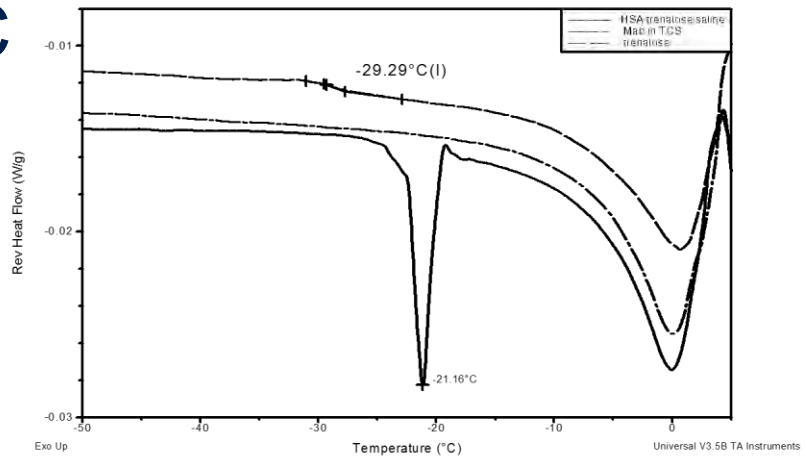
- Process Variables: Temperature (shelf and condenser), time, vacuum
- Product Variables: Formulation, container, closure type, volume of fill,

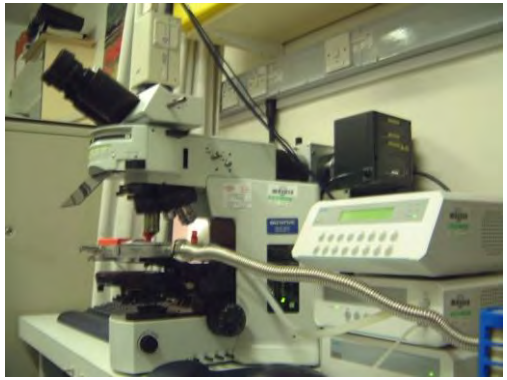
Freeze drying cycle development

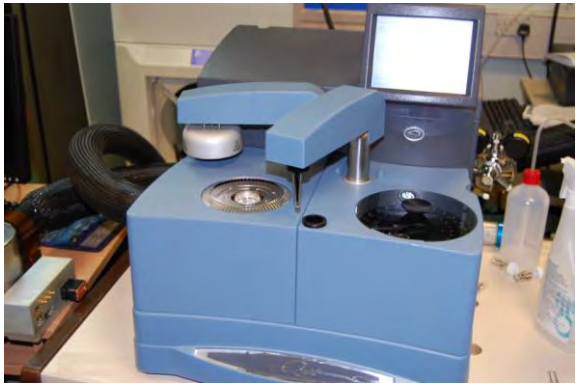
FDM



mDSC







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Non invasive measurements
Oxygen content

Laser IR into headspace gas
Measures oxygen and moisture (in equilibrium between product and atmosphere)

NIST traceable oxygen standards in each
Commonly used container format
Re-calibrated every 2 years

QC tests- Residual Moisture

coulometric KF

Dry mass
Homogeneity CV of fill
Bioburden

Bioactivity
Integrity
Stability – ATD

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Accelerated thermal degradation testing

Stressing samples post drying at elevated temperatures (-20° through to 56 °C) over 3-12 months to determine predicted stability

Pre-peak

Oxygen %	0.23	0.6	1	4	8	17
+56°C	1.4	1.0	1.2	3.5	4.4	6.2
+37°C	2.4	2.9	3.4	7.3	3.6	5.8
-70°C	1.2	1.3	1.1	1.2	1.3	1.3

Frozen baseline showed low pre-peak throughout oxygen levels
Pre-peak increased at higher oxygen levels at elevated temperature stations
Pre-peak still low at low oxygen

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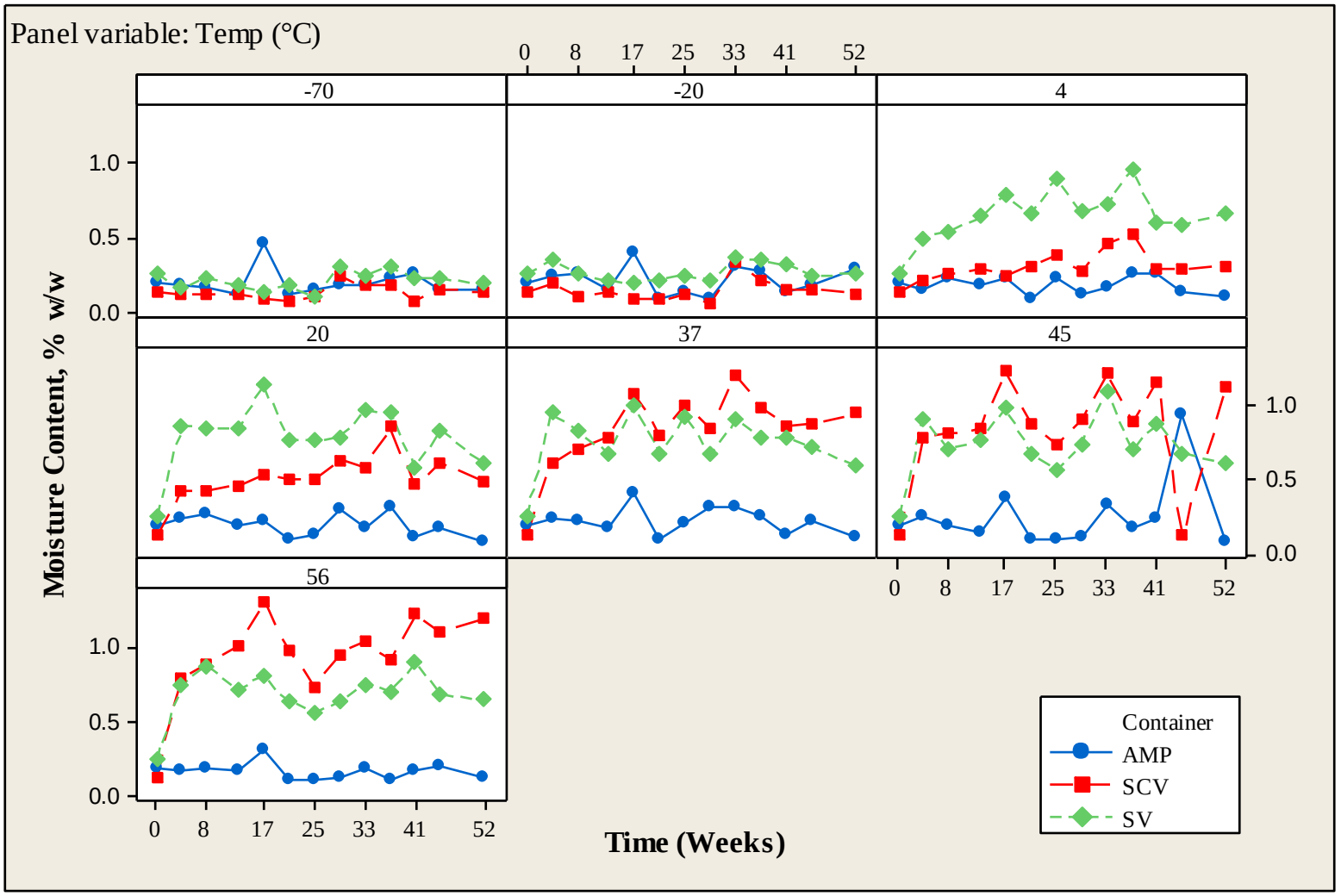
Superiority of ampoule to vial format for long term storage



Matejtschuk et al , Biologicals 33, 63-70. 2005

Application of formats

Ampoules

Primary standards e.g. WHO IS

Long shelf storage

Product shelf life - many years

Less familiarity in some markets

Issues in high containment facilities

Often useful for small batch, high dose (e.g.CRS) or specialist applications

Stoppered vials

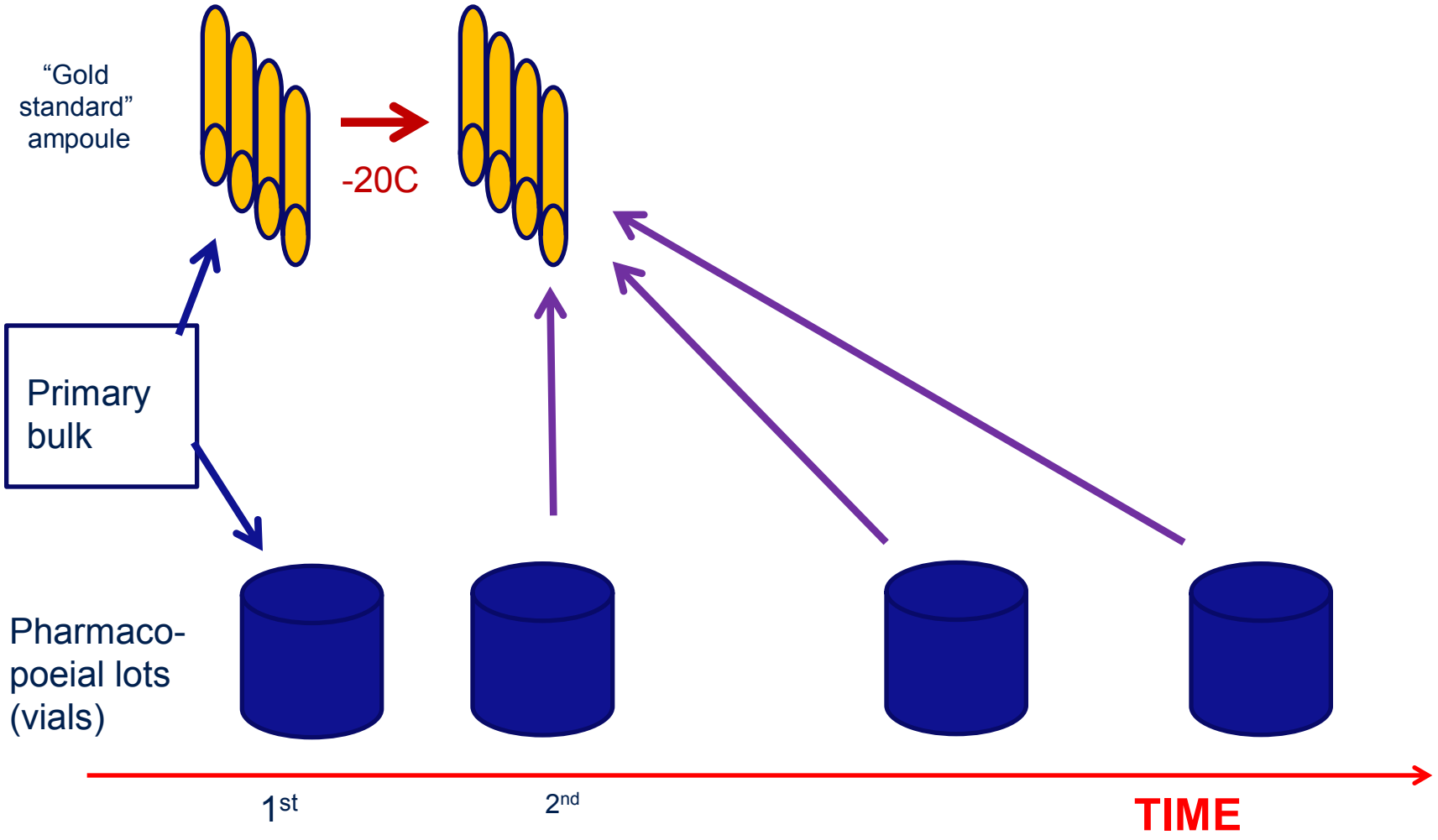
Widely used in diagnostics and therapeutics

Less good for long term stability

Often used up quickly-

- Pharmacopoeial standards
- In house working standards
- Run controls

Strategy for use of ampoule “gold” standard



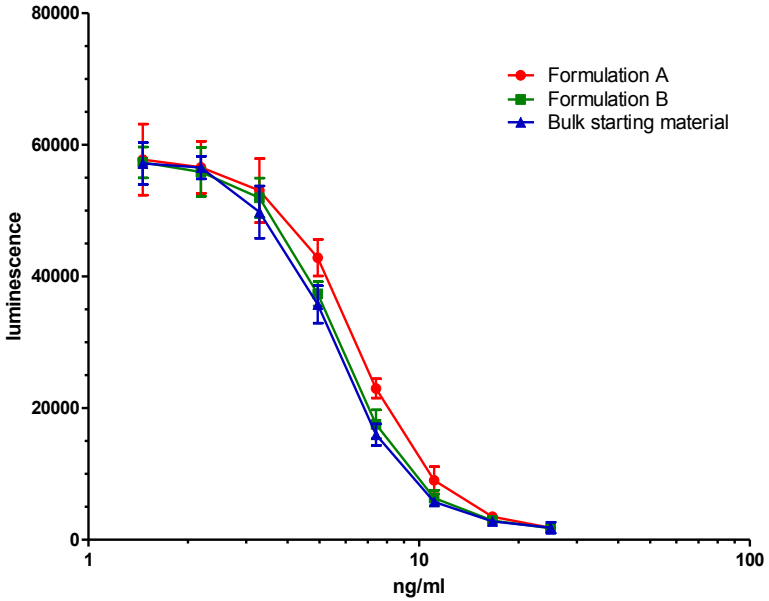
WHO International Standards (IS)

Establishment and Implementation

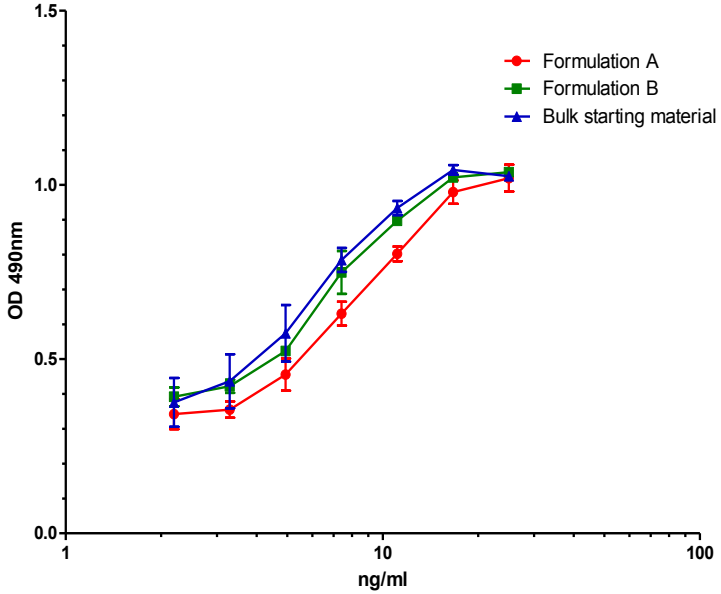
- Collaborative study report for ECBS
  - Introduction/rationale, Bulk material and processing, Stability studies, collaborative study data and statistical analysis
  - Formal recommendation
  - Also include limitations on use, evaluation of the continuity of the IU
  - Draft Instructions for use
- **Report to Participants for comments**
- **With participants agreement, submission to ECBS (July).**
- **ECBS (October) – formal establishment.**
- NIBSC catalogue & adoption by end-users.

Selection of Formulation for Infliximab standard

RGA



Cytotoxicity Assay

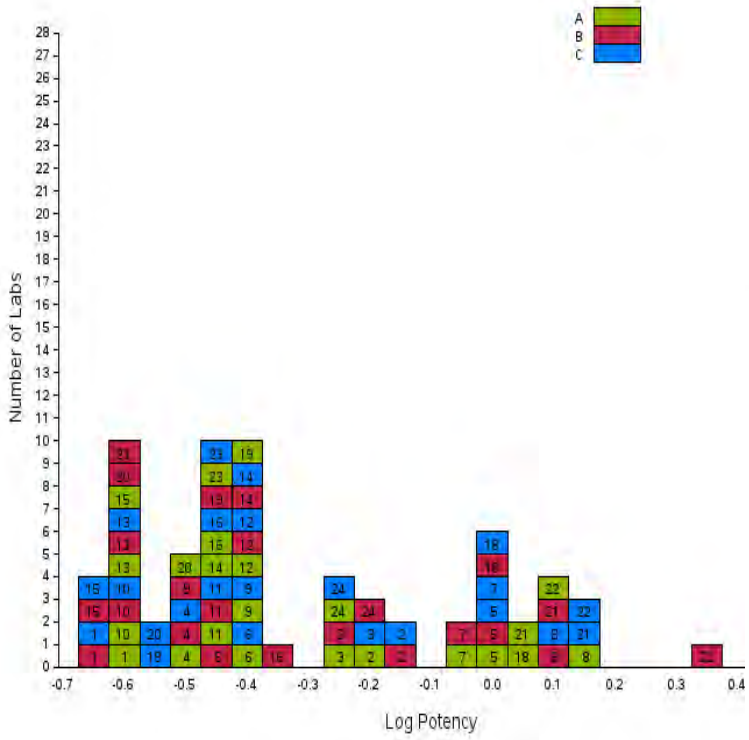


Potency relative to bulk starting material		
Formulation	**Reporter gene assay**	**Cytotoxicity assay**
A	**87.5%**	**75.5%**
B	**96.2%**	**92.4%**

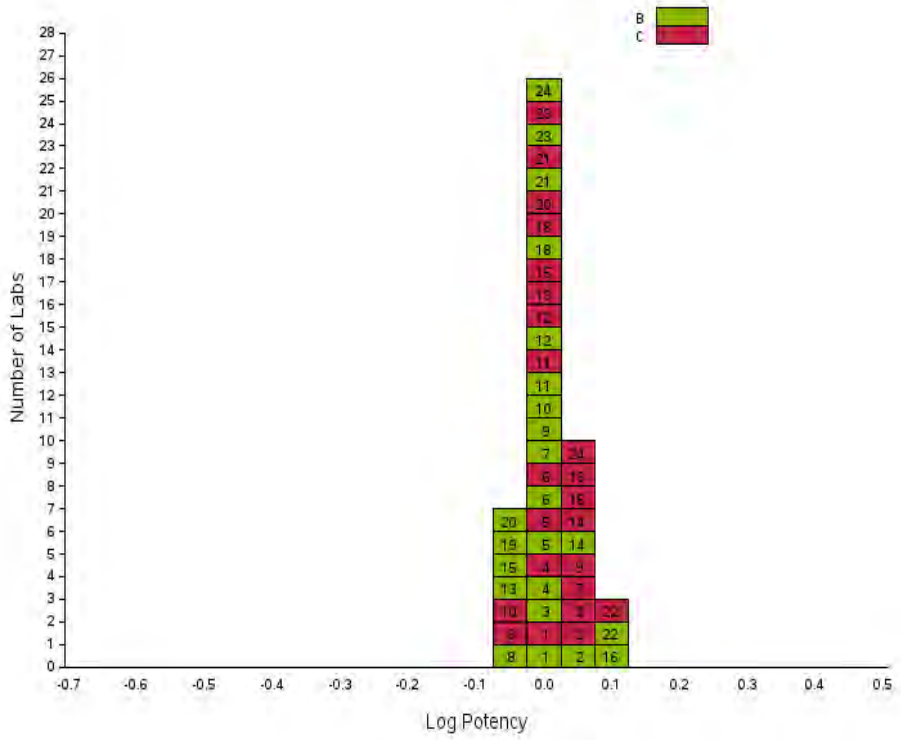
B selected for development of the reference standard

‘Like *versus* Like’

Potencies of Peg-G-CSF samples A, B and C relative to G-CSF IS (09/136)



Potencies of Peg-G-CSF samples B and C relative to A (Peg-G-CSF)



WHO IS for Peg-G-CSF established for Peg-G-CSF products

International Units or SI units

- Based on an increased understanding linking structure with function
  - Development of physicochemical methods such as HPLC have replaced bioassays
  - Steroids, thyroid drugs, antibiotics, some peptides and small proteins
  - Polysaccharide antigens (Q-NMR as well as bioactivity)
  - No longer defined by the biological reference preparation but defined by a stated specific activity for the pure substance (e.g., GH is 3IU/mg and there are similar figures stated for insulin, oxytocin and calcitonin)
- Requires careful planning and broad consultation
  - Changes to labelling and dosing regimens require consensus among all stakeholders and must be supported by scientific evidence
  - May require an equivalence statement describing the relationship between the SI unit and the IU to permit continued labelling and dosing formulated preparations in IU
  - **Retention of the IU remains important for labelling and dosing in many biologics**

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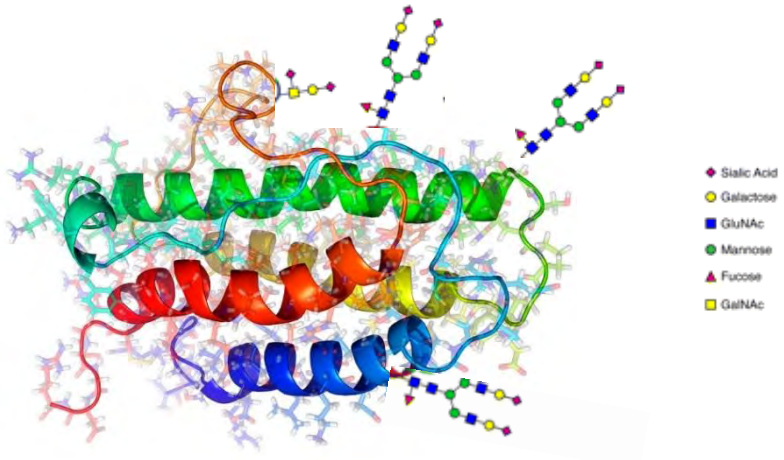
Erythropoietin International Standard

50 years history of establishment and Use

- 165 aa, 30 kDa protein; complex glycosylation critical for bioactivity & pharmacokinetics
- Role in erythrocyte maturation,
- Approved for renal anemia in CKD & chemo therapy treated patients. Dose controlled in IU



1st IRP EPO

In vivo bioassay

2nd IRP 67/343
10 IU/ampoule

urinary

EPO extracts diagnostic (‘60s)

1st IS 87/684
86 IU/ampoule

In vivo bioassay

2nd IS 88/574
120 IU/ampoule

3rd IS 11/170
~1550 IU/ampoule

recombinant

Recombinant EPO products (mid ‘80s)

Biosimilars (since 2006)

WHO IS used to calibrate *in vivo* bioassays and assign potency in IU

WHO International Standards

- Just like for EPO, development of NIBSC/WHO bioassay standards for traditional biologicals (e.g. IFN's, CSF's) is well established.
- This is not the case for non-natural "engineered" molecules or monoclonal antibodies

  Approved without IS and dosed in 'mass

  Target-specific (often sole product)

Intense 'biosimilar development'

WHA 67.21 resolution at the 67th WHA (2014)

'Access to biotherapeutic products including biosimilars and ensuring their quality, safety and efficacy'

WHO recognised global need for standardisation of biotechnology products to ensure safety, quality and efficacy*

BUT progressing cautiously

* Biologicals 39, 5:349-357, 2011 ; WHO informal consultation on future directions for international standards for biotherapeutic products (Geneva, Sep 2015) WHO Technical Report Series, 66th Report, 999:12-20, 2016); www.wikipedia.org

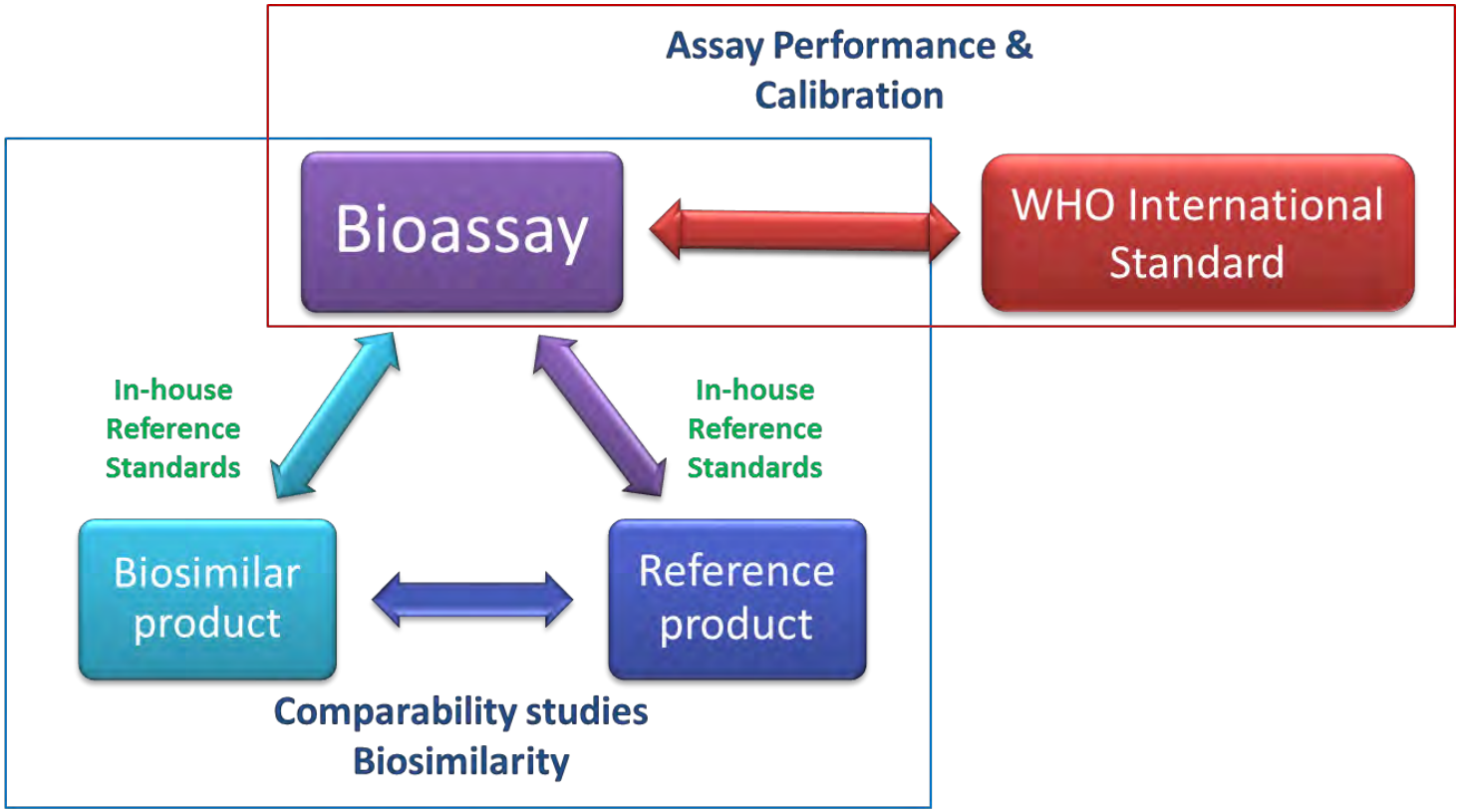
So what role is there for reference materials for non-natural bisimilars?

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."**

Evolving Role of IS



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

Requirements in analytical support

-Analytical methods must be validated according to international harmonized criteria, ICH Q2B

“An investigation of specificity should be conducted during the validation of identification tests, the determination of impurities, and the assay. The procedures used to demonstrate specificity will depend on the intended objective of the analytical procedure”.

In the absence of commonly available reference materials defining specific impurities, independently elaborated methods that are fully validated to ICH Q2b, cannot necessarily be assumed to give equivalent performance

Reference Standards: Limitations

With Biologicals, system validation reagents are often provided by treating the CRS using defined procedures

-Oxidised products	Hydrogen peroxide or chloramine-T treatment
-Aggregates	Agitation or heat treatment
-Deamidation	High pH treatment

Such procedures:

- are non-defined and irreproducible ("vortex for about 30s)

- are one of the most frequent sources of "it doesn't work" user-feedback

- Cannot support defined limits of detection

Reference Standards

There is a case that the portfolio of publicly available reference standards for biologicals should be extended to include such performance validation standards :

To support pharmacopeia monographs for biologicals

To support the validation of alternative methods

To support demonstration of bio-similarity by facilitating validation of method performance

Potential Scope of System Suitability standards for biologicals

Test	Standard	Function
Size –exclusion HPLC	Stable dimerized preparation	Demonstrate column performance (separation and/or Limit of detection)
Ion-exchange HPLC	Deamidated preparation	Demonstrate column performance (separation and/or Limit of detection)
Reverse Phase HPLC	Oxidised preparation	Demonstrate column performance (separation and/or Limit of detection)
Peptide mapping	Single amino-acid mutants Target peptides	Demonstrate system resolution Support quantitative applications
Glycan analysis	Glycan preparations High/Low pI preparations	Demonstrate system resolution Support quantitative applications of Z number

e.g. EPO

Bioassay IS standard – low level of EPO (1µg) in excess protein (HSA) and stabiliser (trehalose). Low concentration of very stable protein often in a milieu containing other non – specific proteins. Not suitable for chemical characterisation methods

CRS Pharmacopoieal standard - purified protein (100 -500µg in protein-free formulation of sugar, buffer and arginine) For chemical characterisation methods, too much material for many bioassays - inefficient to deliver 1,000 fold excess material and can lead to poor practice

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So do we have a different approach to the two RM types?

Common factors:

Low CV of fill

Low moisture content

Inert atmosphere

Stable container (glass ampoule vs stoppered vial)

Potency defined by collaborative study using one or more analytical method

Stability defined by ATD

Differing factors:

Defined content of protein supplied

Formulation of protein

Tests applied to characterise the material

Format - vial vs ampoule

Application - working reagent vs primary standard

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IMPURITY STANDARDS

General principles 1: types of impurity

Process related

Endotoxins
Host cell proteins
Active process agents
(eg cytokines, protein A)
Host cell DNA
Adventitious agents
Etc, etc

Product related

Variants of:

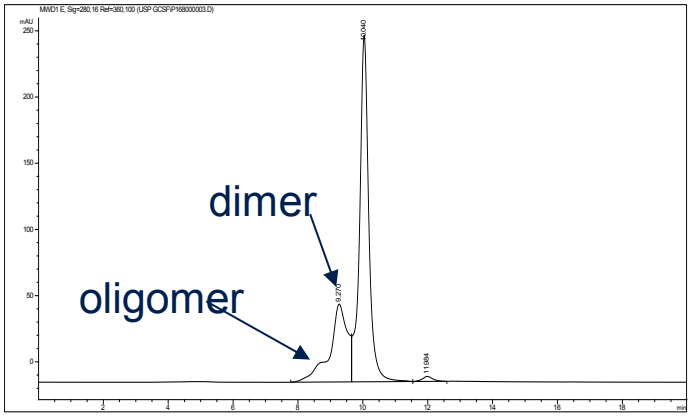
-Structure
-Aggregation state
-conformation

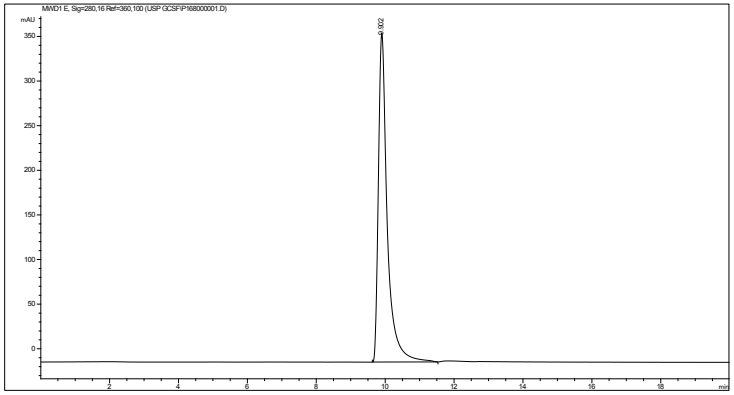
Intrinsic heterogeneity

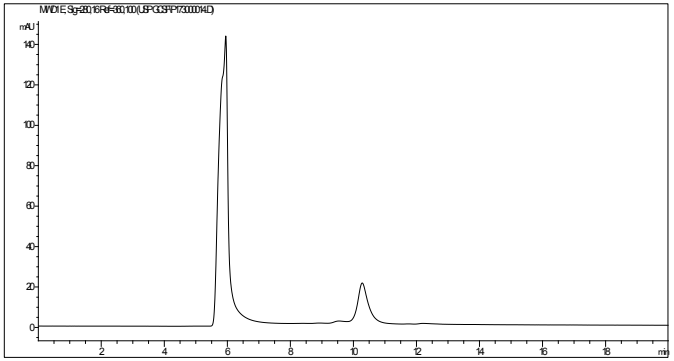
Glycoprotein glycoforms

Standard to Support a size-exclusion test for a recombinant protein

Consider a size exclusion test for dimers and aggregates







If your reference material looks like this, It really isn't much use to validate the test system in terms of its analytical target

However if the prescribed system suitability test (70degC, 1h) produces this, it probably still isn't much use

Size exclusion suitability reference material

Trial Preparation: A highly dimerised EPO made following optimisation of the glutaraldehyde to protein ratio and incubation period and temperature and this was then diluted into monomeric EPO to give approximately 2% dimer and 100µg EPO in 3% trehalose, 0.3% arginine, 0.01% Tween20, 0.45% NaCl, 20mM NaP buffer pH 7.4.

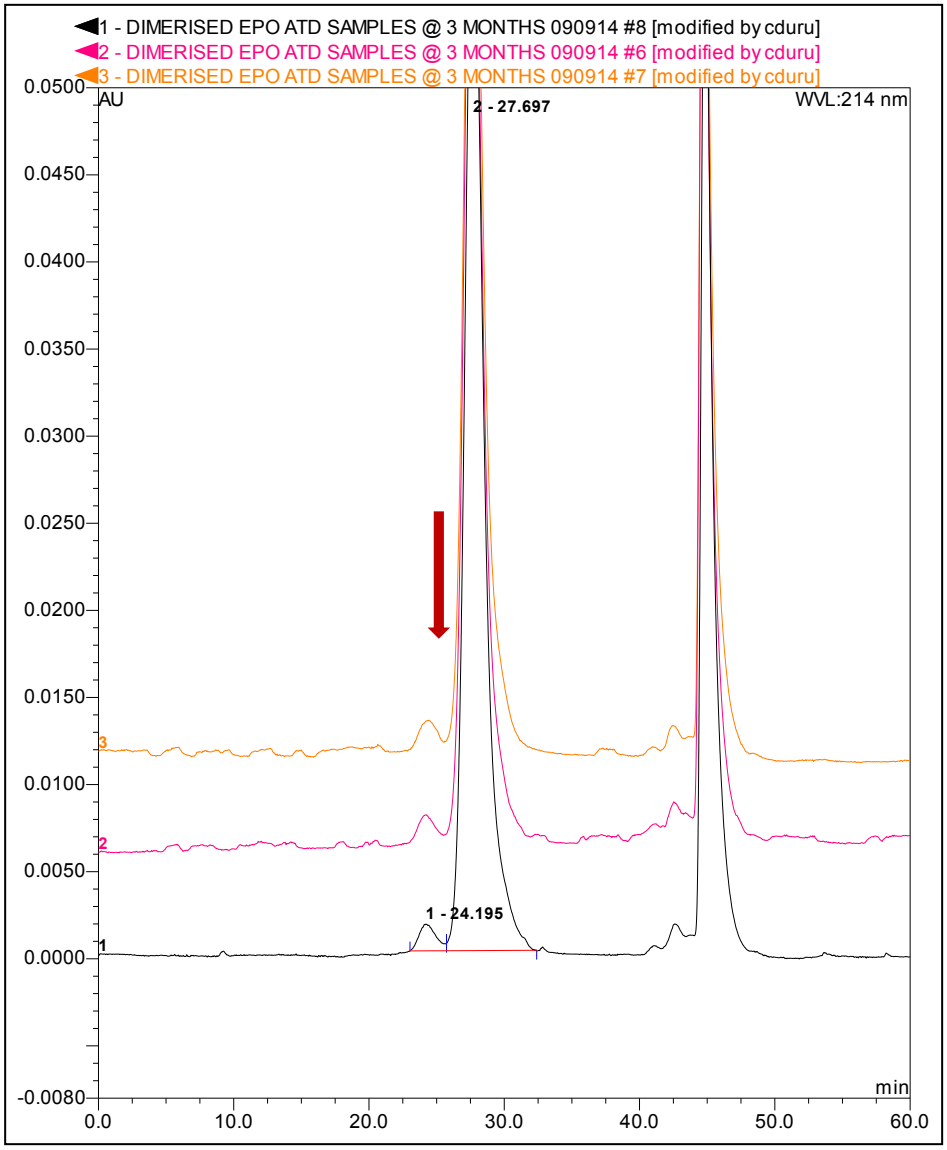
This material was successfully freeze dried and the dimer content seemed unaffected.

Following the success of the small scale study, it was agreed to make a a larger scale production of EPO dimer.

Definitive Batch (15/120): EDQM had supplied sufficient amounts to fill about 5,000 ampoules of EPO (a mixture of α and β forms) at 100µg/vial , with a target of approximately 2% dimer by HPLC, SPD batch code of 15/120.

Thermal stressing was undertaken with storage at elevated temperature to assess the impact on the lyophilised material.

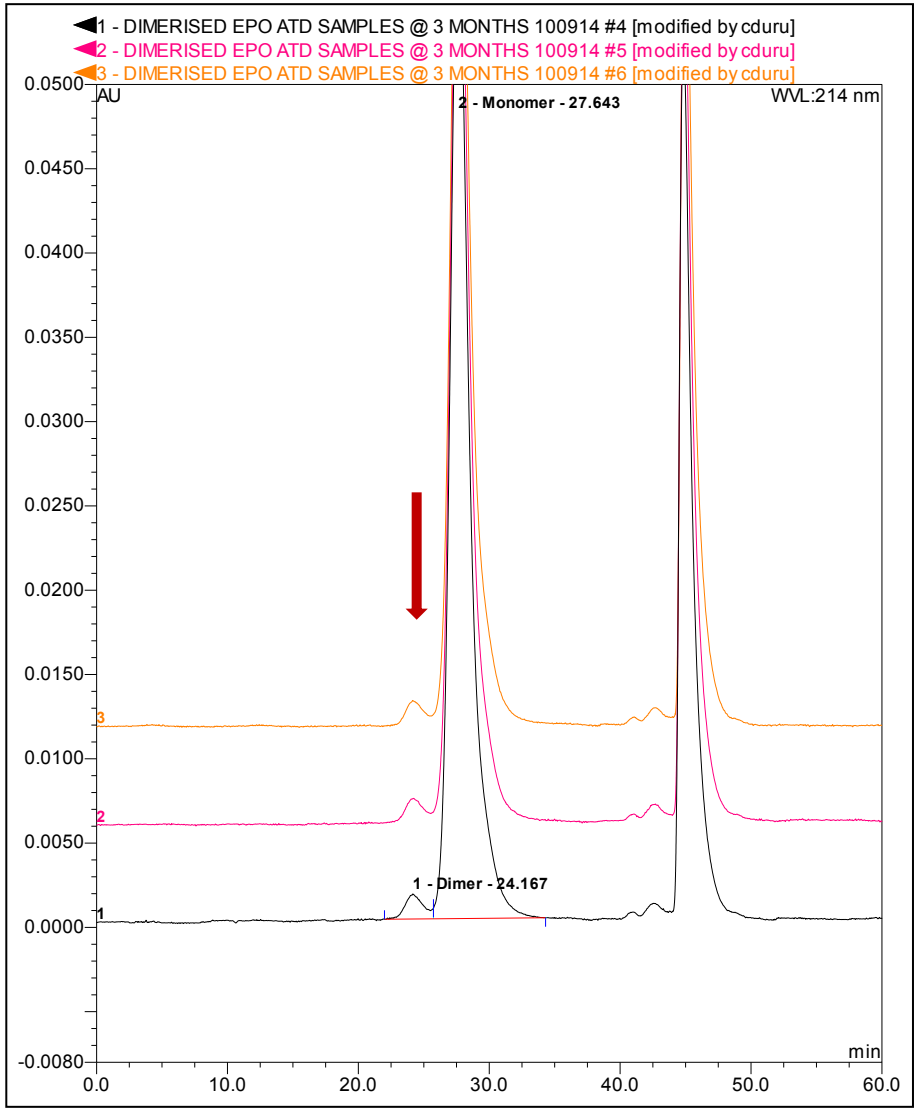
A collaborative study was also performed for this product with NIBSC acting as project lead and six laboratories participating.



EPO Preparation

Spiked with chemically modified dimerised material to a given concentration

Profile after 3 months storage lyo at +45°C
Mean dimer content 2.19% by area (3 hplc runs)



EPO Preparation

Spiked with chemically modified dimerised material to a given concentration

Profile after 3 months storage lyo at -20°C
Mean dimer content 2.17% by area (3 hplc runs)

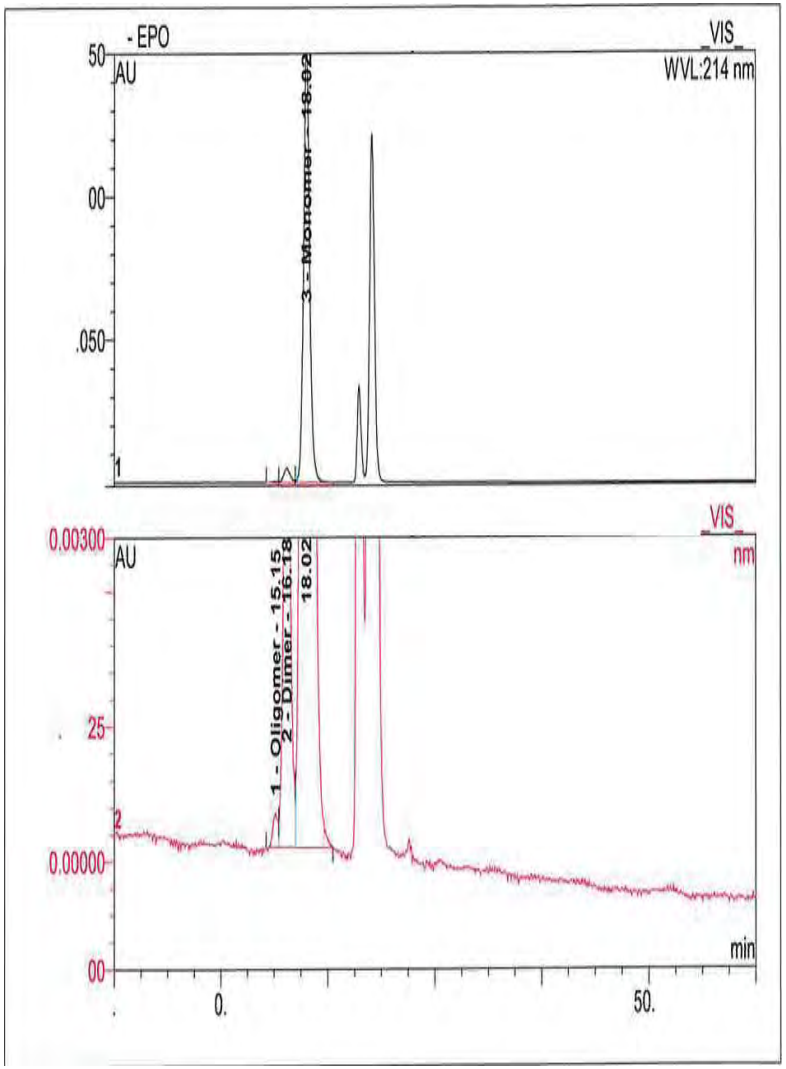
Properties of candidate reference material

Number filled	Mean Fill weight & CV	Residual moisture w/w%	Headspace oxygen (%)
5,318	**0.412g (0.59%)**	**1.51%**	**1.22%**

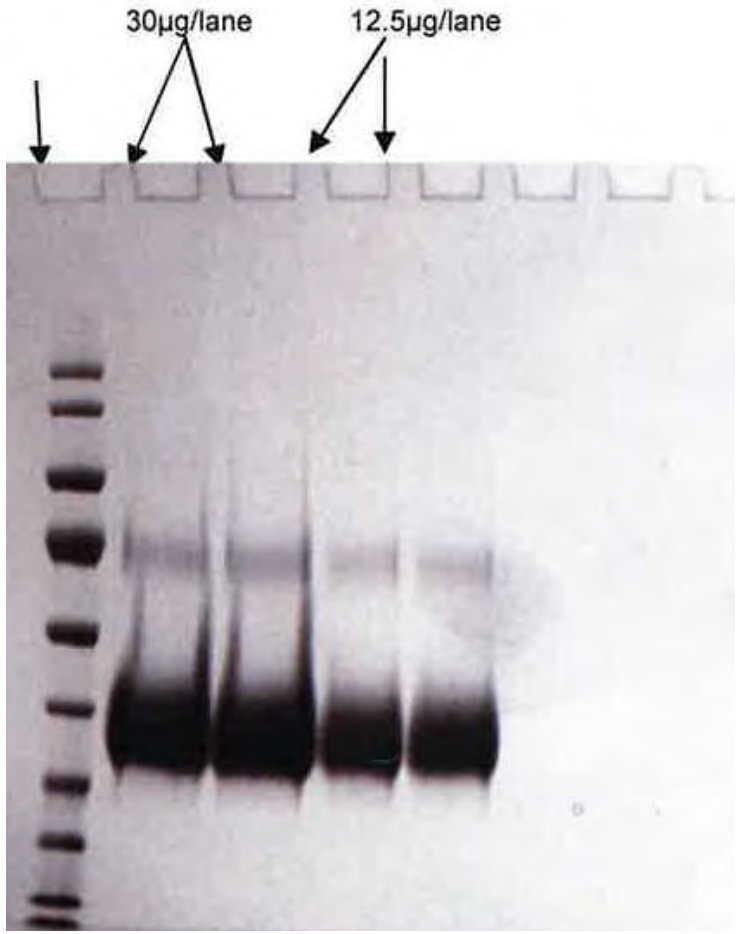
Table 1: Properties of 15/120 cCRS dimerised EPO lyophilised preparation

Sample	Dimer area %	Height mAU	Area mAU* min	Monomer peak area %	Height mAU	Area mAU* min
Run 1	3.14	15.06	633.01	96.86	463.26	1.95e4
Run 2	3.85	17.39	781.69	96.15	465.28	1.95e4
Run 3	3.91	17.50	810.63	96.09	466.92	1.99e4
Mean	**3.63**	-	-	**96.37**	-	-

Table 2 : SEC HPLC of definitive EPO Dimer using the Agilent 1200 system. Summarised λ = 214nm New TSK 3000SWXL column, col. Number: Y02437 100µl inject



1. DePaolis AM et al (1995) Characterization of erythropoietin dimerization. *J Pharm Sci* Nov;84(11):1280-4
2. Erythropoietin concentrated solution, monograph 1316, *Ph. Eur. 8th edition*, Strasbourg, France: Council of Europe; 2013.
3. Migueault I, Dartiguenave C, Bertrand MJ, Waldren KC (2004). Glutaraldehyde behaviour in aqueous solution, interaction with proteins and applications to enzyme cross-linking. *Biotechniques* 37;790-802.



Reducing SDS-PAGE of cCRS (containing around 4% dimer)

A standard has been developed for assessing the suitability of a SEC HPLC using chemically cross-linking dimer.

A six-laboratory collaborative study, organised by EDQM with NIBSC as lead. Dimer content of the preparation was 3.51% (CV=6.0%, range 3.33-3.83%) .

A range of column formats were all used by different laboratories. All laboratories were able to resolved the dimer from the monomer well with a resolution factor of 1.4– 1.9.

The symmetry factor of the monomer peak was also important and values of 1.0-1.2 should allow suitable discrimination of dimer and monomer peaks.

The stability of the dimer:monomer ratio was demonstrated. The freeze dried material showed excellent stability with a predicted loss of dimer content of 0.05% p.a. at –20°C.

The report has been endorsed by the Ph Eur Group 6. It was proposed to establish the cCRS as the Ph. Eur. EPO for SEC system suitability CRS batch 1. This is currently awaiting adoption into the monograph in 2017. by EDQM later in 2017.

Are there opportunities for the pharmacopoeias to produce reference materials to support impurity testing?

SEC validation, through controlled dimer generation

Peptide mapping, by preparation of mutants

IEF/CZE validation, through controlled chemical modification of charged amino-acids

RP-HPLC validation through controlled oxidation

Glycan analysis through the preparation of high/low Z number preparations

Horizontal standards such as glycans, and contaminants e.g. protein A

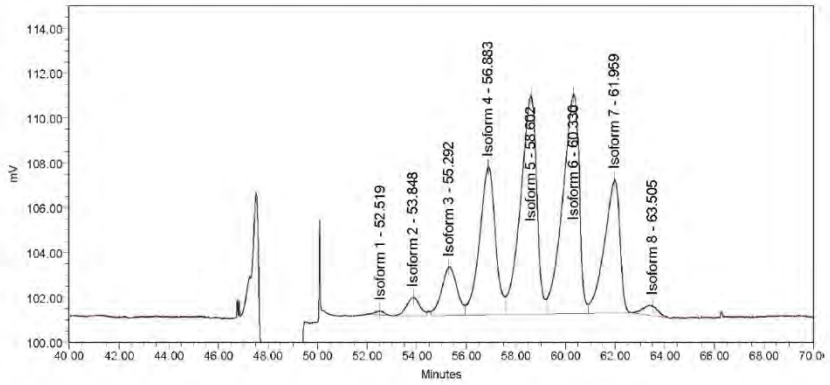
Intrinsic heterogeneity

Glycoprotein such as Erythropoietin exhibit a range of charge variants (glycoforms, arising form variations in the degree of terminal sialylation

These variants may be analysed by semi-quantitative electrophoretic methods, such as CZE

Capillary zone electrophoresis :

The isoforms are identified by their retention time in minutes: Isoform 1: 52.519 - Isoform 2: 53.848 - Isoform 3: 55.292 - Isoform 4: 56.883 - Isoform 5: 58.602 - Isoform 6 : 60.330 - Isoform 7: 61.959 - Isoform 8: 63.505

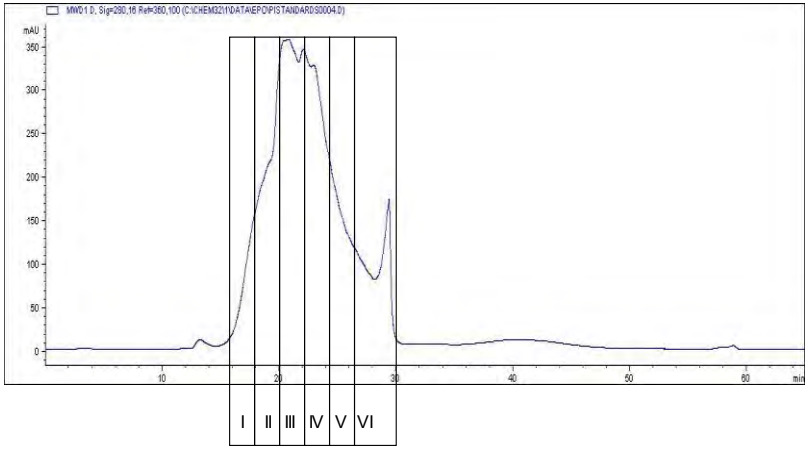


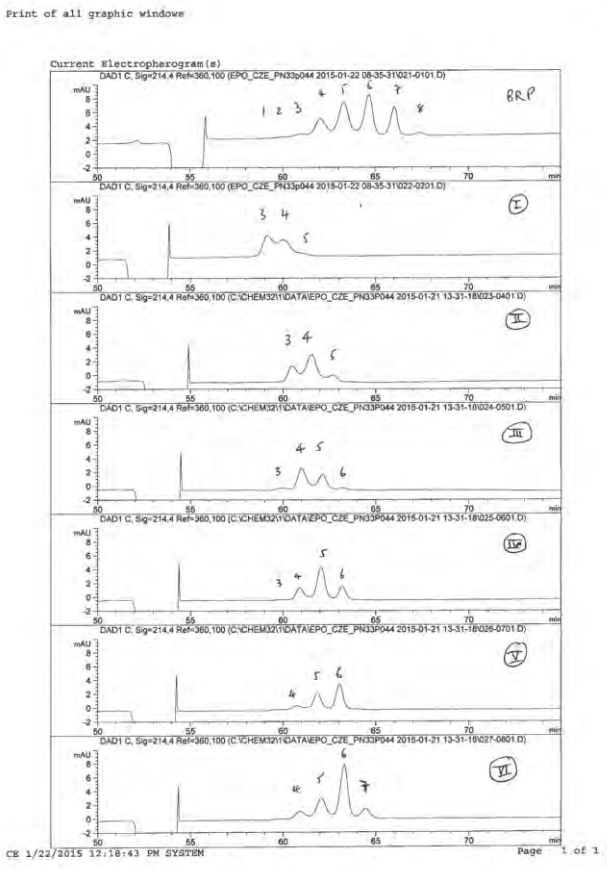
They may also be analysed by quantitative glycan analysis, which produces a Z number, generally held to be a measurable variable related to the distribution of iso-forms

Any glycan analysis method needs to be validated to demonstrate the relationship between Z number and distribution. In practice this can really only be done with a representative range of reference materials

EPO partially fractionated by semi-preparative IEX

Mono-Q
20mM tris pH 7.2
Salt gradient





These fractions exhibit a progressive enrichment in the acidic isoforms in later eluting materials as expected

Summary

Physical reference materials continue to play a vital part in the characterisation and control of biological medicines

With advent of biotech products without “equivalents in nature” the role for these standards may have changed

Examples of reference materials to control bioassay activity and system suitability standards to support assay method development and QA

Such reference materials need to be prepared using the same stringent requirements and established through multi-centre evaluation

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