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Challenges for International Standardisation and Traceability: Biologicals

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What are Biologicals?

World Health Organization (WHO) definition:

"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**."**

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Measurement of Biologicals - 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 material

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J H Burn. The errors of biological assay. 1928:

"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."

Thank goodness for WHO, we now have:

International Unit - IU

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International Standards, majority of which are assigned with IU

WHO biological reference materials established by NIBSC Number > 400, including:	Analytical methods supported Include:
Clotting factors and inhibitors Thrombolytics Hormones Cytokines and growth factors Enzymes Vaccines Micobiological antigens Toxins Antisera and immunoglobulins Genomic DNA, cDNA and RNA	In vivo bioassay In vitro bioassay Enzyme assays Receptor binding assays Function assays Microbiological assays Gene amplification methods

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WHO Principles

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

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Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on in vitro diagnostic medical devices European Directive 98/79/EC on in vitro diagnostic medical devices, which included the requirement for the traceability of values assigned to calibrators and control materials for in vitro diagnostic devices to be assured through available reference measurement procedures and/or reference materials of higher order.

In vitro diagnostic medical devices – Measurement of quantities in samples of biological origin – Metrological traceability of values assigned to calibrators and control materials

prEN ISO 17511

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prEN/ISO 17511 requires:

1. Single method studies (where possible reference method or conventionally agreed)
2. SI units rather than IU (where possible)
3. Traceability to previous standard, with defined uncertainty

WHO guidelines:

1. Calibrated in a multi-method study (rather than a single, reference method)
2. With values assigned in International units (rather than mg/mol)
3. With no imprecision assigned to the ampoule content

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Why can't we measure biologicals based on metrological principles with SI traceability?

- Biologicals usually have multiple targets, measurand difficult to define and can be a combination of components
- Biological activity cannot be measured directly by primary methods
- In most cases, not possible to isolate different active components within a biological
- Unclear path of traceability – relies on comparison with reference standard, the unit of which is related to a physical existence of the standard itself
- Undefined uncertainty of measurement

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Mass content do not always relate to activity

Glycan Mapping of rhEPO

- N-linked glycan chains were released from the protein using the enzyme N-glycanase
- The glycans were reductively aminated with a fluorescent label
- The glycans were separated by charge on a DEAE column
- These fractions were further separated on a reversed phase column
- The identities of the individual glycans was established by MALDI mass spectrometry

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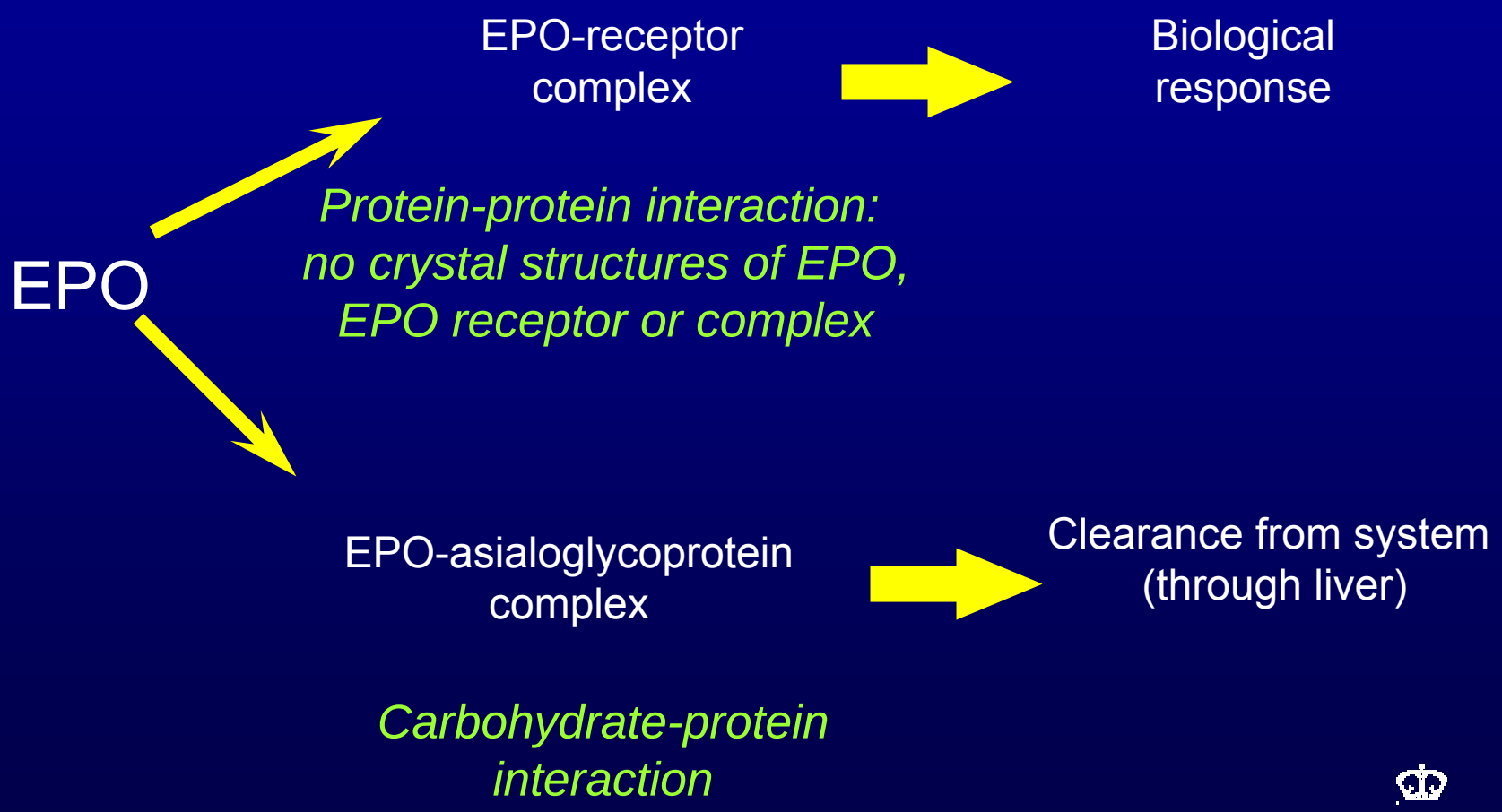
Processing pathways for EPO



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Glycan chains on rhEPO from CHO cells

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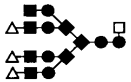
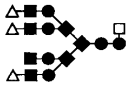
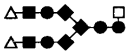
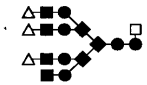
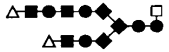
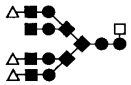
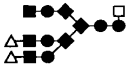
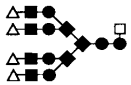
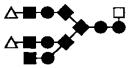
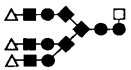
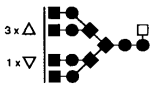
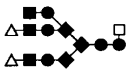
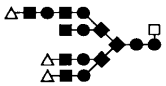
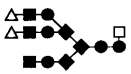
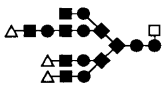
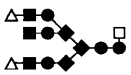
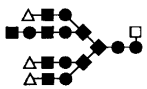
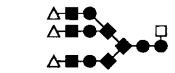
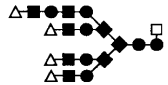
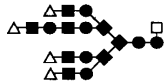
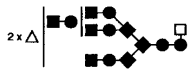
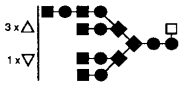
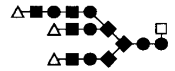
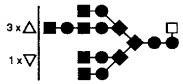
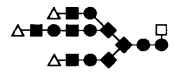
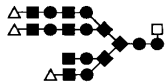
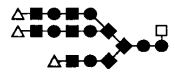
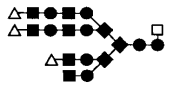
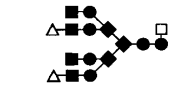
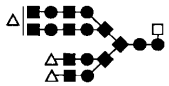
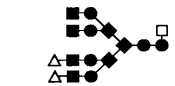
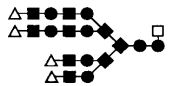
	N1.1A	< 1		N3.7.1A	5
	N1.1B	< 1		N3.7.1B	1
	N2.4	7		N3.7.1C	1
	N2.6A	< 1		N3.7.2A	3
	N2.6B	< 1		N4.4	19
	N2.6C	< 1		N4.2	2
	N3.4	2		N4.5	2
	N2.7.2A	2		N3.9.2A	2
	N2.7.2B	1		N3.9.2B	1
	N2.7.3	1		N3.10	1

	N3.5	9		N4.6.1	5
	N3.2	< 1		N4.6.2	5
	N2.10.3	1		N4.7A	< 1
	N3.7.2B	3		N4.7.B	< 1
	N3.7.3	2		N3.12A	< 1
	N3.9.3	2		N3.12B	< 1
	N2.10.1	<1		N3.12C	< 1
	N2.10.2	3		N4.8	7

Ron Hokke, Ph.D. Thesis, Utrecht, 1993

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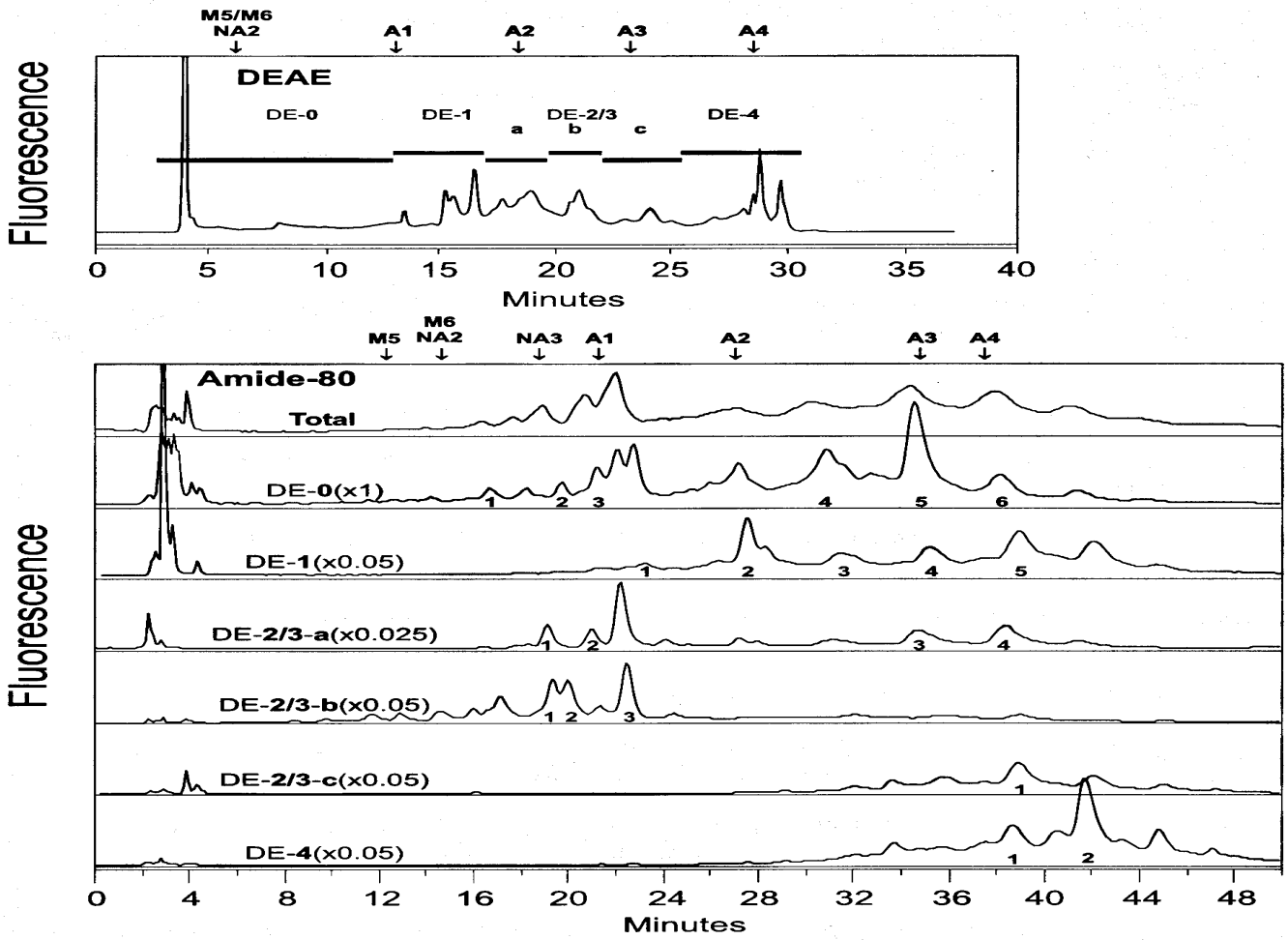
Glycan mapping of rhEPO



Yuen et al., *Brit. J. Haematol*, 2003, **121**, 511-526

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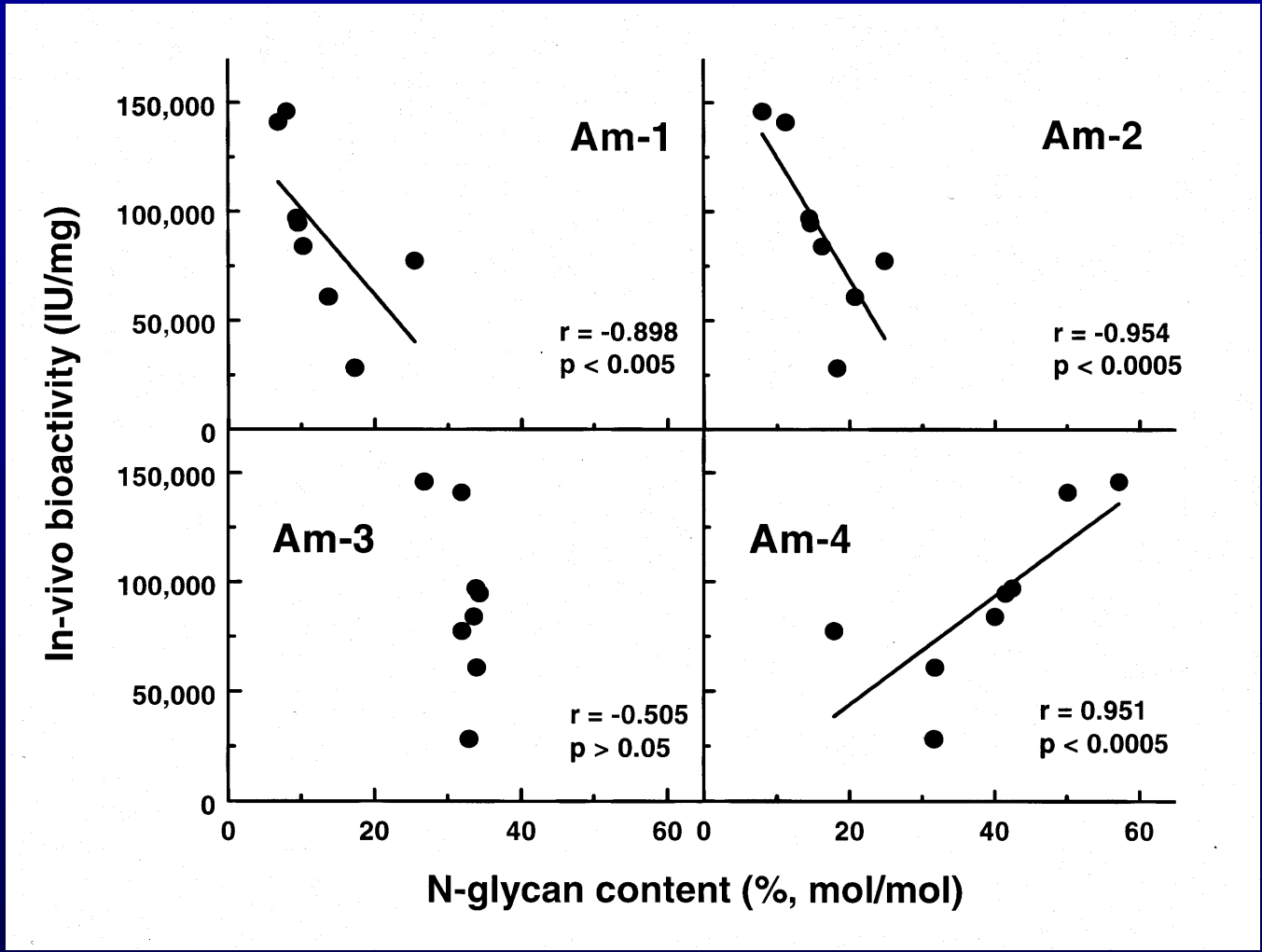
rhEPO: Correlation of glycosylation with *in vivo* activity



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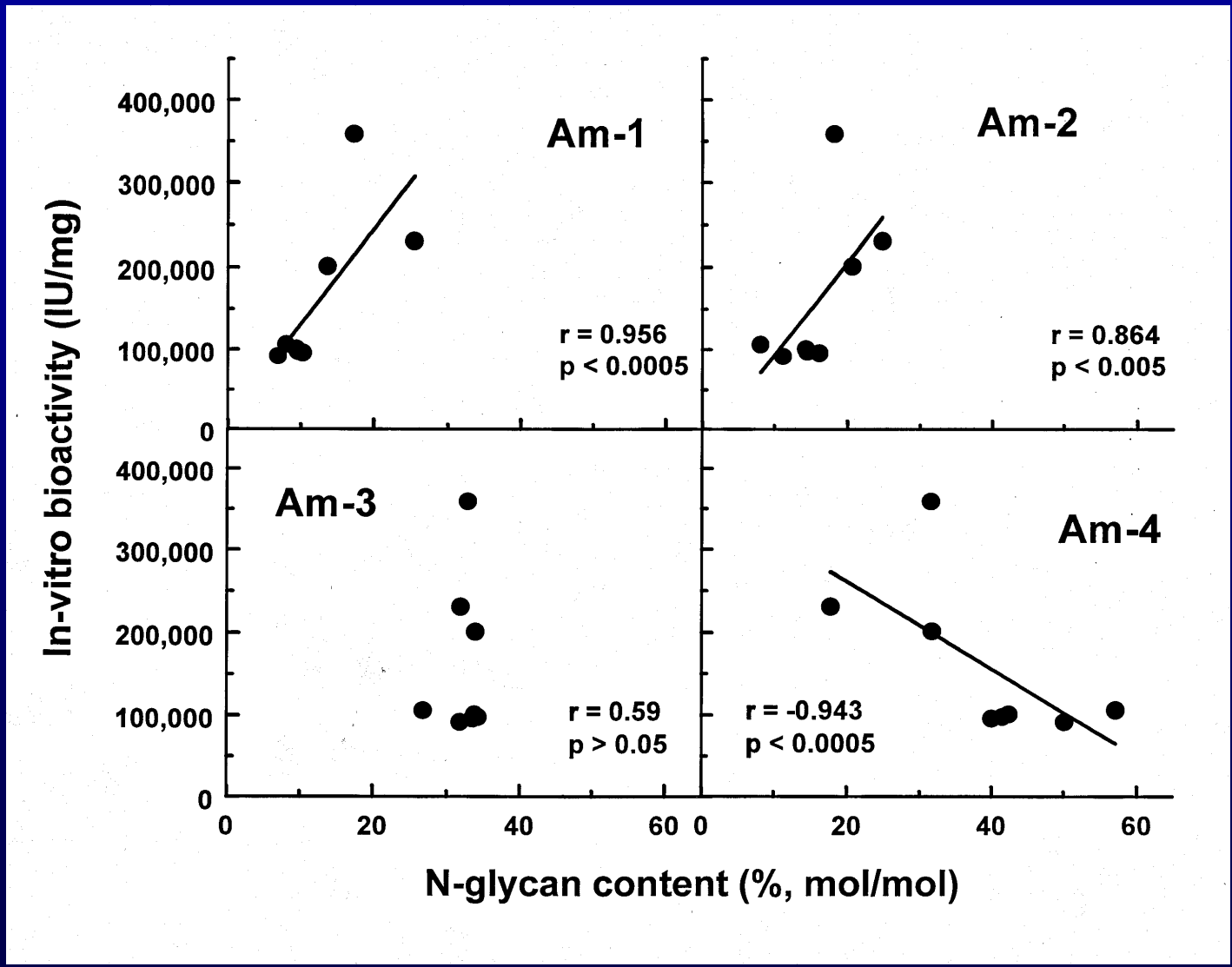
rhEPO: Correlation of glycosylation with *in vitro* activity



Yuen et al., *Brit. J. Haematol*, 2003, **121**, 511-526

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The traceability issue

Consider calibration of a reference material, rm for an analyte A, where a new reference is being set up to replace an earlier one

A(rm)-1 ⟶ A(rm)-2

1 The non-biological case

Rm-2 can be shown to be identical to rm-1 by analytical methodology
The analyte A is defined by this analytical methodology
Principles of metrological traceability apply

- Calibration of rm-2 in terms of rm-1
- Use of the single, most metrologically precise assay
- Traceability from rm-2 back to rm-1
- Formal statement of uncertainty

2 The biological case

Rm-2 cannot be shown to be identical to rm-1 by analytical methodology
The analyte A is defined by the reference material rather than the analytical methods
Principles of metrological traceability are difficult to apply

- Rm-2 cannot be calibrated in terms of rm-1
- No single “best” assay can be identified
- Since rm-2 now defines the analyte there is no traceability from rm-2 to rm-1
- “uncertainty” of rm-2 in terms of rm-1 is a can of worms ie highly complicated

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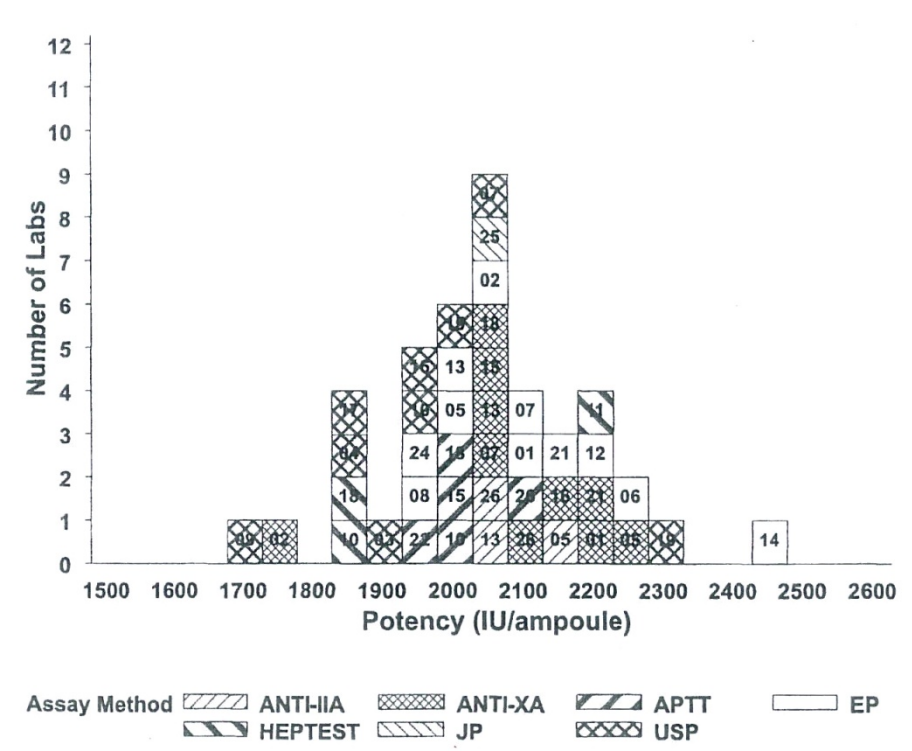
Uncertainty of measurement: Method bias, and single method vs multi method calibration

Calibration of the current International Standard for Unfractionated Heparin



Deviation from the mean of any assay result is composed of two elements:
the assay imprecision, and the 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”

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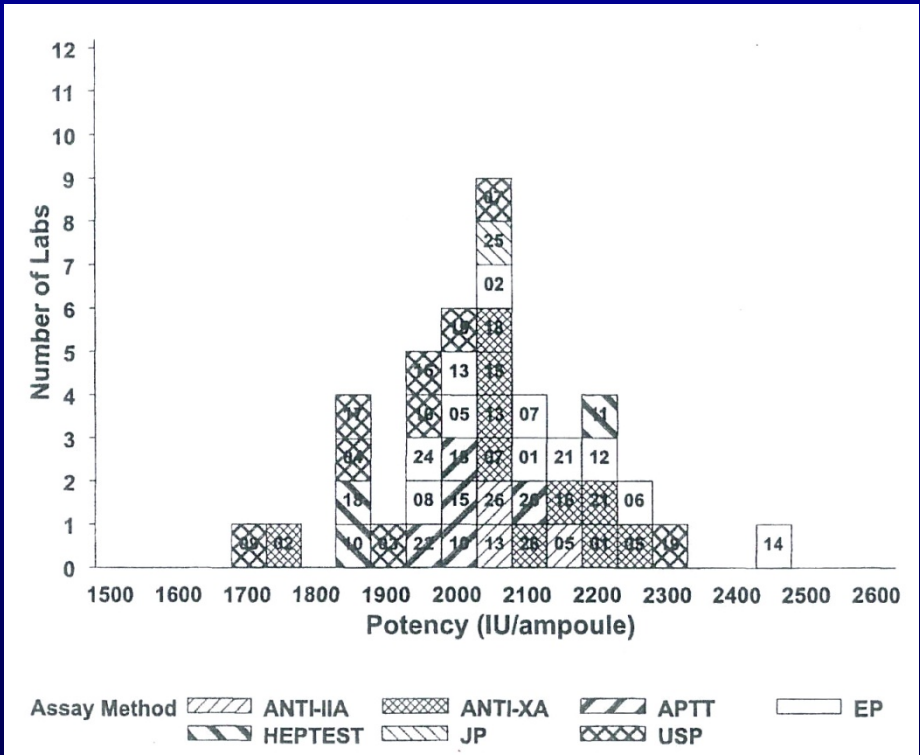
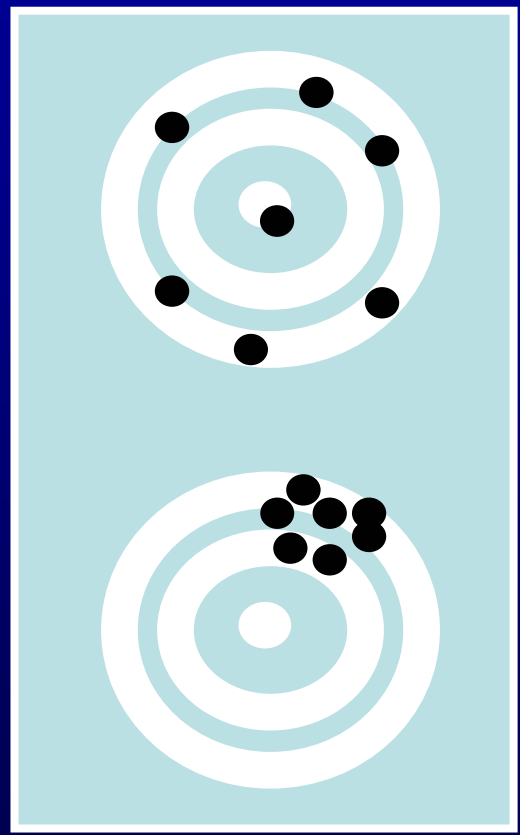
Uncertainty of measurement: Method bias, and single method vs multi method calibration

Calibration of the current International Standard for Unfractionated Heparin





Accurate, but not precise

Precise, but not accurate

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The principle of multi-method calibration is frequently modified,

For example

- Separate standards are frequently calibrated and established using “immunoassays” and “bioassays”

- Nonetheless, specific reference immunoassays/bioassays are not usually established

- Separate biological activities in the same reference standard: eg anti Xa and anti-IIa activity in low molecular weight heparin are separately calibrated

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Non-biological

Single-method calibration reflects a metrological imperative, where minimising the imprecision is considered the most important factor

Biological

Multi-method calibration reflects a biological approach, where the “true, overall value” is considered more important than the imprecision

Which is correct?

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The WHO/NIBSC standpoint has been that:

Assignment of imprecision of the estimate is inappropriate

It also follows that:-

For assignment in IU, minimising the imprecision by the use of single-method calibration is also inappropriate

For assignment in SI, both defined methods/calibration Protocols and assignment of imprecision may be appropriate

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There is light at the end of the tunnel

- WHO recognise the need to value assign well characterised reference standards with SI traceability

For example:

The 2nd International Standard for Somatropin, 98/574 calibrated against the 1st International Standard for Somatropin, 88/624 by a combination of SE HPLC, bioassays and immunoassays

88/624 contained 2.0mg per ampoule as defined by amino-acid analysis and UV-spectroscopy

Direct SI traceability from the 1st IS to the 2nd IS

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International standards and reference reagents with SI traceability

- 1st International Standard for Parathyroid Hormone 1-34 Recombinant, Human, 04/200 – traceable to primary calibrant PRS0404
- 1st WHO Reference Reagents for Chorionic Gonadotropin, Intact, Human, 99/688
- 1st WHO Reference Reagents for Chorionic Gonadotropin, Nicked, Human, 99/642
- 1st WHO Reference Reagents for Chorionic Gonadotropin, α subunit, Human, 99/720
- 1st WHO Reference Reagents for Chorionic Gonadotropin, β subunit, Human, 99/650
- 1st WHO Reference Reagents for Chorionic Gonadotropin, Nicked β subunit, Human, 99/692
- 1st WHO Reference Reagents for Chorionic Gonadotropin, β –core fragment, Human, 99/708

The primary calibrants PTH and all the HCG preparations were both value assigned by amino acid analysis and UV-spectroscopy. Correlation with bio- and immuno- assays values have been established

- Proposed 1st International Standard for Insulin-like Growth Factor –I, 02/254 – traceable to primary calibrant PS01

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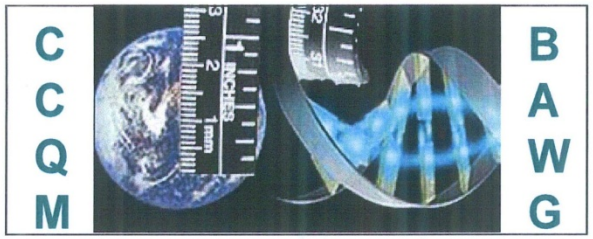
There is light at the end of the tunnel

- WHO recognise the need to collaborate with metrological orientated organisations such as the CCQM and IFCC
- BAWG – CCQM – road map to assist in calibration of basic building blocks of biologicals such as amino acids and peptide .....

NIBSC



BAWG Routemap (ii) Protein Measurement

CIPM - traceability to SI for protein measurement

CMC in complex protein measurement

Trigger

Target

CMC protein identification & quantification 1°

CMC protein characterisation & quantification 2°-

CMC protein structure/function/potency measurement 2° /3° / 4°

KC

pilot

Intercomparison activity

Proteome quantification (multiparametric)

PTM quantification

Protein potency

Protein interactions

Protein quantification

Protein RM

peptide quantification

Peptide RM (SI)

Protein function e.g immunoreactivity

peptide (P55) quantification

Protein (P58) structure

Under-pinning bioscience

Extraction/digestion

Measurement technology

Uncertainty estimation

Unit harmonisation

ELISA (P59) fluorescence

2005

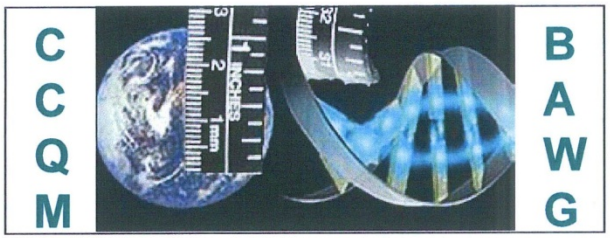
2007

2009

2011

2013

2015



BAWG Routemap (iii) Biomeasurement RMS

CIPM - traceability to SI for biomeasurement

Develop [si] traceable reference measurement system(RMS) for biological assays

CMC in complex biomeasurement

Trigger

Target

Intercomparison activity

KC

pilot

Gene RMS

Protein RMS

Cell/ bioassay RMS

[SI] Calibrated NA/gene RMs

Gene profiling diagnostic / identity

Calibrated protein RMs

Cell/cell interactions

Cell binding & transporter mechanisms

"standard" cell RM

Relative (NAT / expression array) quantification

Specific biomarker panel RM

Macromolecule / cell interaction

bioassay RM

Absolute DNA quantification

Biomarker quantification

Inter/intra cellular function

NA identity/ quantification

Protein identity/ quantification

Protein structure / function

Under-pinning bioscience

Uncertainty estimation

Sample prep'n

Unit harmonisation

Measurement technology

2005

2007

2009

2011

...2020

...2020

Acknowledgment

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- LGC
  - Helen Parks

The National Institute for Biological Standards and Control

Assuring the Quality of Biological Medicines

NIBSC