Units and traceability in biological reference materials

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In the context of Biological Standardization, WHO has defined a biological substance as "a substance which cannot be completely characterized by physico-chemical means alone, and which therefore requires the use of some form of a bioassay". The 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, and since the 1920's the International Standards, currently supplied by WHO, have, in many cases served as the international biological reference materials for such procedures.

It had been proposed that analytes measured in diagnostic procedures may be divided in two broad categories:

Category A: those where results of measurement are *traceable* to SI units, i.e. mole/L.
They are chemically well-defined compounds, and for many, reference measurement systems (RMSs; reference measurement procedures and primary reference materials) are in place. Examples are: glucose, cholesterol, urea, steroid hormones, thyroid hormones. To this category belong some 100 compounds.

Category B: those where results of measurement are NOT *traceable* to SI units but are expressed in terms of arbitrary units e.g. WHO International Units. Tests for such analytes may be based on one of three principles: biological function (e.g. clotting assays; enzyme catalytic activity assays, infectivity assays), immunoprocedures (e.g. ELISA) and nucleic acid amplification techniques. "Biological substances" fall into this category. Group B comprises ~ 500 substances/ analytes.

Biological standardization and control: developments in clinical endocrinology

	Therapy	Quality control	Diagnostic measurement
1920's	Use of crude tissue extracts (eg pancreas, thyroid, pituitary)	Potency determined by biological assay in animals	Limited use of bioassays
		Biological standards	
1050's	Identification of active principles		
1970's	Production of purified hormones from tissue	Introduction of highly selective analytical methods such as HPLC	Development of immunoassays
	Production of small peptides by chemical synthesis		Use of WHO standards in immunoassay calibration
		Reduced dependency on bioassays	
1980	Production of proteins by recombinant DNA technology	Pressure to reduce animal use	Availability of pure, well characterised assay reagents
1990	Production of complex glycoproteins by recombinant DNA technology	Single European market	Dramatic increase in number of substances to be measured
1994		Removal of batch release orders from hormones	Trend away from the use of units
		Pressure to eliminate use of activity units	

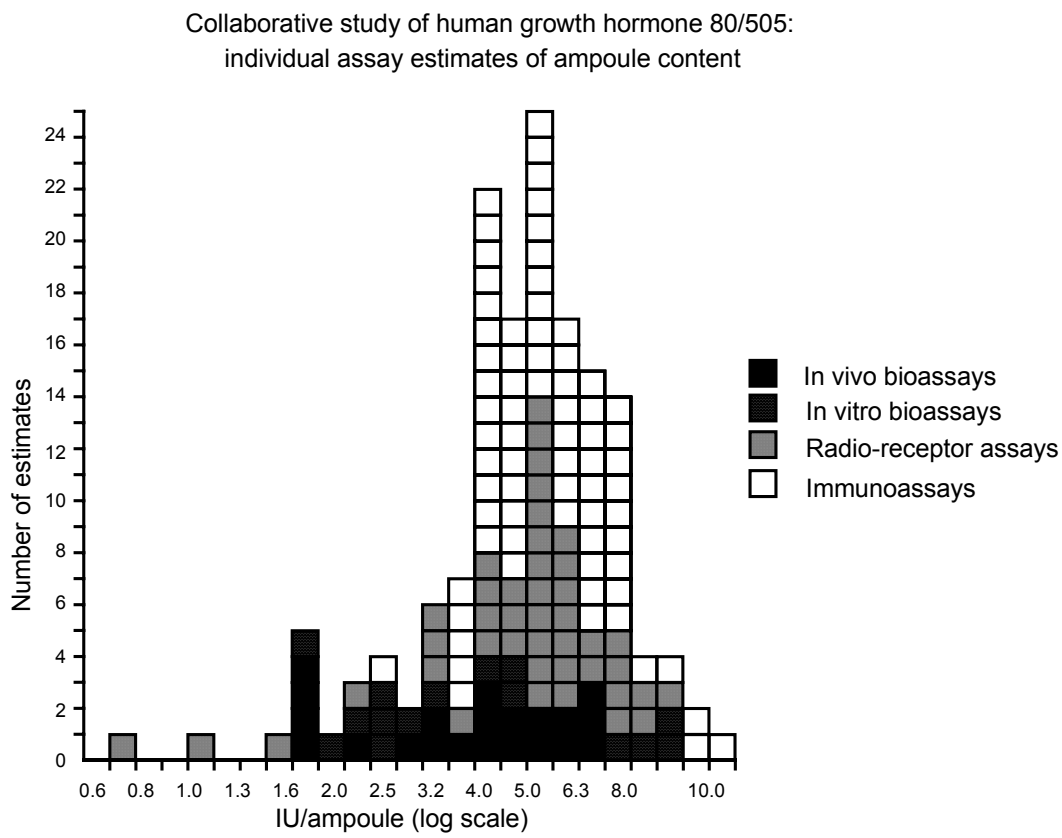
"Dudley had, by that time, succeeded in preparing insulin by precipitating as a picrate and recovering as a hydrochloride in the form of a dry powder. My intervention accordingly took the form of insisting that it was complete nonsense to try to define any unit of any remedy in absolute terms of reactions in limited numbers of animals; and that, from the international point of view, the only sensible thing was to obtain the remedy in perfectly stable form, and define the unit in terms of an absolute quantity of such a standard sample internationally accepted, leaving the method of its determination to be the subject of indefinite possibilities of Experimental improvement. McLeod replied that he had no doubt that such a policy would be ideal, but that he had no reason to believe that the preparation of a sample of insulin in a dry and indefinitely stable form was a practical possibility. At that point I was glad to be able to take from my waistcoat pocket a small tube of the preparation which was to become the first international standard, and roll it across the table to McLeod with the statement 'Well there it is'"

Sir Henry Dale, writing on the Edinburgh conference of 1923

Dale's principles:

- Arbitrary rather than absolute units
- Traceability to a standard with a physical existence
- Unrelated to a method of determination

Figure 1



(Data from Bristow, Gaines-Das, Jeffcoate and Schulster, 1995)

The first IS for hGH, 80/505

Purified human pituitary hGH

Calibrated by International collaborative study using in vivo bioassays, in vitro bioassays, receptor assays, immuno-assays

Mean of all assay estimates - 4.4 IU/ampoule

Assigned content 4.4 IU/ampoule

Nominally, 1.7mg/ampoule

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

prEN ISO 17511

1 Cases with primary reference measurement procedure and primary calibrator(s) giving traceability to SI units

- Cases with International conventional reference measurement procedure (which is not primary) and international conventional calibrators without traceability to SI
- Cases with International conventional reference measurement procedure (which is not primary) but no international conventional calibrators and without traceability to SI
- Cases with International conventional calibrator (which is not primary) but no international conventional reference measurement procedure and without traceability to SI
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The reference system and biological analytes: Problems that require solutions

- The reference method

2 The SI unit and its “absoluteness”

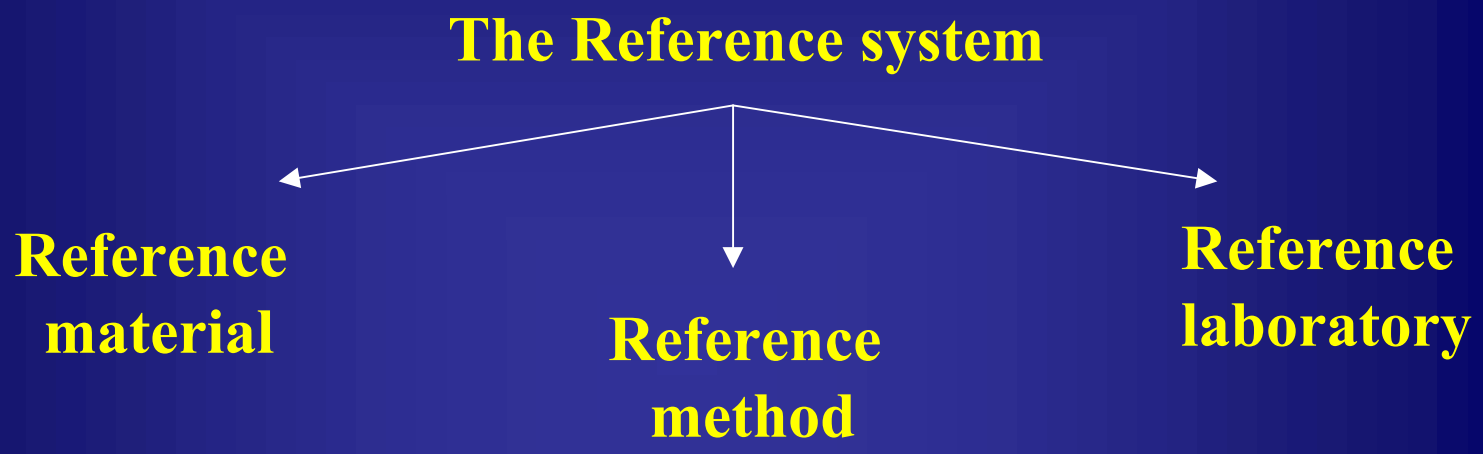
3 Method bias, and single method vs multi method calibration

4 Calibration and the assignment of imprecision

5 Biological units and the metrological hierarchy

1 The reference method and biological analytes

Assignment of a numerical value (calibration) to a standard must be done within the context of a Reference system



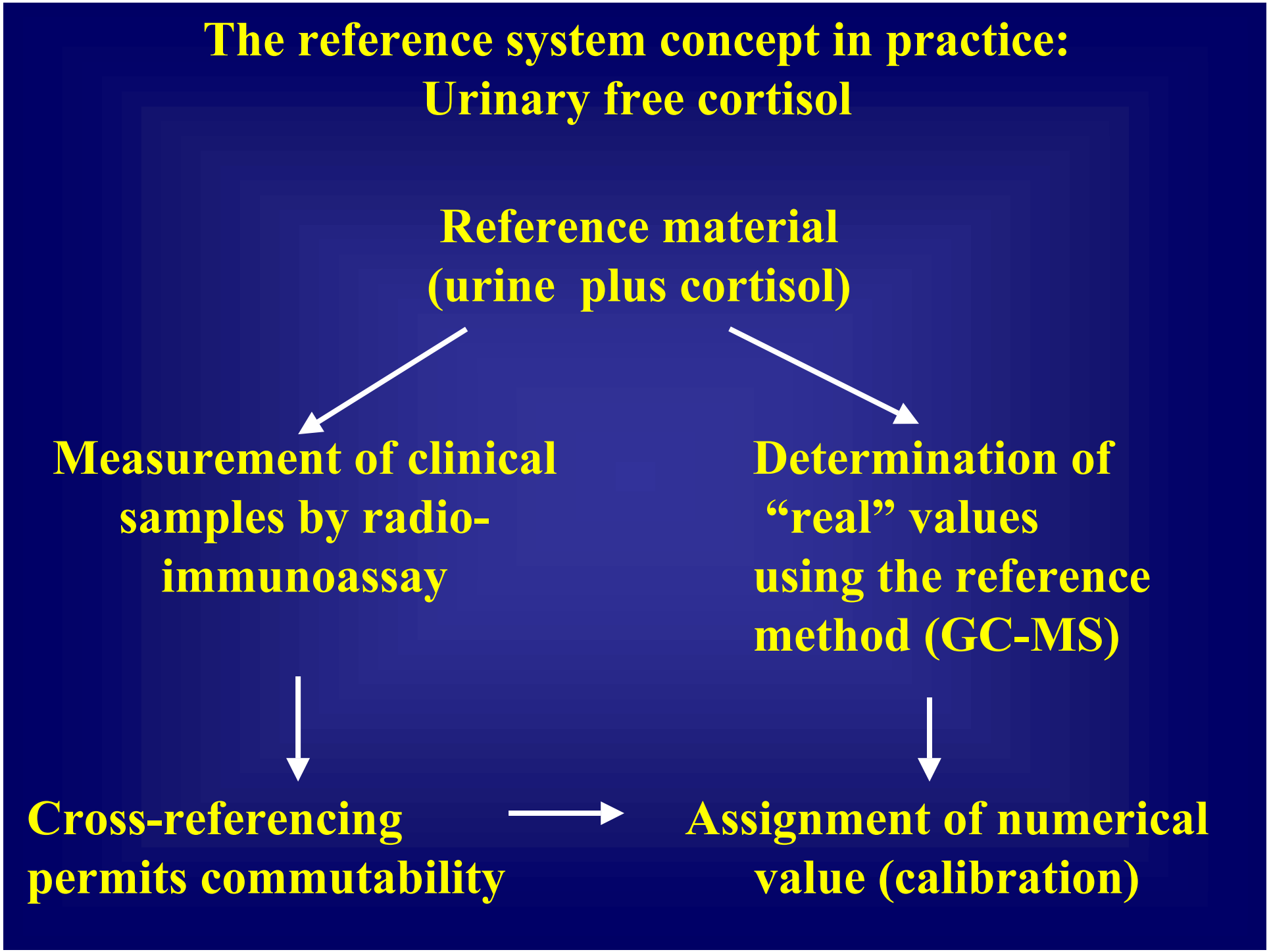
The application of the reference system is intended to to provide:

traceability

quantification of uncertainty

commutability

The reference system concept in practice: Urinary free cortisol



The Performance of the Reference Method should:

be completely definable by a written standard

be capable of measuring, in absolute terms, levels of the analyte in clinical samples

be stable between laboratories and over time

provide data in SI units with a quantifiable uncertainty

provide a complete physico-chemical description of the analyte as a unique, homogeneous chemical entity

be distinct from the routine immunoassays used in the clinic

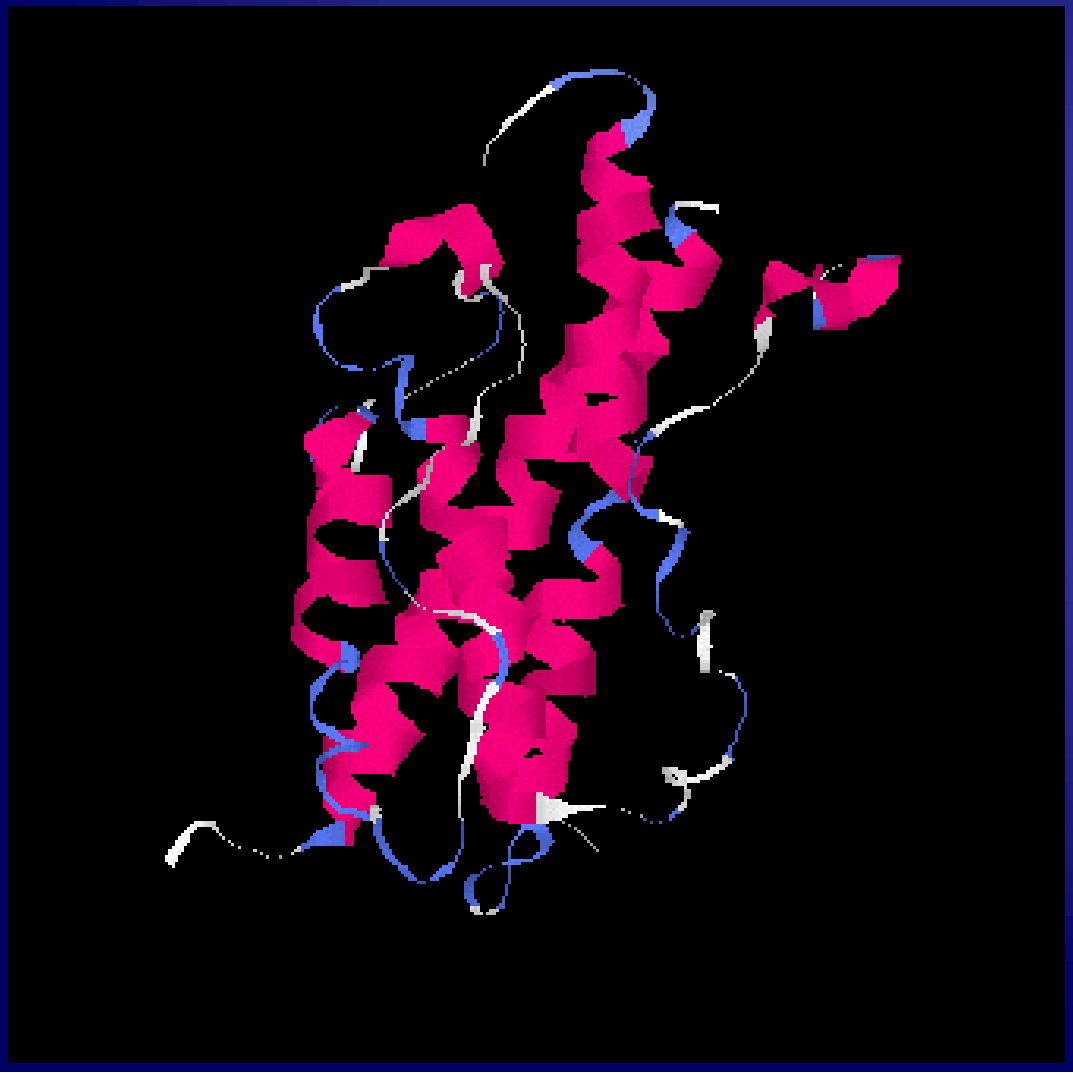
Biological analytes

1 Where calibration of the reference material is traceable to SI units

2 Where calibration of the reference material is not traceable to SI units

1 Where calibration of the reference material is traceable to SI units

Human growth hormone:



Molecular weight 22,000
191 amino-acids
3087 atoms

The second IS for somatropin: assignment of ampoule content

Specified HPLC method

Laboratory	Laboratory mean (mg/ampoule)
1	1.86
2	1.94
3	1.91
4	1.96
5	1.93
6	2.02
7	1.96
8	1.96
9	1.82
10	1.95
11	1.91
12	1.94
13	2.00
14	1.93
15	1.97
16	1.85

Overall mean
1.933mg/amp
RSD 2.69%

The physico-cemical assay does not stand alone. It is only valid in the context of a specification which includes:

RP,SE, IEX HPLC
Peptide mapping
electrophoretic properties
LC-MS
Bioassay

The Performance of the Reference Method should:

be completely definable by a written standard

be capable of measuring, in absolute terms, levels of the analyte in clinical samples

be stable between laboratories and over time

provide data in SI units with a quantifiable uncertainty

provide a complete physico-chemical description of the analyte as a unique, homogeneous chemical entity

be distinct from the routine immunoassays used in the clinic

The Performance of the Reference Method should:	hGH
be completely definable by a written standard	Yes
be capable of measuring, in absolute terms, levels of the analyte in clinical samples	No
be stable between laboratories and over time	Yes
provide data in SI units with a quantifiable uncertainty	Yes
provide a complete physico-chemical description of the analyte as a unique, homogeneous chemical entity	No
be distinct from the routine immunoassays used in the clinic	Yes

Biological analytes

1 Where calibration of the reference material is traceable to SI units

2 Where calibration of the reference material is not traceable to SI units

Thyroid stimulating hormone

A 30kD heterodimeric glycoprotein

- ? and ß subunits
- Glycosylated
- Sulphated
- no possibility of assigning reference preparation content in SI units

Calibration of the current International Reference Preparation for TSH

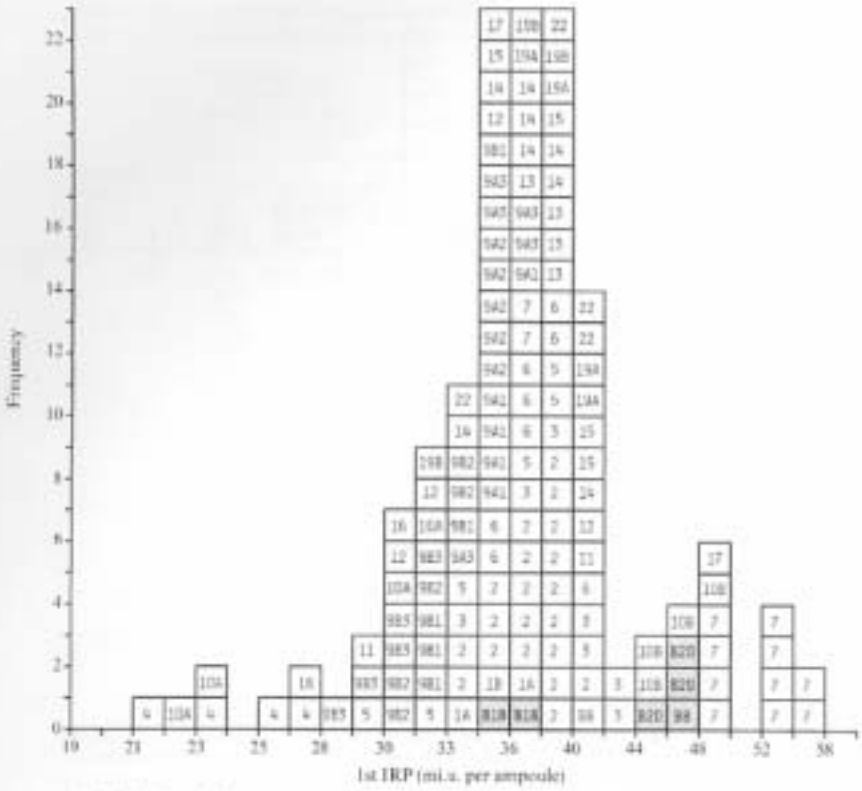


FIGURE 1. Calibration by immunoassay of ampoules coded 80/558 and H, its coded duplicate, as mi.u. of the First International Reference Preparation (IRP) of TSH per ampoule, and by bioassay (shown shaded and with laboratory numbers preceded by the letter B) as mu. MRC Research Standard A.

Variability of estimates Is derived from:

- Assay imprecision

- Assay bias

Current dogma would require the selection of one of These assays methods as a “reference method”, Thereby eliminating assay bias in the calibration

The Performance of the Reference Method should:	hGH (SI units)	TSH (IU)
be completely definable by a written standard	Yes	No
be capable of measuring, in absolute terms, levels of the analyte in clinical samples	No	No
be stable between laboratories and over time	Yes	No
provide data in SI units with a quantifiable uncertainty	Yes	No
provide a complete physico-chemical description of the analyte as a unique, homogeneous chemical entity	No	No
be distinct from the routine immunoassays used in the clinic	Yes	No

“Reference methods” for:

-Chemical analytes
-Biological analytes in SI units
-Biological analytes in arbitrary, international units

-are not the same thing.

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Primary structure
(amino-acid sequence)

Secondary structure
(helix)

Tertiary structure
(3D conformation)

Quaternary structure
(subunit interaction)

Analytical methods will be differentially affected by structural modifications.

For example: proteolysis would affect size-exclusion chromatography, but would not be recognised by amino-acid Analysis

For complex macro-molecules, SI units are not, in practice, absolute, but are method-dependant

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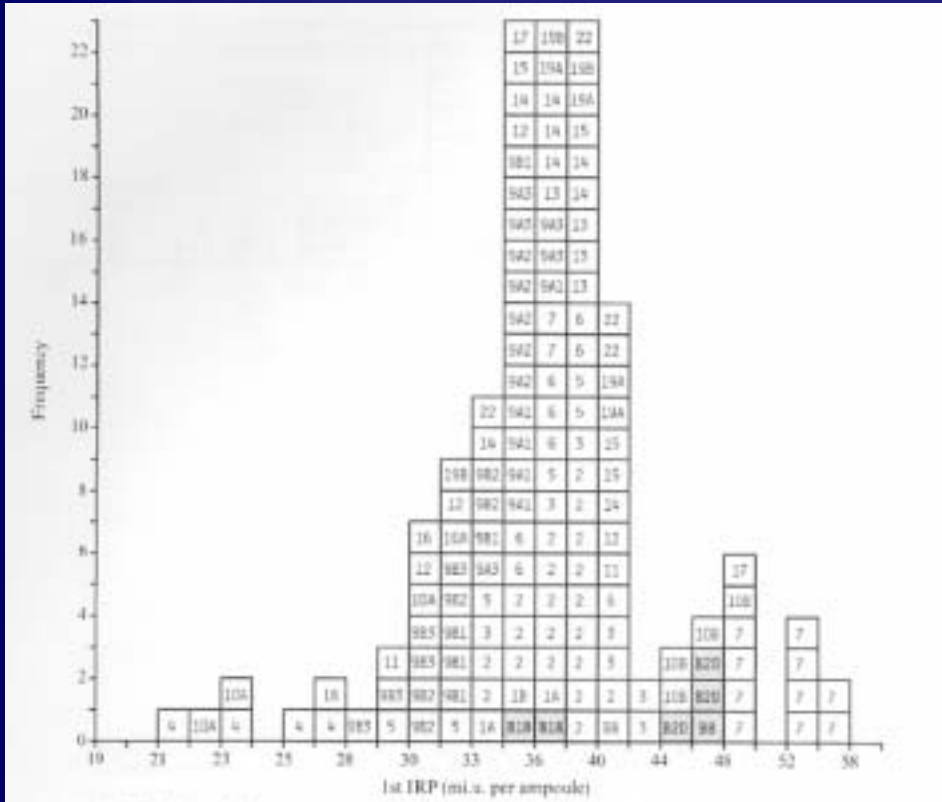

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Deviation of any assay result from the mean is composed of two elements; the assay imprecision, and the bias:

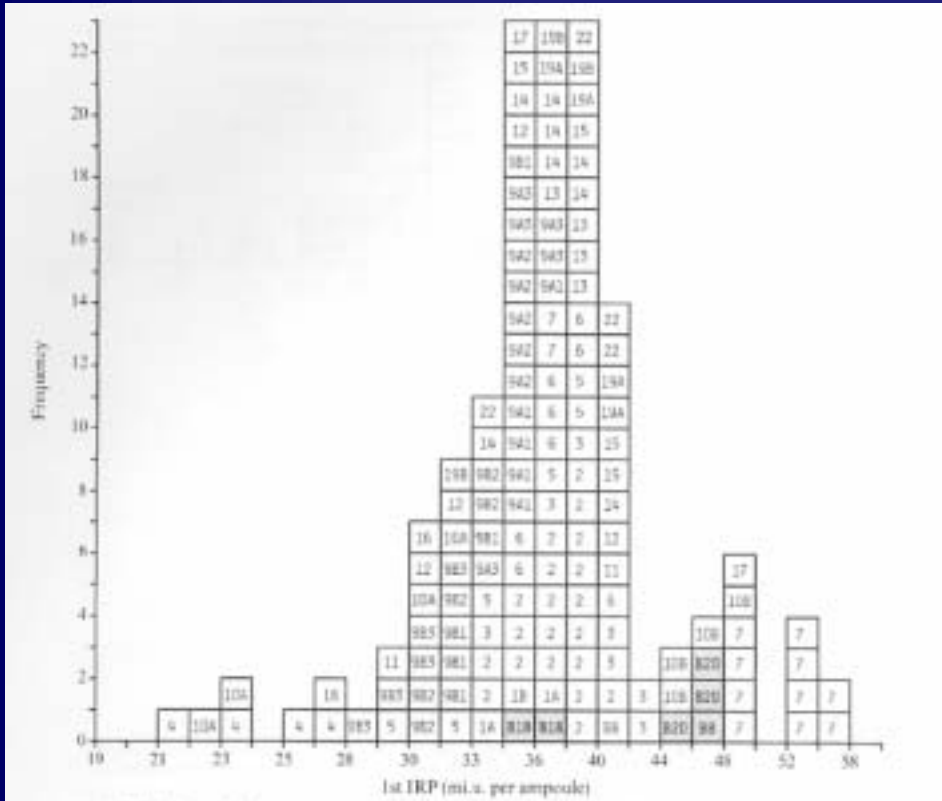
The WHO multi-method approach, by including all assays, seeks to average out, and therefore eliminate the bias effect.

The WHO approach will provide an estimate which is "accurate" but not "precise"

The "reference-method approach will provide an estimate which may be "precise", but not "accurate"

3 Method bias, and single method vs multi method calibration

Calibration of the current International Reference Preparation for TSH



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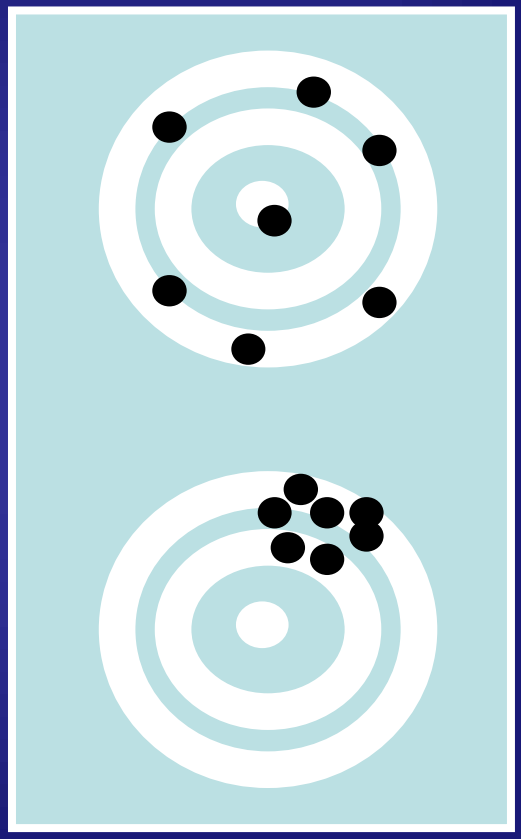

Accurate, but not precise

Precise, but not accurate

Single-method calibration reflects a metrological imperative,
Where minimising the imprecision is considered the most
Important consideration

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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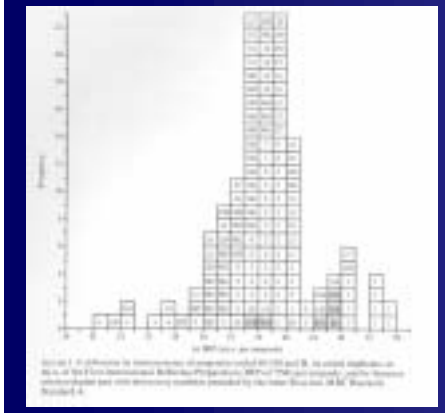
Thyroid stimulating hormone: International reference preparations for immunoassay

WHO 68/38 → 150 mIU/ampoule



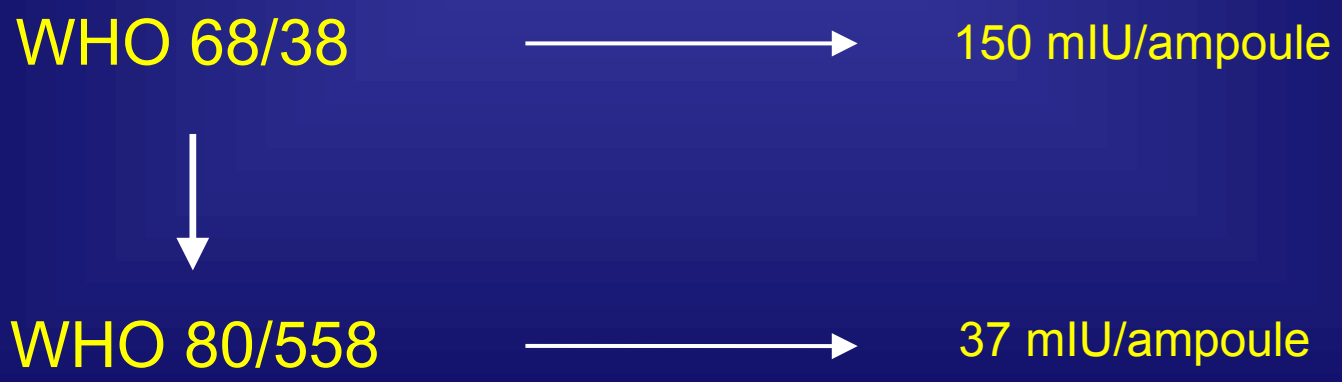
Calibration of 80/558 in terms of 68/38

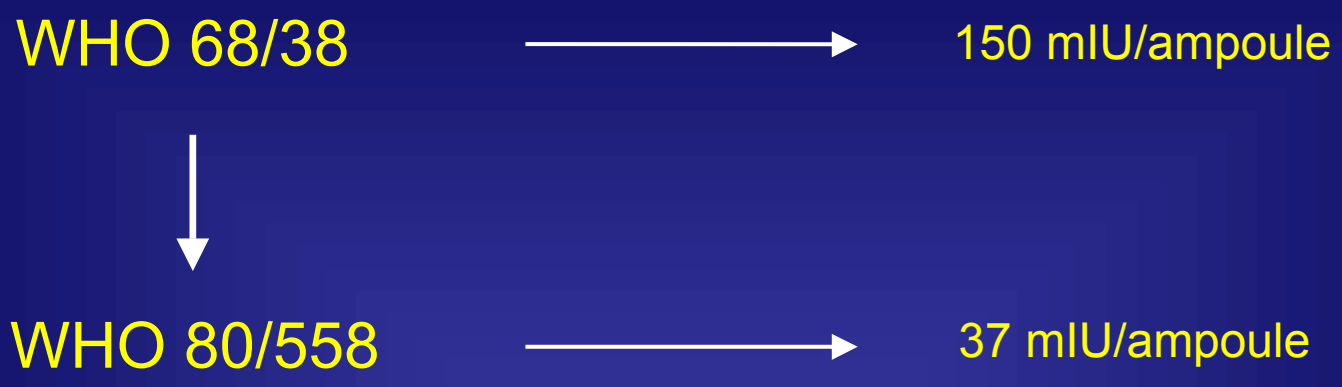
37mIU/ampoule
± 21%

WHO 80/558 → 37 mIU/ampoule

Should the second IRP, 80/558, be assigned a content of 37 mIU/ampoule, or 37 ± 21% mIU/ampoule?

The International Unit is, by definition, a fraction of the International Standard





Units expressed in terms of 80/558 cannot be expressed in terms of, or traced to units of 68/38, as 68/38 does not exist as a standard.

The unit as defined by 68/38 ceases to exist, and is re-established defined by 80/558

The unit has no existence independent of the standard, in the way that SI units do.

The WHO/NIBSC standpoint has been that:

Assignment of imprecision of the estimate is inappropriate

Minimising the imprecision by the use of single-method Calibration is also inappropriate

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prEN ISO 17511 appears to assume a metrological "hierarchy", in which SI units are of a higher metrological order than International units.

This would imply that, where possible, procedures reporting SI units should be used to calibrate reference preparations

This may be a flawed assumption. Many biologicals exist in both active and inactive states in plasma, where the activity reflects the clinical situation of the patient

Calibration in less precise biological units would be more appropriate than calibration in more precise, clinically irrelevant SI units

The SI unit should not be considered to be metrologically superior by virtue of its greater precision

Summary

1 WHO/NIBSC supply international standards for assays of biological analytes

- The international unit is defined as a fraction of the International standard and is thus traceable to the current international standard
- Replacement of an international standard re-defines the unit and therefore its traceability
- Current developments in analytical methodology offer the potential for better traceability to SI units, however current clinical chemistry principles may be incompletely applicable to biologicals
- NIBSC/WHO is committed to participating in the rational elaboration of principles and procedures for ensuring the traceability of the international unit.