Role of primary and secondary reference materials

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Traceability: a fundamental concept in QA

- **Traceability to a common standard (ideally up to the SI) ensures a high quality and (long term) equivalence of measurement results obtained by different methods**
- **Traceability is not a purpose on its own (or formal claim) and has to be correctly implemented**
- **Traceability concept embedded in relevant standards on requirements and competences of laboratories (ISO 17025, ISO 15189), in vitro diagnostics calibration (ISO 17511) and reference materials (ISO Guide 30-35 series, ISO 15194)**

Applicability of the traceability concept

Quantities:

- **The traceability concept applies. Traceability of measurement results is achieved by an unbroken (unbiased) sequence of calibrations (using reference materials), each contributing to the uncertainty.**
- **Traceability of measurement results can be achieved to the SI via primary and eventually secondary CRMs or to conventional measurement standards (e.g. WHO International Standards), the latter establishing an arbitrary scale**

Applicability of the traceability concept

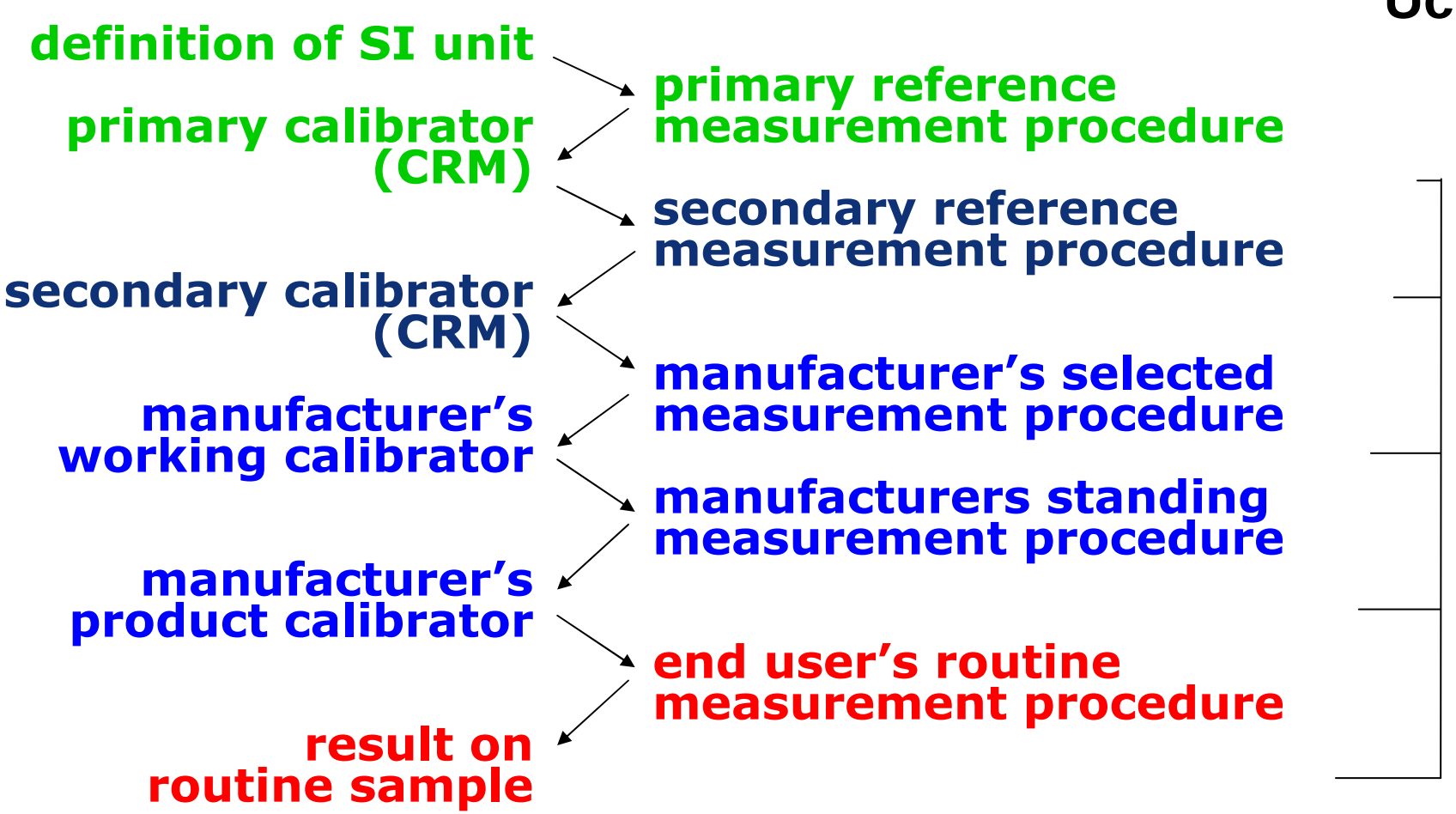
Nominal properties:

- **No international consensus on what traceability means for nominal properties and how to establish**
- **Traceability to a material standard defining the nominal property technically does not make sense (i.e. the identity or sequence would not be defined by a reference material of 'higher order')**
- **Rather a matter of metrological quality of the method(s) used to attribute the nominal property**

Use of certified reference materials

- **Certified reference materials play an important role as calibrators in traceability chains and related calibration hierarchies**
- **IVD Directive 98/79/EC requires traceability of values assigned to product calibrators to reference materials and reference measurement procedures of higher order**
- **Can be used for overall quality assurance and for method validation purposes**

ISO 17511


definition of SI unit
primary calibrator (CRM)
secondary calibrator (CRM)
manufacturer's working calibrator
manufacturer's product calibrator
result on routine sample
primary reference measurement procedure
secondary reference measurement procedure
manufacturer's selected measurement procedure
manufacturers standing measurement procedure
end user's routine measurement procedure
Uc

Definitions

- **Primary reference measurement procedure:**
  - Reference measurement procedure having the highest metrological qualities, whose operation can be completely described and understood, for which a complete uncertainty statement can be written down in terms of SI units, and where results are, therefore, accepted without reference to a measurement standard of the quantity being measured (or to a calibrator of the same quantity).
  - It shall be based on a principle of measurement proved to be analytically specific, …, and having a low uncertainty of measurement.

Definitions

- **Primary reference material:**
  - Reference material having the highest metrological qualities and whose value is determined by means of a primary reference measurement procedure
  - The primary calibrator shall have its value assigned either directly by a primary reference measurement procedure or indirectly by determining the impurities of the material by appropriate analytical methods. The material usually is highly purified containing a physico-chemically well-defined analyte, ….

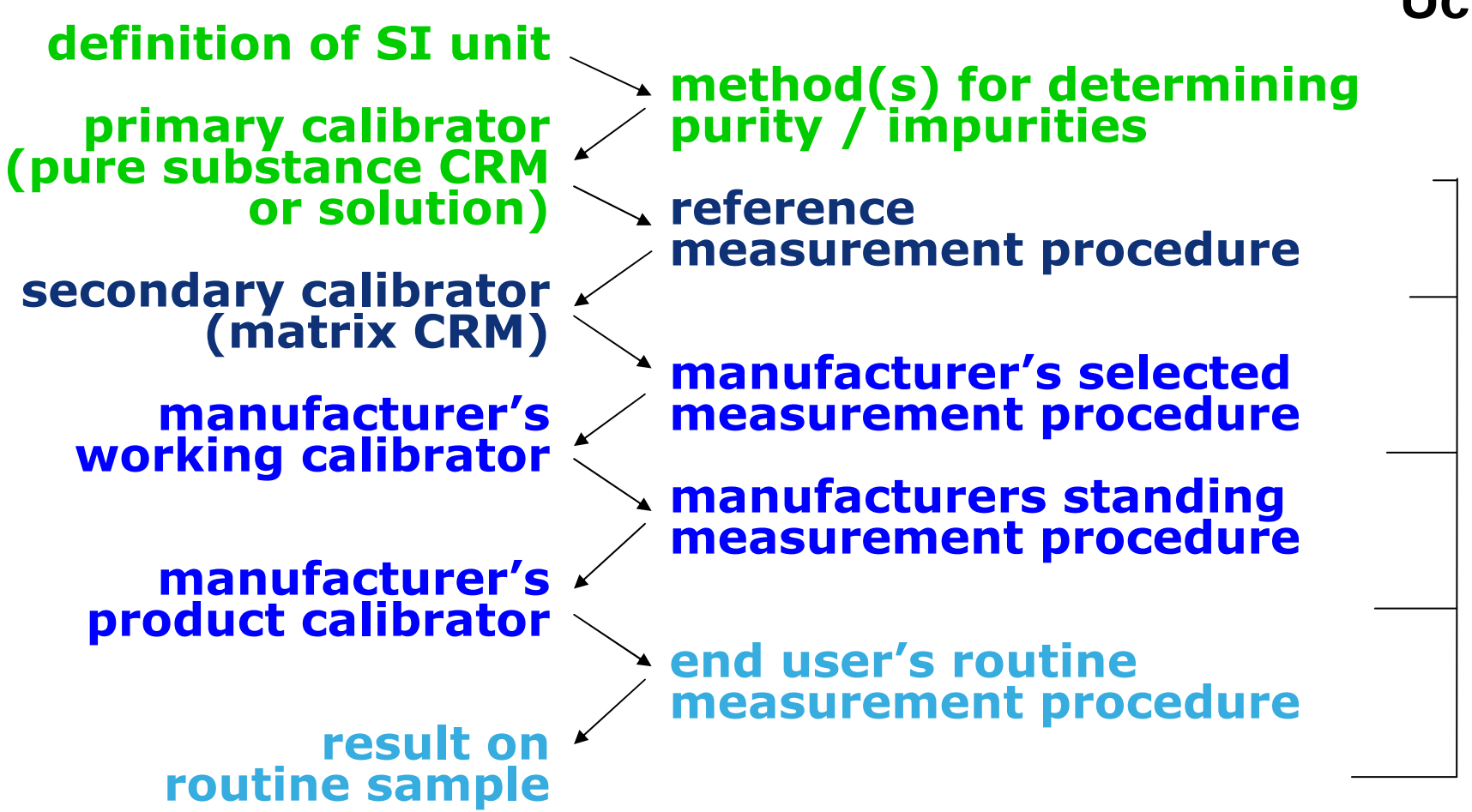
Definitions

- **Secondary reference material:**
  - Secondary calibrator shall have its value assigned according to one or more secondary reference measurement procedures and is usually accompanied by a certificate.
- **Secondary reference measurement procedure:**
  - Secondary reference measurement procedure shall describe a measuring system which is calibrated by one or more primary calibrators.

Technical requirements for establishing a traceability chain (ISO 17511:2003)

- Definition of the measurand (particular quantity subject to measurement (VIM2))
- Maintain the measurand throughout the traceability chain (for which primary and secondary CRMs certified)
- Elimination of bias
- Commutable calibrators / CRMs
- Otherwise traceability chain will be broken and no equivalence of measurement results will be achieved

Common situation



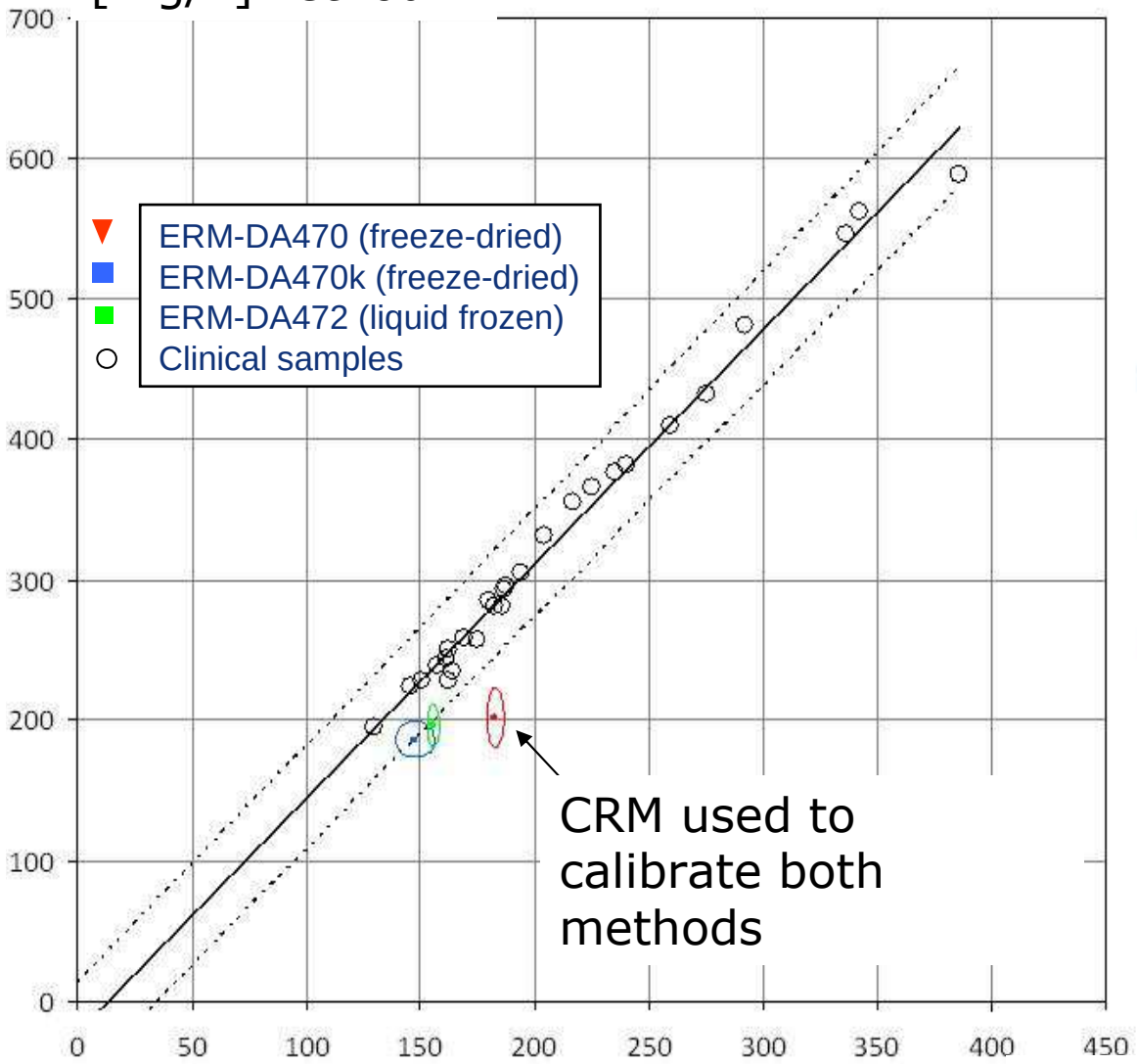
Typical traceability chains in laboratory medicine

- The measurand (particular quantity subject to measurement) is not maintained throughout the traceability chain and usually defined by the methods applied
- Relationship between measurands must be known
- Values for clinical samples and calibrators obtained by different methods in a traceability chain should correlate closely (calibrators are commutable)
- Works well for small molecules. For large molecules there are more pitfalls

Role of primary and secondary RMs

- **Primary and secondary reference materials (calibrators) play an important role as anchor points in a calibration chain**
- **The term primary and secondary reference materials relates to their position in the calibration hierarchy rather than to the metrological quality of the measurement procedures used for their value assignment**

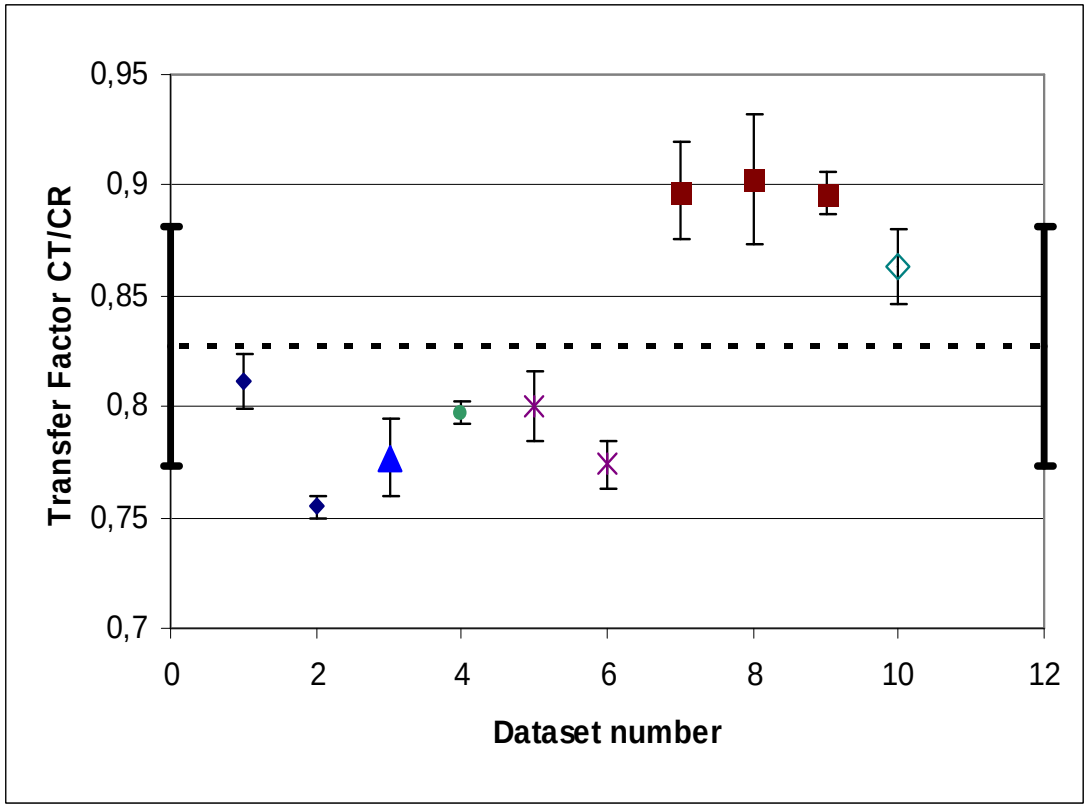
Ceruloplasmin



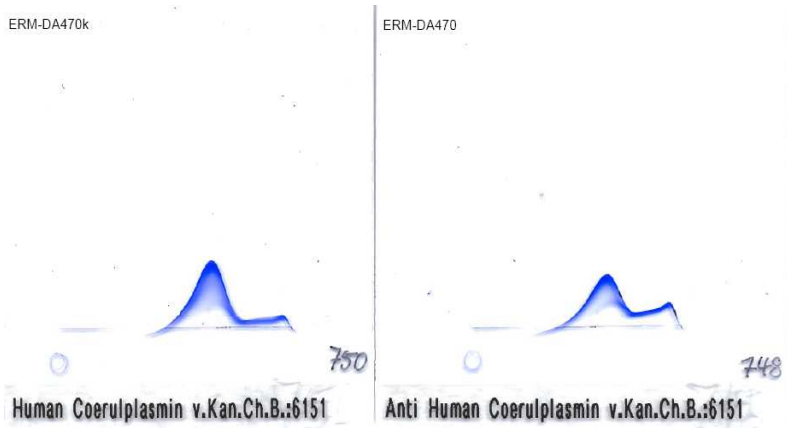
- Both methods were **using ERM-DA470** for calibration
- **Result** for ceruloplasmin in ERM-DA470 is reasonable with both method 1 and 2 (certified concentration 205 mg/L)
- **ERM-DA470 is not commutable** for this combination of methods

Discrepant results for clinical samples

Ceruloplasmin

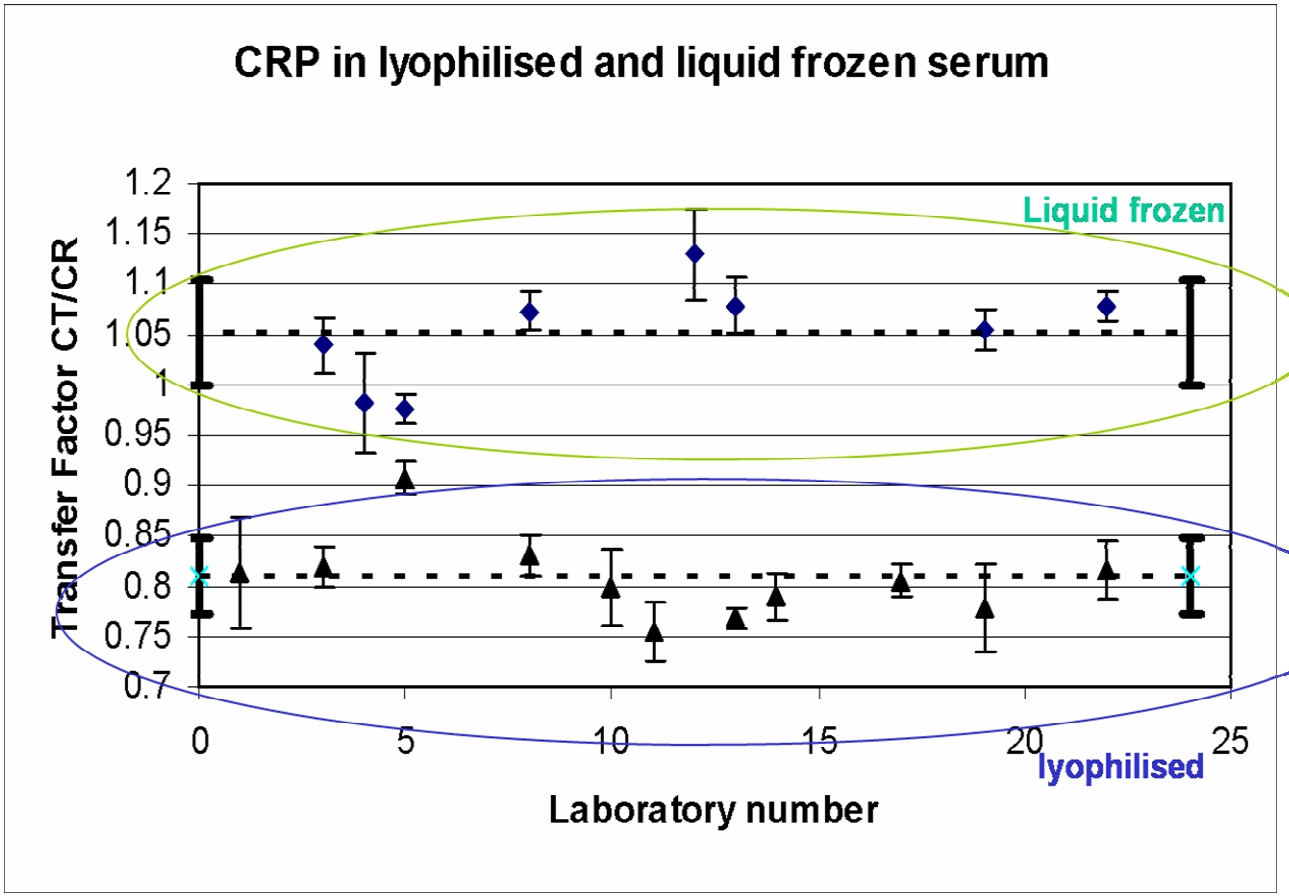


ERM-DA470k/IFCC ERM-DA470/IFCC



C-reactive protein

European Commission



The issue: **Lyophilisation results in a loss of about 20 % of measurable CRP compared to the non-lyophilised material and both formats appear commutable!**

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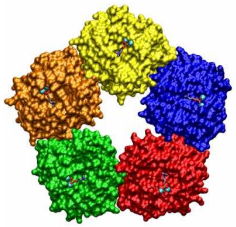
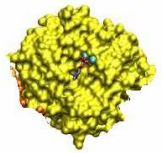
CRP oligomeric state



Pentamer in serum matrix and buffer



Monomer in buffer

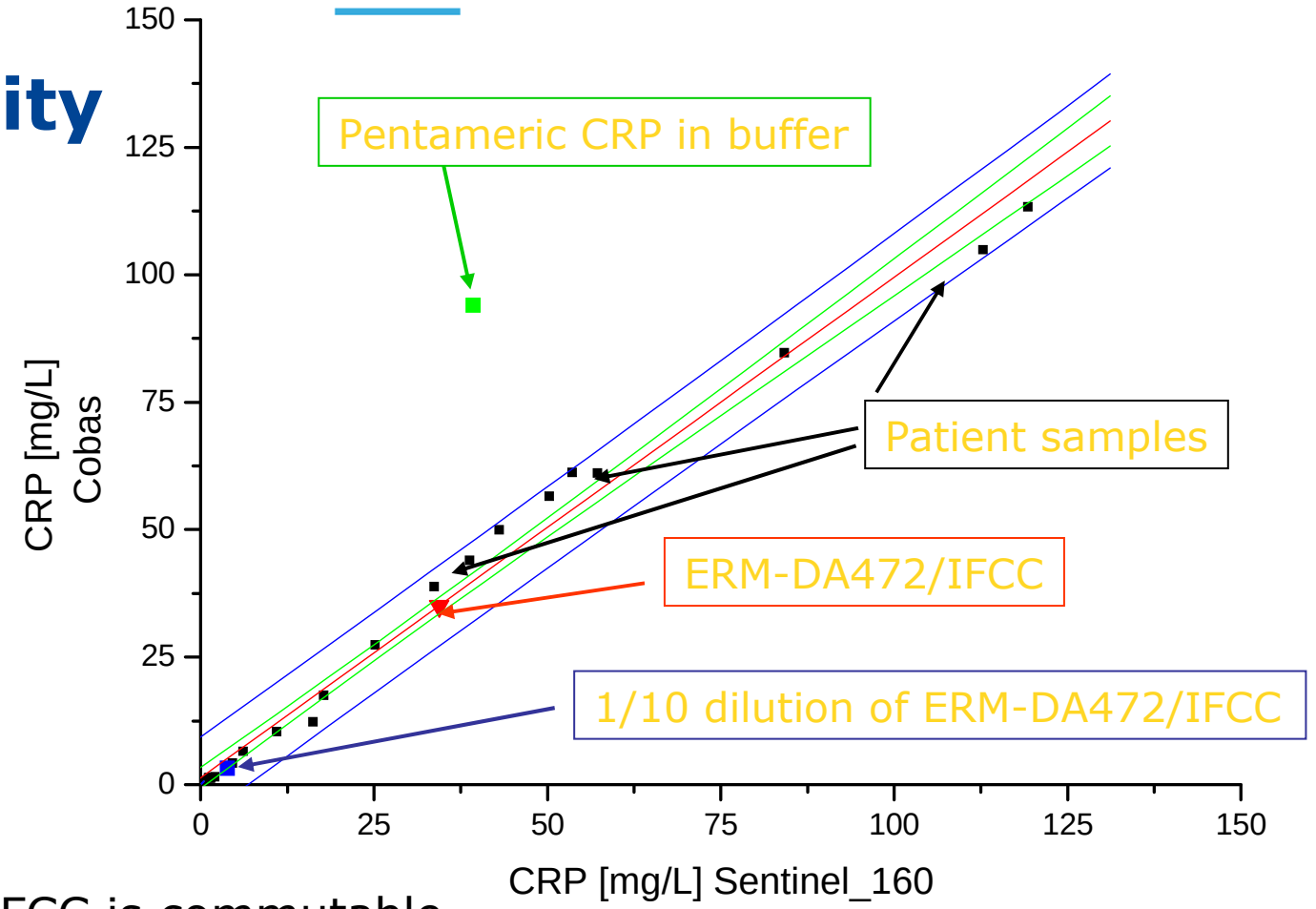
Material	nominal conc	CRP mass concentration measured with different methods (n=3), relative to the nominal concentration							
	%	IA1 %	IA2 %	IA3 %	IA4 %	IA5 %	IA6 %	IA7 %	IA8 %
ERM-DA472/IFCC	100	101	89	107	100	89	98		100
Pentamer In buffer	100	103	49	59	79	96	118		100
Monomer In buffer	100	12	1	<1	<13	<8	2	3	2

- mCRP < 10 % response in homogeneous immunoassays compared to pentameric CRP

CRP commutability

Method or instrument:

Beckman Immage
Dako
Sentinel
Abbott Architect
Cobas
Integra
BN ProSpec

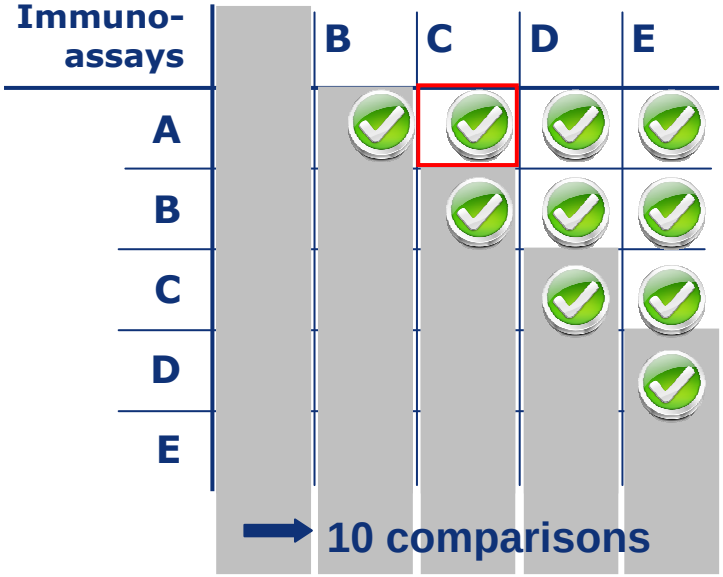


ERM-DA472/IFCC is commutable
A 1/10 dilution of ERM-DA472/IFCC is commutable
CRP without matrix is not commutable

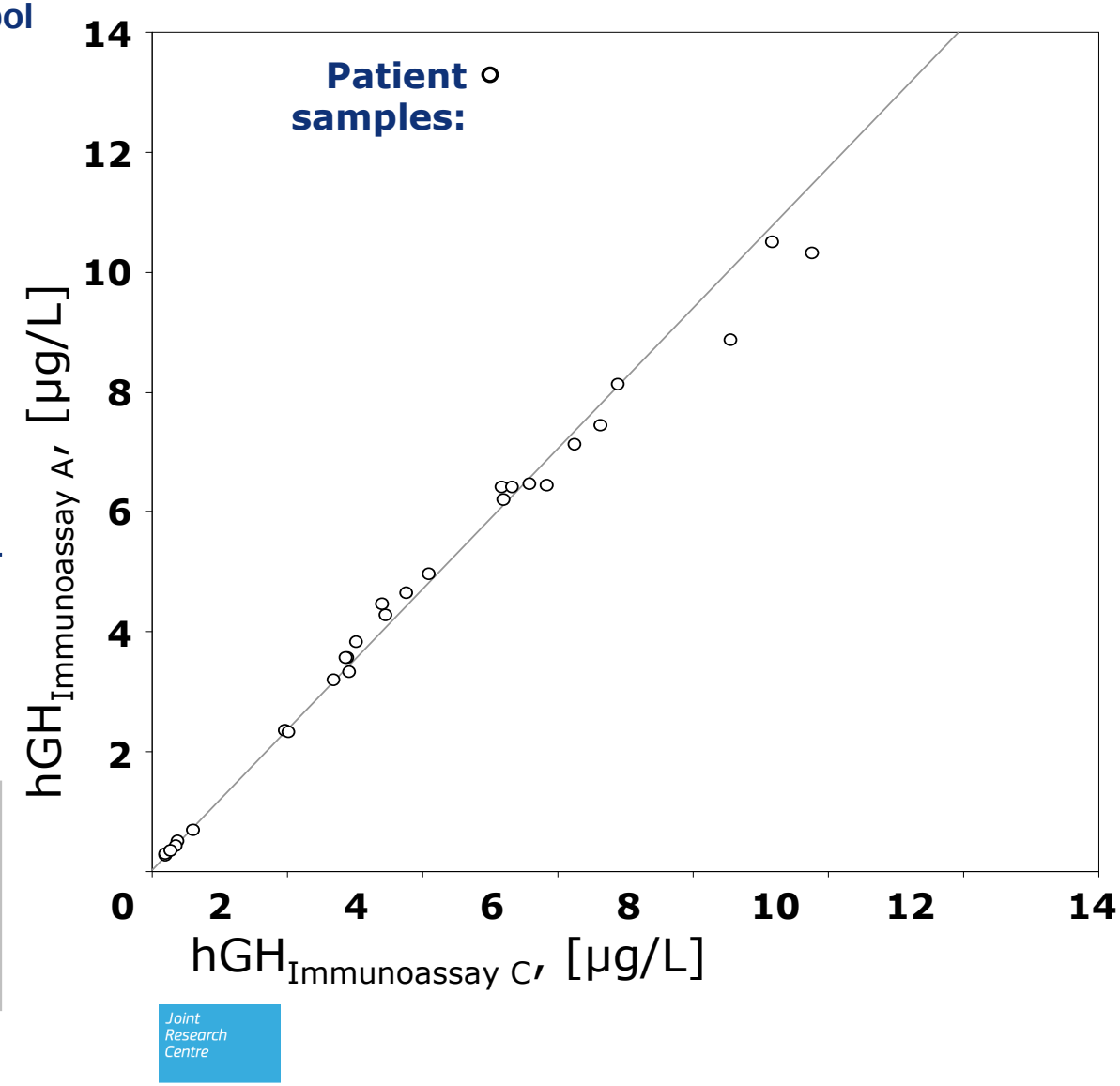
hGH

European Commission

Manufacturer	Calibration	Symbol
Siemens®	WHO 98/574	A
Roche®	WHO 98/574	B
Dia Sorin®	WHO 98/574	C
Mediagnost®	WHO 98/574	D
IDS®	WHO 98/574	E



Immunoassay C *versus* A



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Methods evaluated: Main routine immunoassays

➡ **10 comparisons**

Patient samples: ○

Reconstitution: ■ Phys. buffer

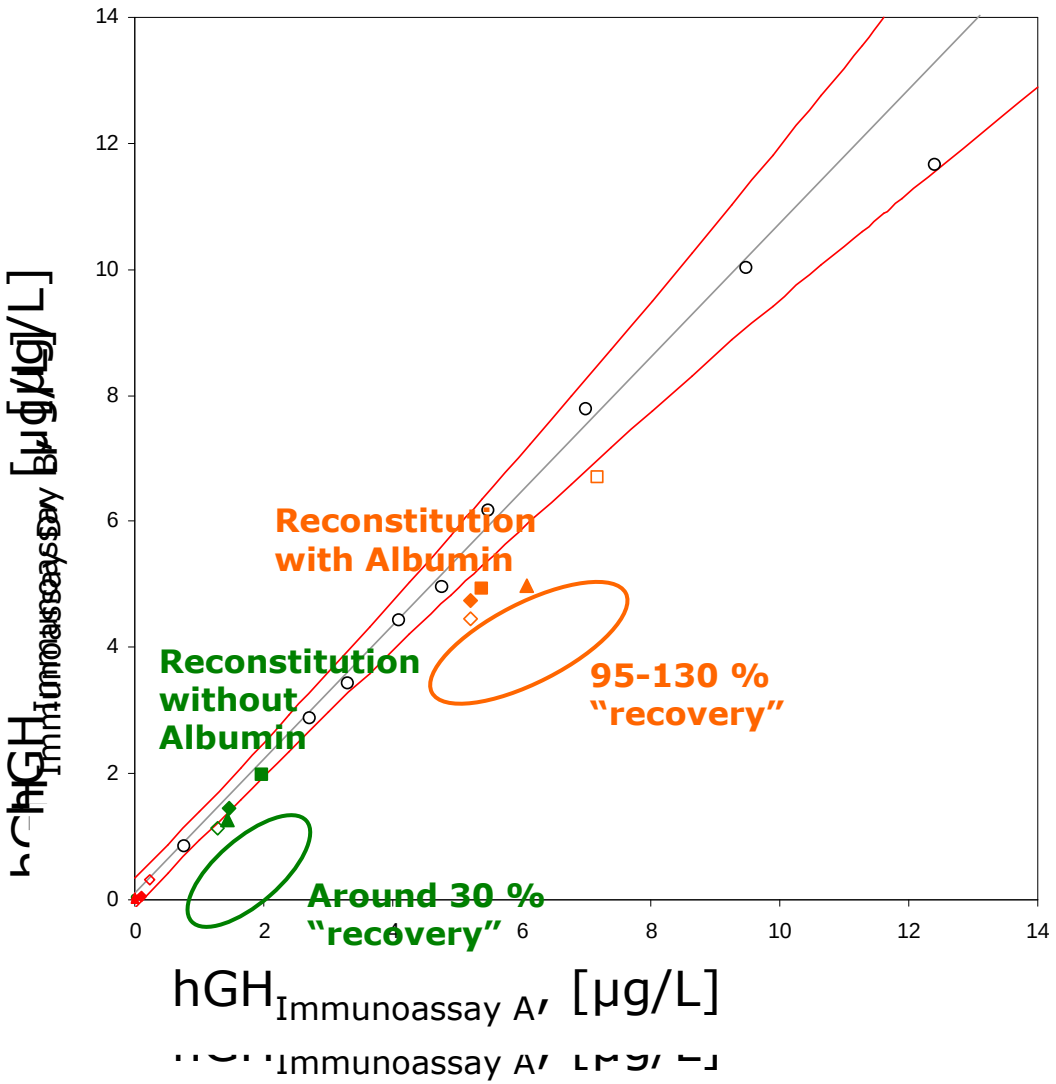
■ Phys. buffer - Albumin

Blank controls:

Spiking:

- ○ Phys. buffer
- □ Phys. buffer - Albumin
- ■ hGH depleted serum
- ◆ Charcoal stripped serum
- ◇ SRM 971 male
- ▲ Sheep serum

Immunoassay A *versus* B



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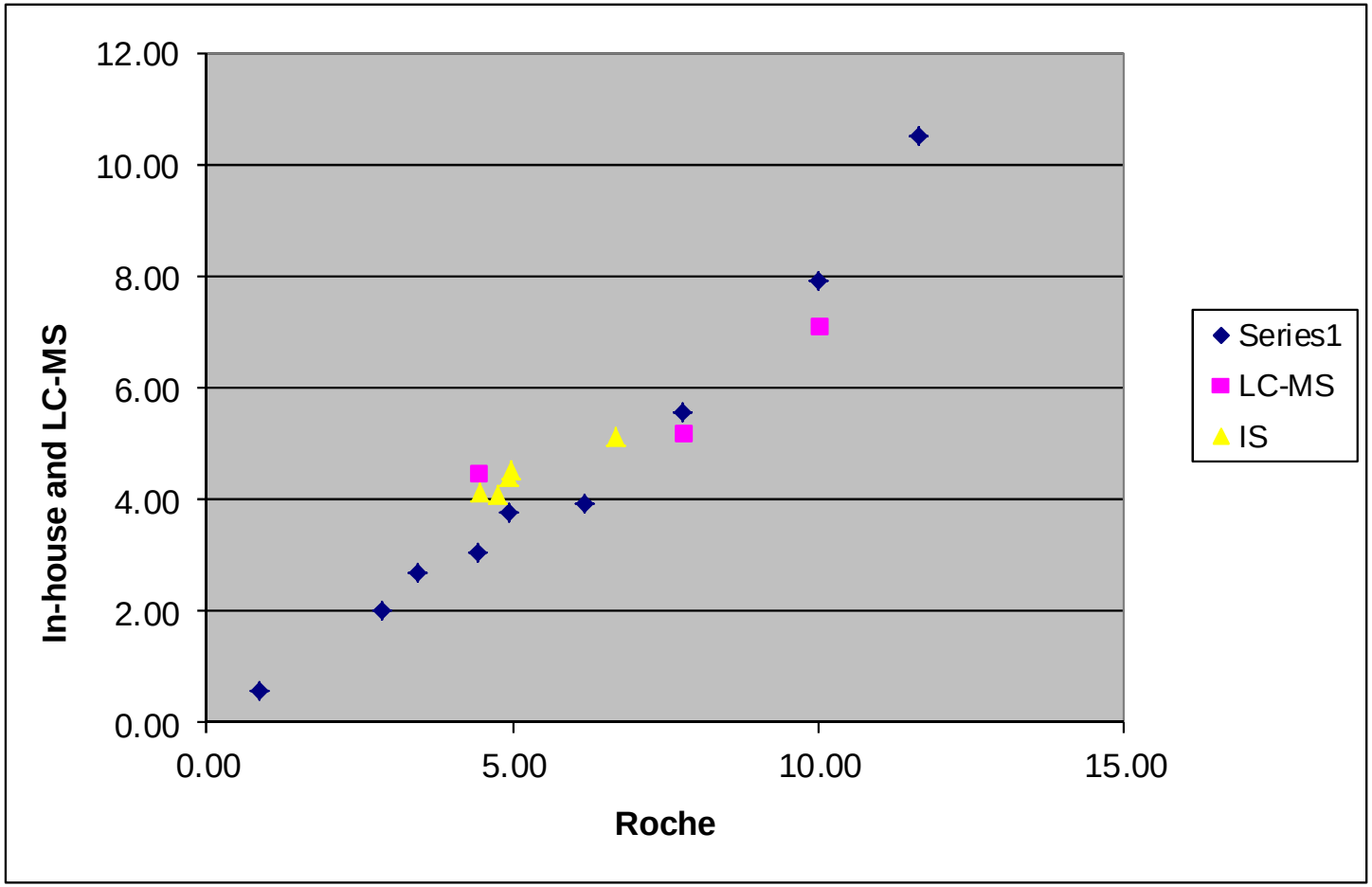

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Lessons learned

- **Commutability absolutely required**
- **Non-commutability will introduce a bias for at least one of the methods**
- **Commutability means that it can be used for achieving equivalent results but it does not guarantee the absence of bias**
- **If necessary (e.g. in case of a non-commutable primary reference material) uniform calibration protocols using commutable calibrators and commutable dilutions thereof need to be developed**

Leasons learned

- **Control of all relevant influence parameters need to be controlled and their impact quantified (with related uncertainties) to reproducibly produce reference materials to use them for calibration in a way that equivalent measurement results can be achieved on long term**
  - **Matrix properties and interaction**
  - **Structure, aggregation, oligomeric state**
  - **Isoform profile**
  - **Metal binding**

- **Careful evaluation of the impact of processing steps on the properties of reference materials is crucial**

Leasons learned

- **Methods determining the amount of substance of a protein only (such as mass spectrometry on peptides or the protein backbone) may not be sufficient to control the traceability chain well enough (see CER, CRP, hGH)**

- **A reference measurement procedure based on well understood immunoassay(s) could be a valid option for value assignment to secondary reference materials since it is likely to show equal selectivity and/or correlating measurands with routine procedures**

Conclusions

- **A higher order reference material or reference measurement procedure cannot stand alone, they are integral parts of reference measurement systems**

- **Application of reference materials outside their validated intended use requires verification of their usability for the extended purpose**

- **A reference material can only serve as primary or secondary calibrator for given and validated applications / traceability chains**

Conclusions

- **Standardisation of complex clinical markers is challenging, however, often possible with reasonable efforts and within acceptable timelines**

- **Correlation is key!**