

## Performance criteria for reference measurement procedures and reference materials



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## How to define performance targets for CRMs and RMPs?

- Consider their intended use and role in calibration hierarchies.
- Understand sources of bias and error, not only at the highest levels of the calibration hierarchy but down to the routine user level.
- Design reference measurement procedures and characterise certified reference materials appropriately to serve the overall goal of measurement result equivalence within analytical performance goals.

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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)**

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## **Role of CRMs and RMPs**

- **Traceability of measurement results is achieved by an unbroken (unbiased) sequence of calibrations, each introducing additional uncertainty contributions on top of the values assigned to CRMs/calibrators at the previous higher calibration hierarchy level (i.e. always increases).**
- **Traceability of measurement results can be achieved via measurement standards (of which values are traceable to the SI or are measurement procedure defined) or to conventional measurement standards (e.g. WHO International Standards), the latter establishing an arbitrary scale lacking reproducibility.**

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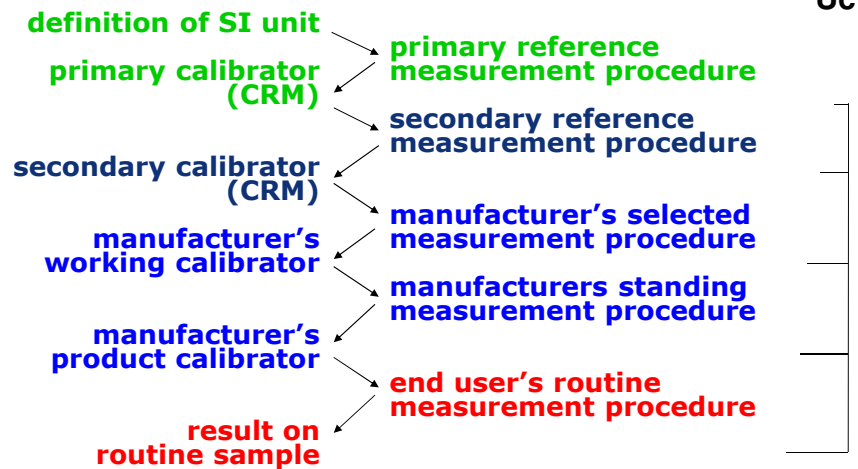


## Technical requirements for establishing a traceability chain (ISO 17511:2003)

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



## ISO 17511





## Typical traceability chains in laboratory medicine

- The measurand (particular quantity subject to measurement, VIM2) is not maintained throughout the traceability chain and usually defined by the methods applied.
- The relationship / correlation between measurands in a given traceability chain must be known.
- Values for clinical samples and reference materials / calibrators obtained by different methods in a traceability chain should correlate closely (CRMs / calibrators must be commutable).

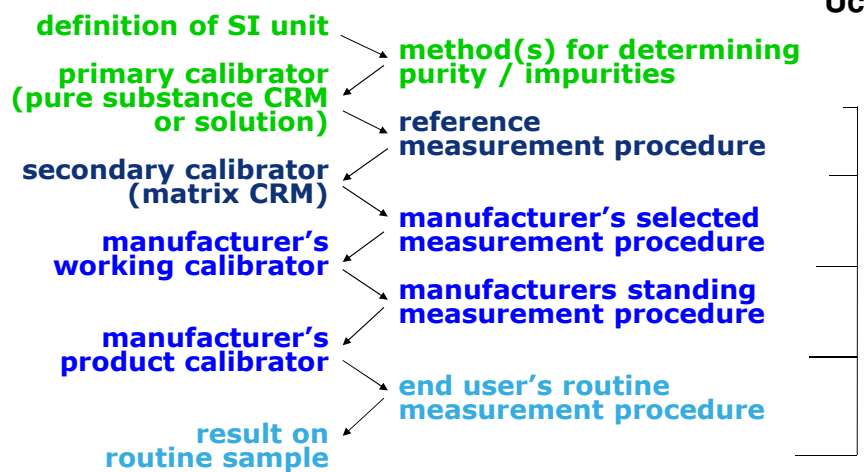
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## Common situation



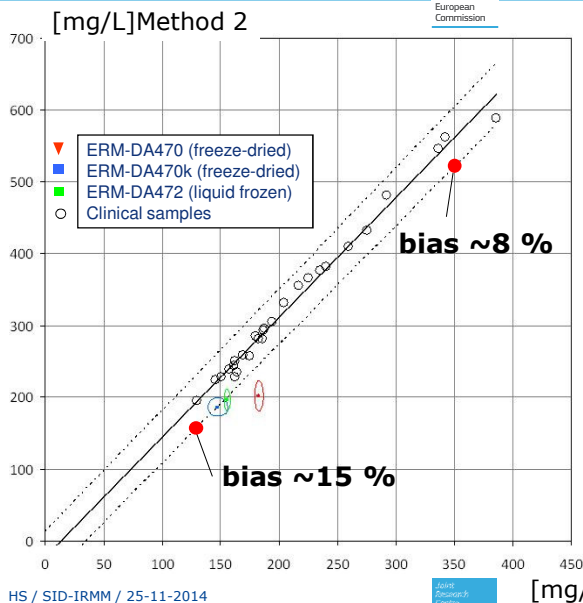
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## Consequences of non-commutability

- **Commutability of reference materials is required to avoid systematic bias between methods calibrated with them. Relative residuals in commutability plots lead to a corresponding systematic bias between the two methods for clinical samples if no correction takes place (the latter related to uncertainty).**
- **Correlation and commutability are key for successful standardisation and fulfilment of stringent analytical performance goals!**

## Ceruloplasmin



The acceptance limits for commutability should be defined by analytical performance requirements rather than data scatter around the correlation line.

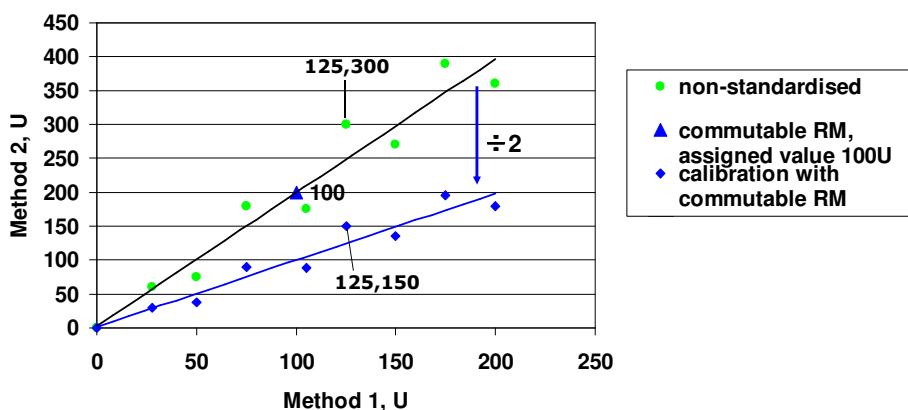


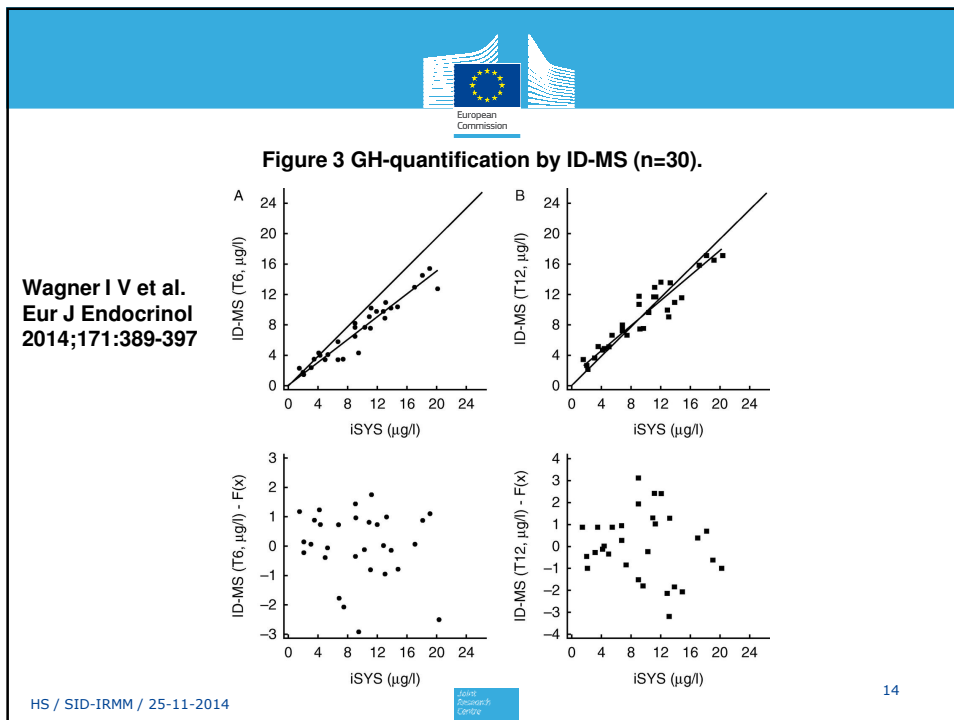
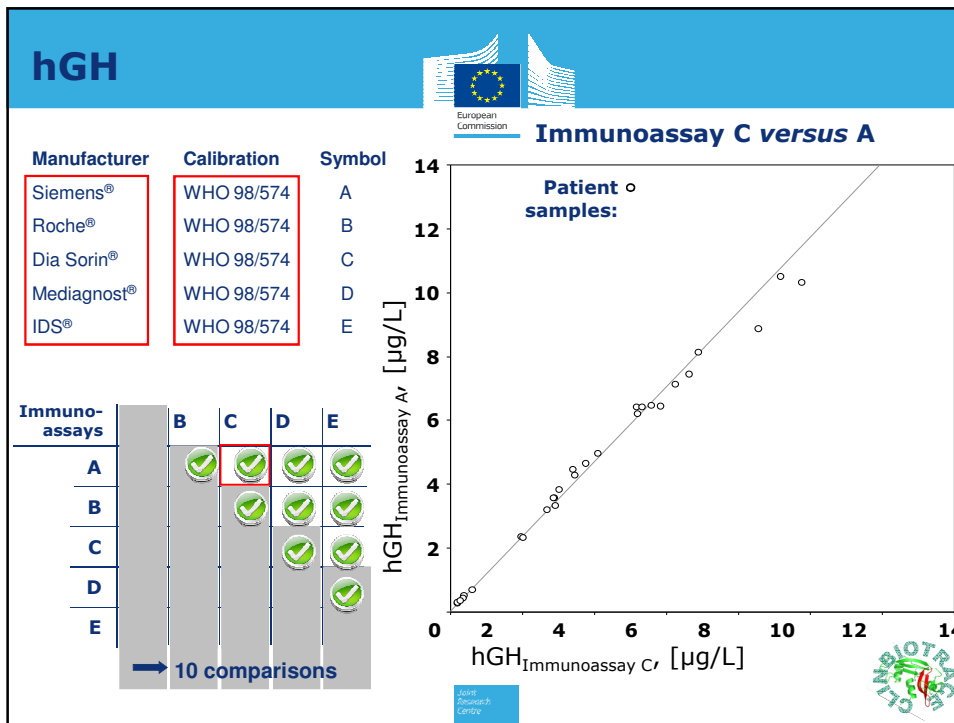
IFCC working group on commutability developing updated guidance.

## Need for commutable reference materials

- Commutability of a reference material means that it can be used for achieving equivalent results but it does not guarantee the absence of bias.
- To guarantee absence of bias identification and control of all influence parameters / quantities is required (eventually multiparametric traceability)
- If necessary uniform calibration protocols using commutable reference materials and commutable dilutions thereof need to be developed (instructions for use and accompanying documentation).

## Consequences of non-correlating methods





## Challenges due to lacking correlation

- **Due to differences in selectivity of measurement procedures non-correlating methods produce biased results on individual clinical samples. The bias cannot be removed by calibration with even perfectly characterised and commutable reference materials.**
- **If bias is too large to meet analytical performance goals preference should be given to the method with higher clinical performance and at least one method would have to be reformulated to remove the bias.**

## Challenges due to lacking correlation

- **A reference measurement procedure for heterogeneous analytes not correlating with routine measurement procedures will be of limited utility (unless results would be of higher clinical significance compared to the routine method(s) and/or lacking correlation would be caused by insufficient method selectivity)**
- **It could only be applied to the characterisation of reference materials and calibrators with great caution.**
- **Introduces arbitrariness and bears the risk that bias is not sufficiently under control, hence may result in reduced analytical performance.**



## Requirements for CRMs and RMPs

- **Measurand (VIM2) / analyte of the reference measurement procedure and characterized in certified reference material must be known, identical or sufficiently closely correlating in calibrators, clinical samples and routine procedures to be calibrated.**

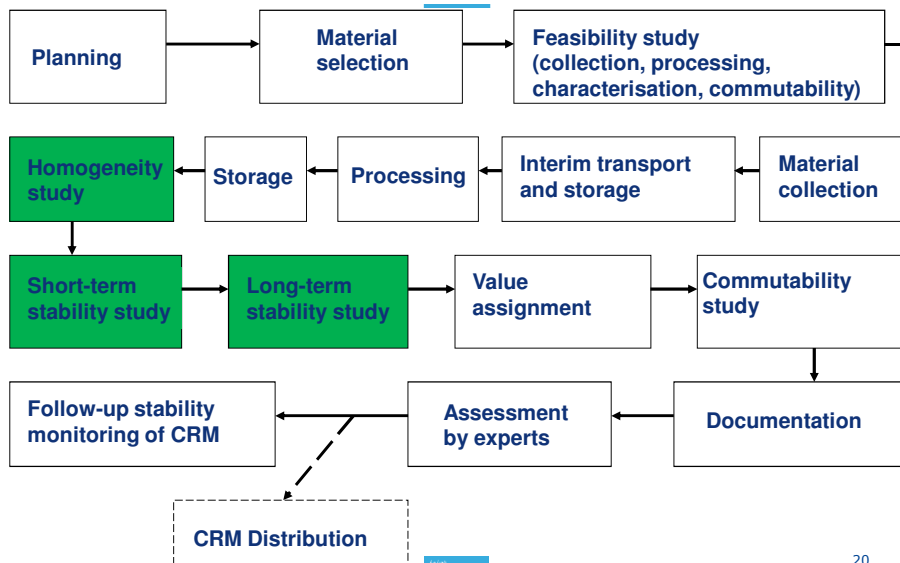
## Requirements RMPs (ISO 15193)

- **Measurement principle and method (reasons for certain steps)**
- **Measurand / analyte definition**
- **Reagents and materials (incl. calibrants)**
- **Pieces and apparatus (incl. calibrated devices)**
- **Influence quantities**
- **Preparation of measurement system and analytical portion**
- **Calibration**
- **Analytical reliability (sensitivity, linearity, recovery,  $u$ ,  $R$ ,  $r$ , LOD)**
- **Validation (incl. inter-laboratory comparisons)**
- **The uncertainty of the measurement results must be sufficiently low for accurately assigning values to reference materials / calibrators.**



## Requirements for CRMs (ISO Guide 34, ISO 15194)

- The reference material must be thoroughly characterized in terms of
  - Homogeneity (u for possibly hidden heterogeneity)
  - Stability (u for possibly hidden instability)
  - Certified property value using adequate and highest quality (reference) measurement procedures.
  - Commutability
- Traceability statement for certified value
- Due to error propagation the uncertainty of the certified value should be significantly lower than the analytical performance goals for routine procedures.

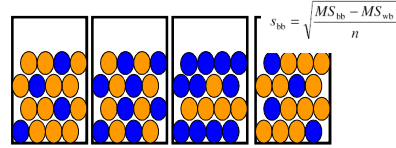


## CRM characterisation (ISO Guide 34, ISO 15194)



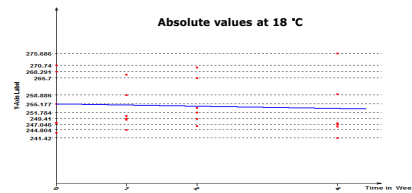
### • Homogeneity

- ✓ 20 samples
- ✓ Triplicates
- ✓ 3 platforms

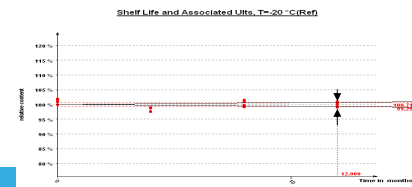


### • Short Term Stability (dispatch conditions)

- ✓ 4 time points (0, 1, 2, 4 weeks)
- ✓ 3 temperatures (-20, 4, 18 °C)



### • Long Term Stability (storage)



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## Uncertainty certified value (ISO Guide 34 and 35 ISO 15194)



### Expanded, combined uncertainty

$u_{c, rel}$

$$U_{CRM} = k \sqrt{u_{char,rel}^2 + u_{dm,rel}^2 + u_{purity}^2 + u_{bb}^2 + u_{ls}^2}$$

With  $k=2$ , corresponding to a level of confidence of approximately 95 %

Certified value [mg/L]	$U_{CRM} (k=2)$ [mg/L]
5.48	0.15



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## ERM-DA471/IFCC Cystatin C



$u_{char, rel}$ [%]	$u_{dm, cal, rel}$ [%]	$u_{purity, cal, rel}$ [%]	$u_{bb, rel}$ [%]	$u_{lts, rel}$ [%]	$u_{c, rel}$ [%]
0.74	0.64	0.3	0.29	0.73	1.29

Uncertainty from characterisation:

$$u_{char} = \frac{s_{mean}}{\sqrt{l}}$$

Uncertainty from dry mass determination of the purified protein

Uncertainty from purity assessment of purified protein

Uncertainty from possible between bottle heterogeneity

Uncertainty from possible long-term instability

Combined standard uncertainty

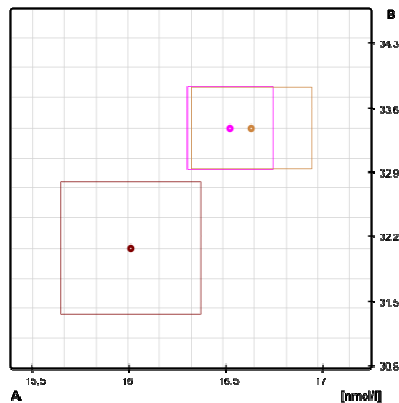


## Reduction of uncertainties

- Underestimation and omission of relevant uncertainty components or neglecting influence parameters / quantities in the calibration hierarchy leads to a lack of bias control and non-equivalence of measurement results within their stated uncertainties and potentially outside analytical performance goals.
- Hence uncertainty estimates should be realistic for reference materials and reference measurement procedures.

## RELA 2013

### Progesterone



## To meet analytical performance goals

- Reducing uncertainties of reference measurement procedures and of certified values of reference materials might be challenging and at present technically not possible or resource demanding.
- Further technical development on reference materials and measurement procedures might be required as well as adaptations of routine methods.
- Reduction of the number of calibration steps and improved value transfer processes e.g. by using reference methods further down in the calibration hierarchy may contribute to achieving analytical performance goals.

## To meet analytical performance goals

- **Underestimation or omission of uncertainty may seemingly produce nice numbers but makes results unreliable and increases chances to introduce unrecognised bias.**
- **Hence not a solution to the meet ambitious performance goals.**
- **Requirements laid down in ISO Guides 34 and 35 and ISO 15194 for reference materials and in ISO 15193 for reference measurement procedures need to be fulfilled for establishing effective traceability.**

## Important to keep in mind

- **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.**