



# Joint Research Centre

Institute for Reference Materials  
and Measurements  
(IRMM)

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<http://irmm.jrc.ec.europa.eu>

# Institute for Reference Materials and Measurements (JRC-IRMM) in Geel, Belgium



- around 250 Staff
- 4 Scientific Units

# Traceability and comparability via reference materials



e.g. ERM-DA470k/IFCC

Certified for the mass fraction of ALB, AAG, AAT, A2M, C3c, C4, HPT, IgA, IgG, IgM, TRF, TTR

Replacing ERM-DA470  
(BCR-470)

Aim of traceability is to ensure comparability of results

## Definition of the SI unit

“Pure” protein

e.g. AAA,  
refractive index

Reference procedure,  
immunoassay ring trial

CRM  
matrix material

Value transfer 1:  
LC-MS, immunoassay

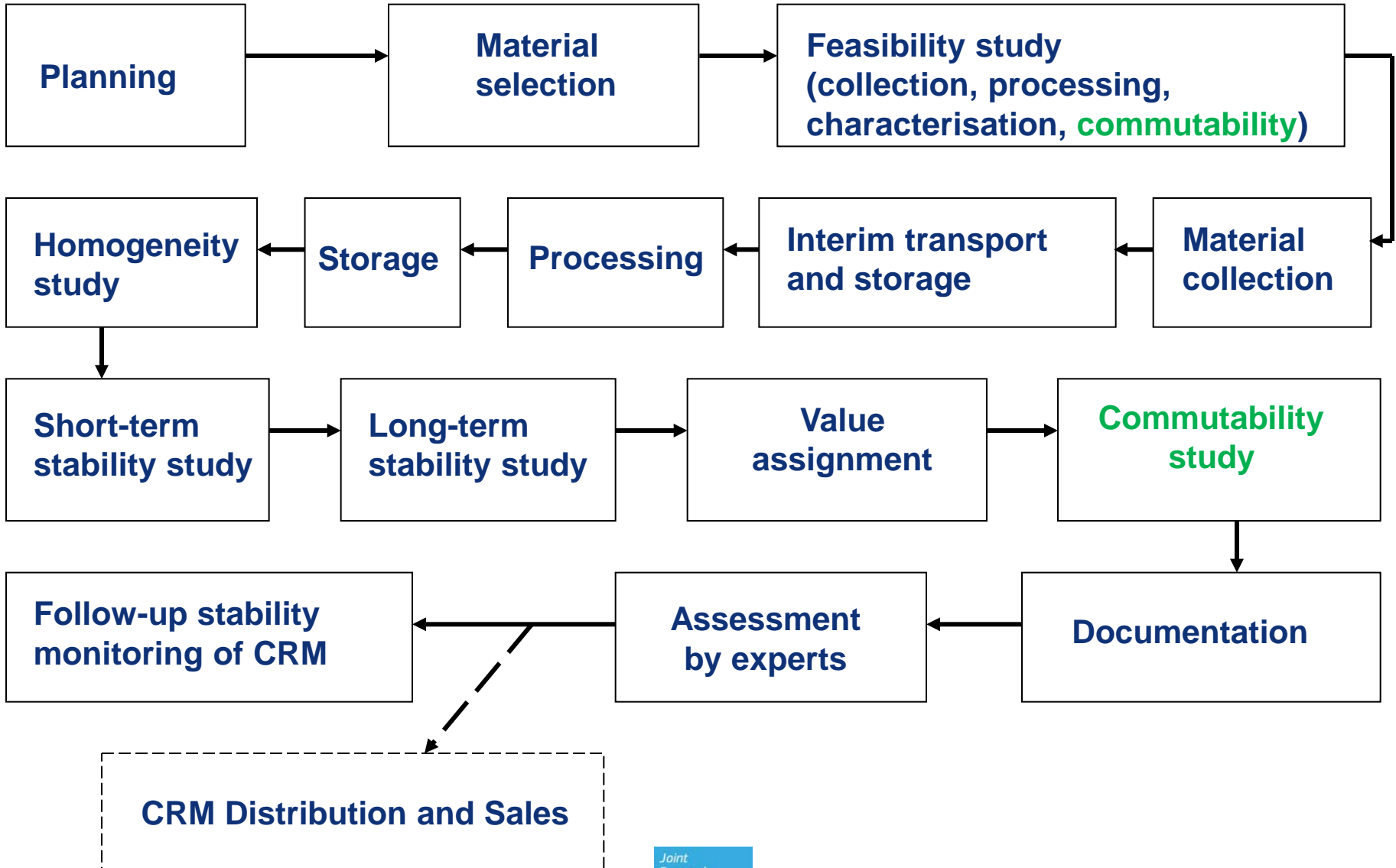
Manufacturer's  
working calibrator

Value transfer 2:  
immunoassay

Manufacturer's  
product calibrator

Immunoassay

Routine sample



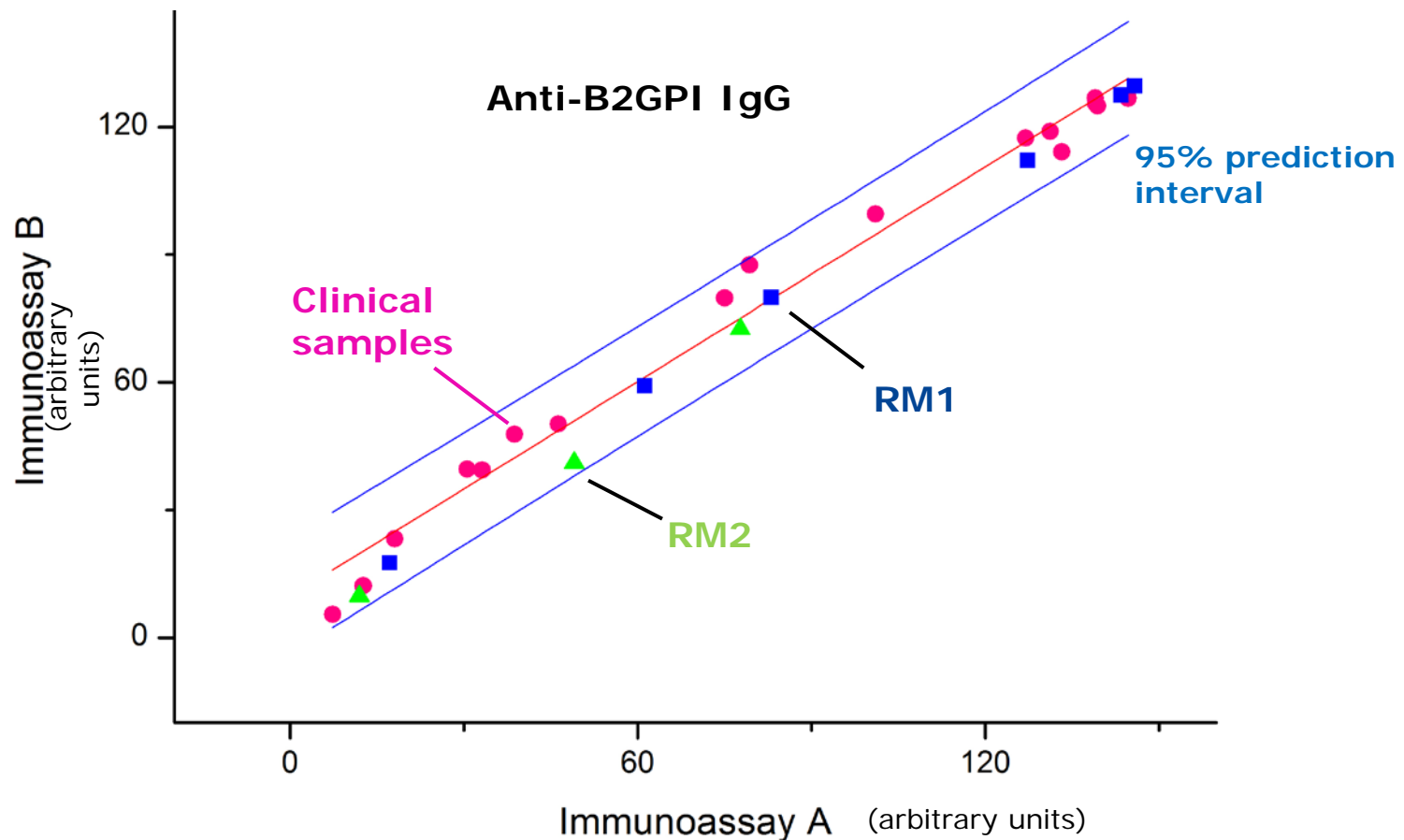
| Analyte                                    | Issues                                | Commutability* |
|--|---------------------------------------|----------------|
| $\beta$ 2-microglobulin (B2M) <sup>1</sup> | recombinant                           | yes            |
| C4   |                                       | yes            |
| ceruloplasmin                              | sample ageing                         | $\pm$          |
| myoglobin                                  | recombinant, <sup>15</sup> N labelled | $\pm$          |
| HbA2 <sup>1</sup>                          | processing                            | yes            |
| HbA1c <sup>1</sup>                         | processing                            | yes            |
| albumin <sup>2</sup>                       | urine/serum analysis ongoing          |                |
| C-reactive protein                         | oligomerisation, matrix               | yes            |
| cystatin C <sup>1</sup>                    | non-linear method correlation         | $\pm$          |
| A $\beta$ 42 <sup>1</sup>                  | spiking, matrix                       | $\pm$          |
| IgG anti-MPO, anti-PR3 <sup>1</sup>        | method correlation                    | yes            |
| IgG anti B2GP                              | monoclonal                            | yes            |
| enzymes (LD, CK, ALT) <sup>1</sup>         | recombinant, isoform, matrix          | $\pm$          |
| human growth hormone                       | recombinant, matrix                   | no             |

<sup>1</sup> in collaboration with IFCC, <sup>2</sup> in collaboration with NKDEP, Infusino et al. CCLM 2011

\* with respect to the selection of methods evaluated

## Commutability studies as part of feasibility studies

- test of method correlation
- selection of reference material format



## Evaluation of routine methods – Comparison for IgG anti MPO

|           | Method 1 | Method 2 | Method 3  | Method 4  | Method 5  | Method 6  | Method 7  | Method 8  | Method 9  | Method 10 | Method 11 |
|-----------|----------|----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| Method 1  |          | Low      | High      | Very high | Very high | Very high | Very high | Very high | Low       | Medium    | High      |
| Method 2  |          |          | Very high | Very high | Very high | Very high | Very high | Very high | High      | High      | High      |
| Method 3  |          |          |           | Very high | High      | Very high | High      | High      | Medium    | High      | High      |
| Method 4  |          |          |           |           | High      | Very high | High      | Medium    | High      | High      | High      |
| Method 5  |          |          |           |           |           | High      | Very high | Very high | High      | Very high | Very high |
| Method 6  |          |          |           |           |           |           | Very high | High      | Very high | High      | High      |
| Method 7  |          |          |           |           |           |           |           | Very high | High      | High      | Very high |
| Method 8  |          |          |           |           |           |           |           |           | Medium    | High      | High      |
| Method 9  |          |          |           |           |           |           |           |           |           | High      | High      |
| Method 10 |          |          |           |           |           |           |           |           |           |           | Very high |
| Method 11 |          |          |           |           |           |           |           |           |           |           |           |

Methods claiming to measure the same analyte may in fact have different selectivities

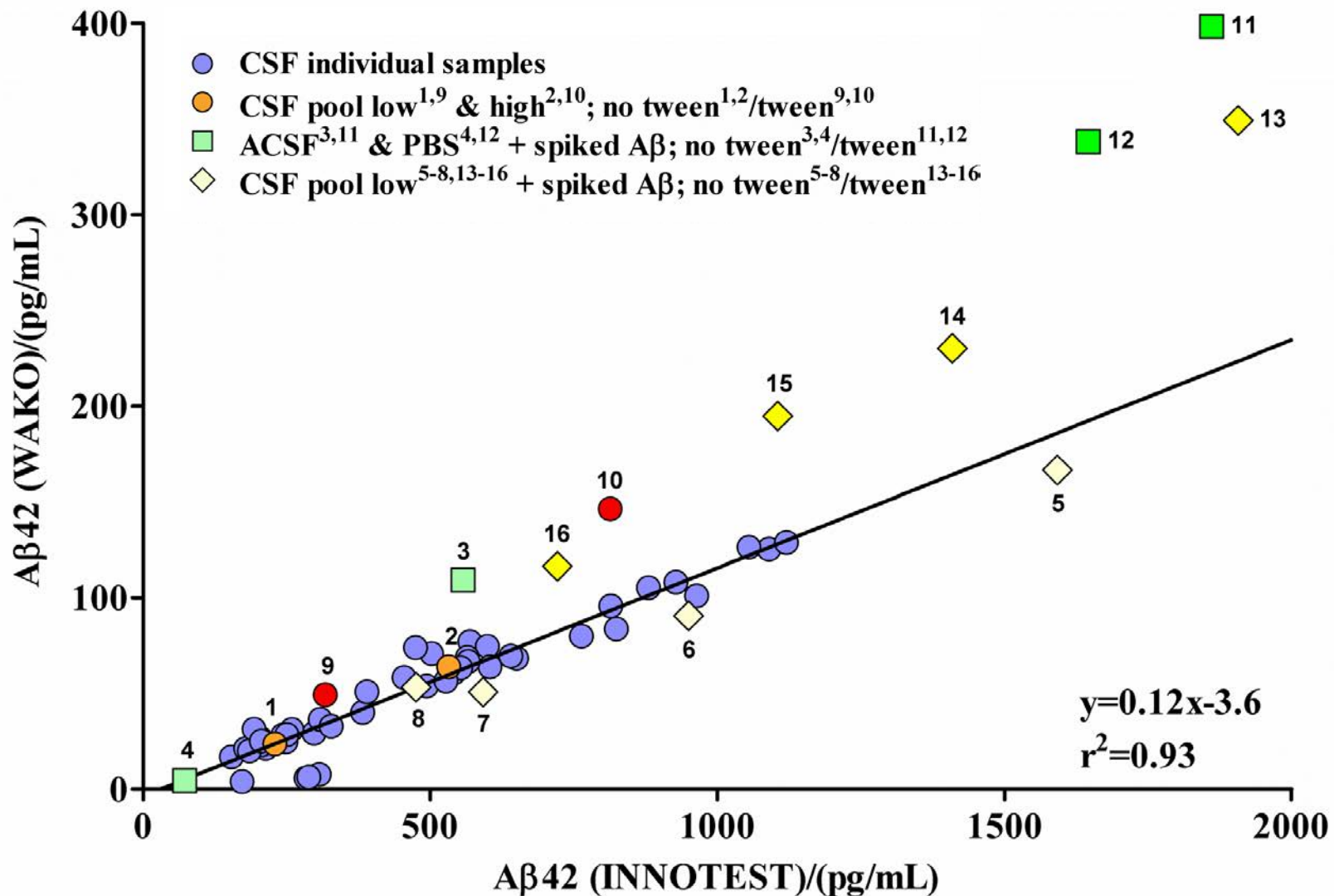
→ clinical significance of different analytes?

→ selection of analyte to standardise

| Degree of correlation |           |
|-----------------------|-----------|
| Low                   | Medium    |
| High                  | Very high |



# RM format evaluation: e.g. Alzheimer marker A $\beta$ 42, results obtained without use of common calibrator



## RMs fit for purpose as calibrators ?

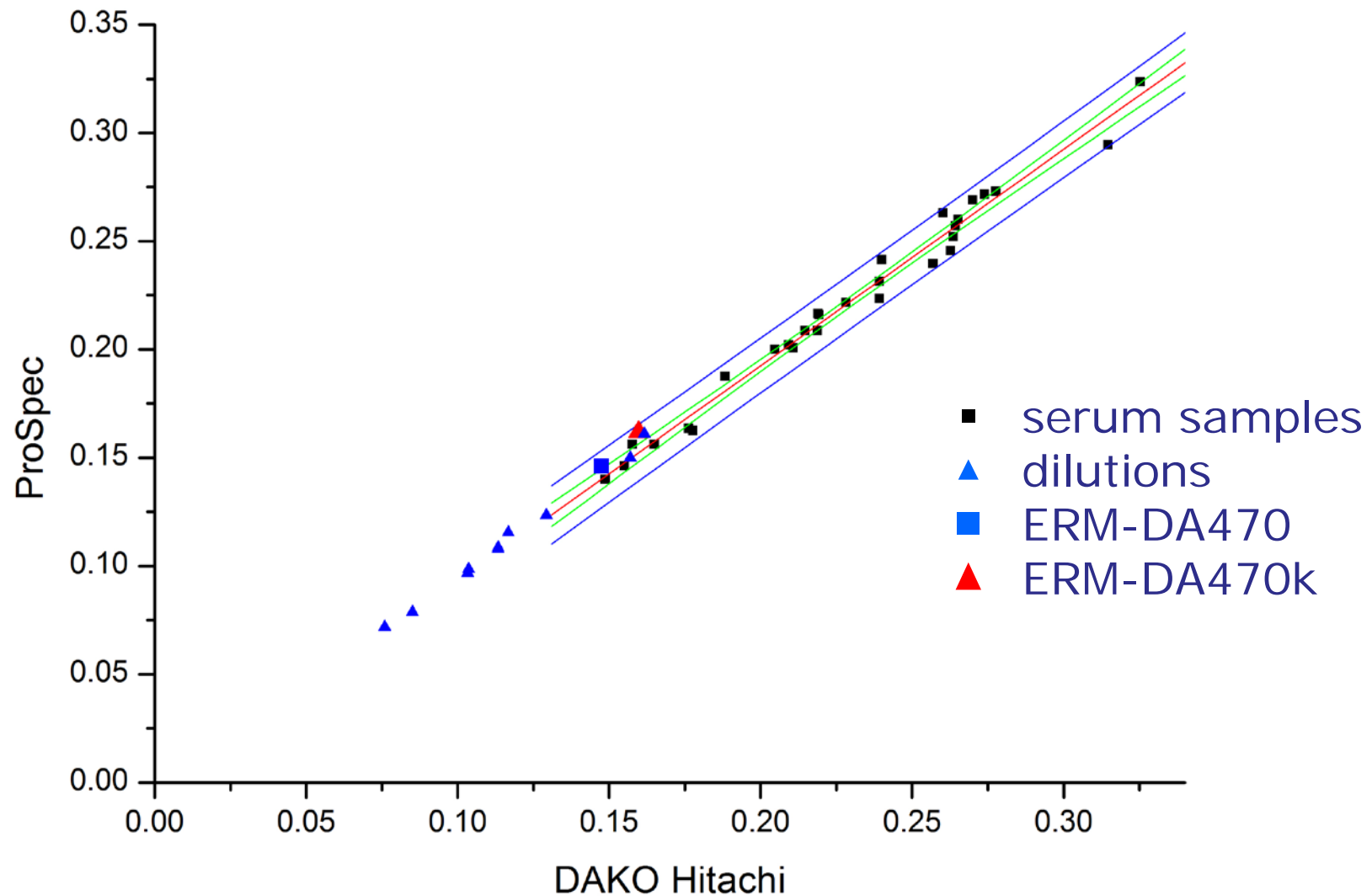
### **Use of CRM 470/RPPHS Has Not Achieved True Consensus for Ceruloplasmin Measurement**

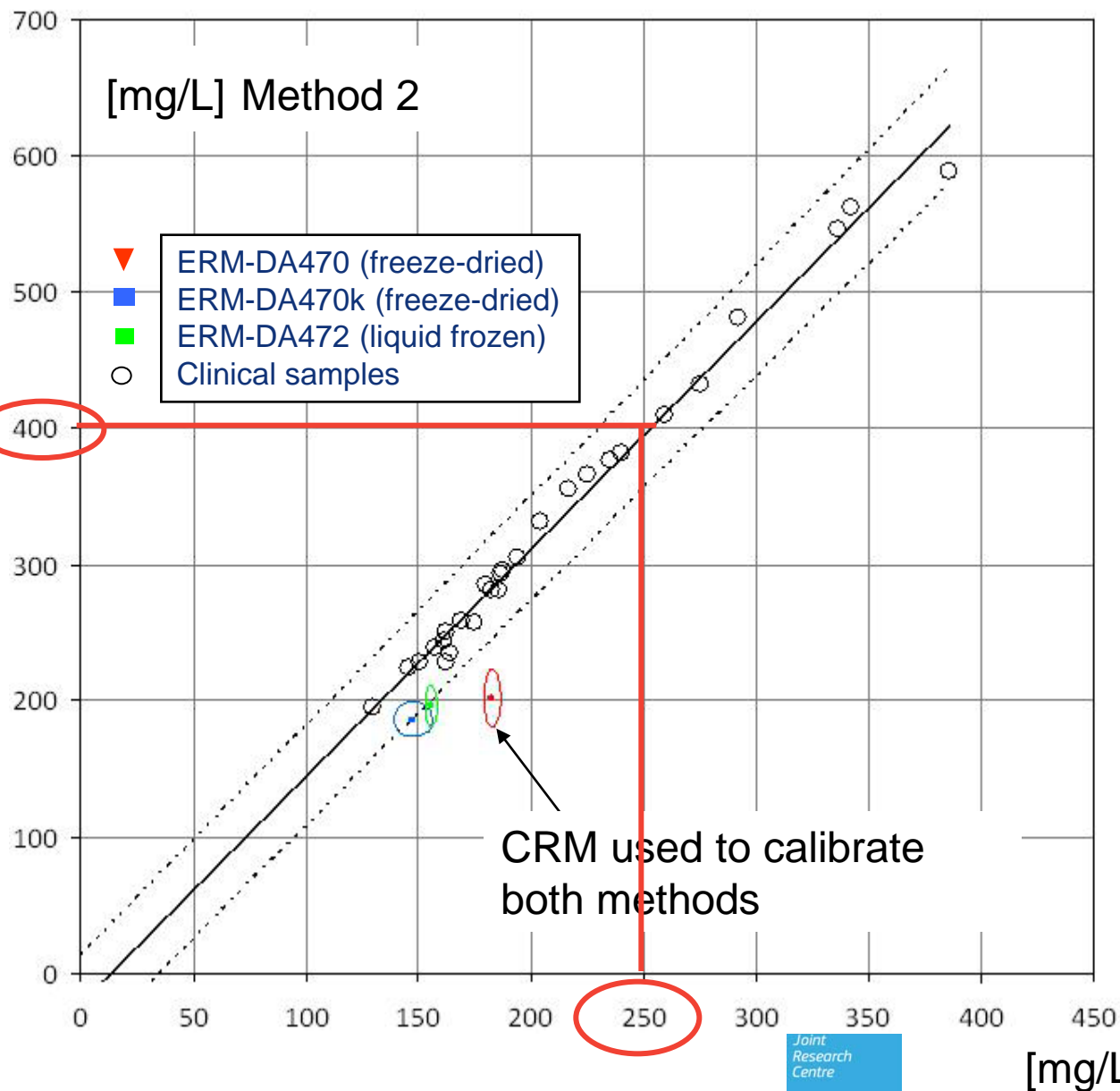
*To the Editor:*

The use of primary protein reference material CRM 470/RPPHS (1) was intended to lead to reduced method-dependent variation in specific protein analyses. Observations from UK NEQAS for Specific Proteins indicate that this is true for most proteins, but not for ceruloplasmin. Because the

R. Beetham, P. White, P. Riches, D. Bullock, F. MacKenzie  
*Clinical Chemistry* 48, 2002

## Commutability study to validate use of CRM for calibration

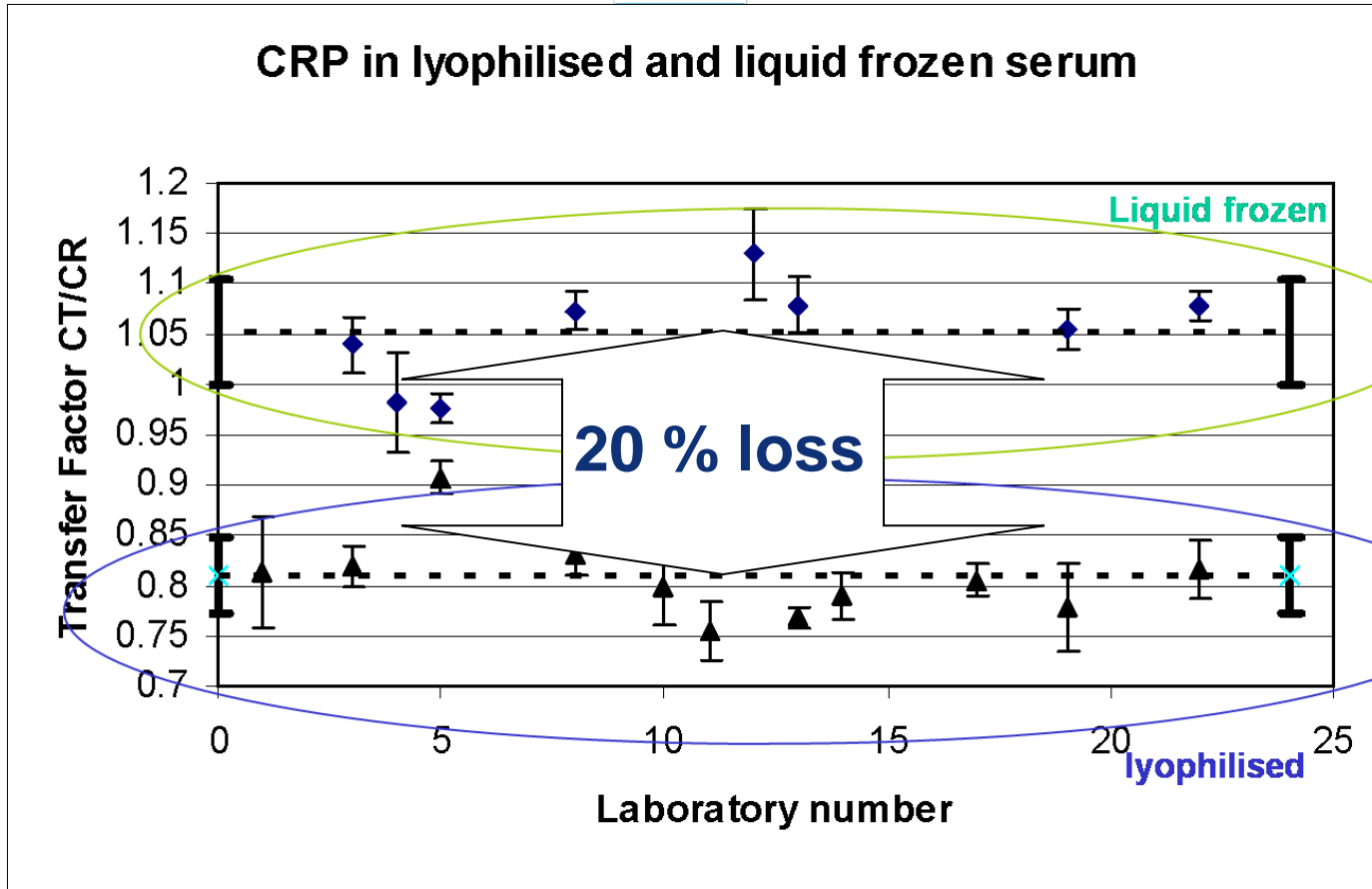




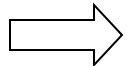
- Results from both methods were traceable to **ERM-DA470**
- **No bias** when ERM-DA470 is measured (certified concentration 205 mg/L)
- **ERM-DA470 is not commutable** for this combination of methods



Discrepant results for clinical samples

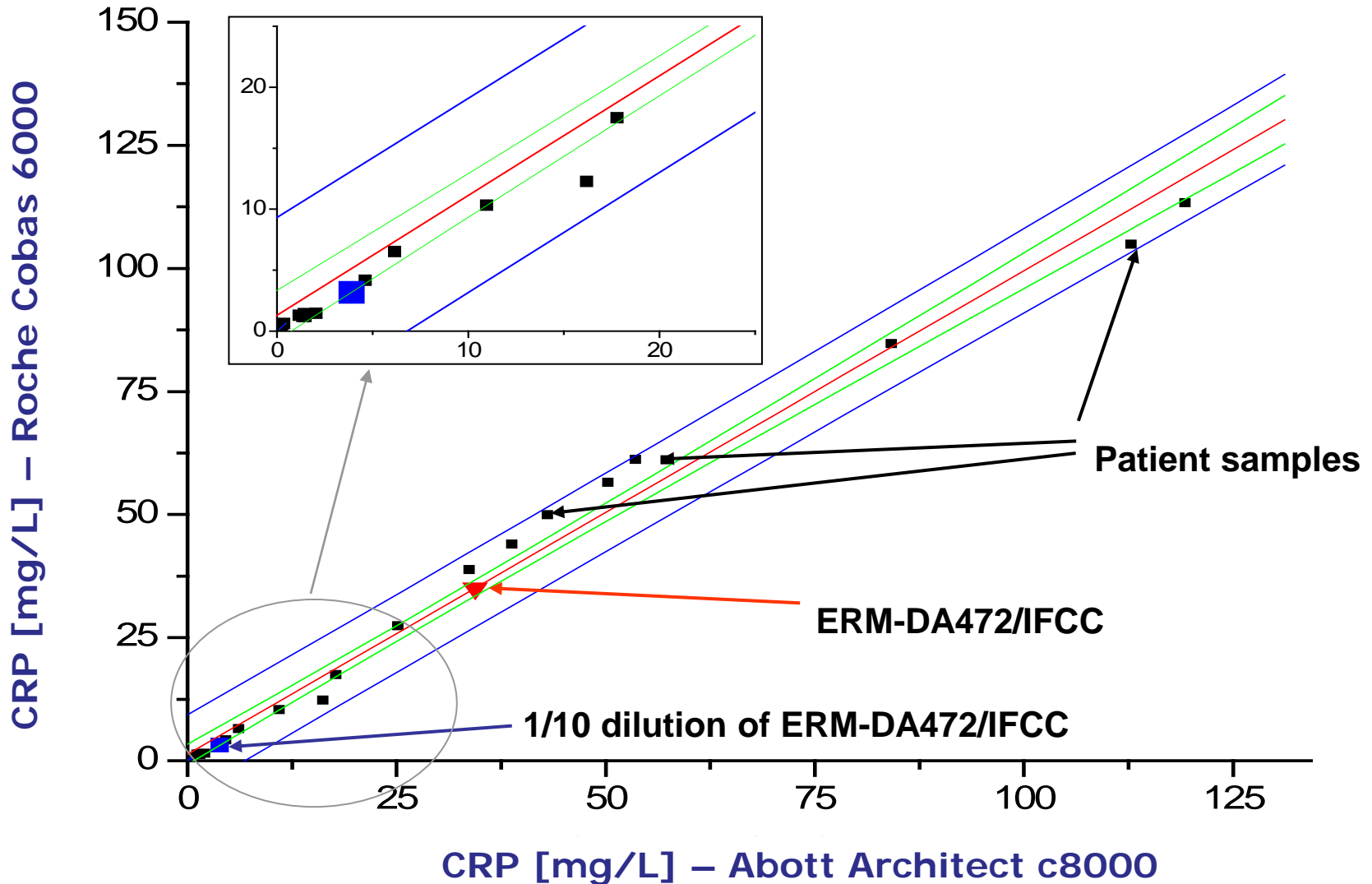


- Lyophilisation results in a loss of measured CRP of about 20 %
- Similar bias present in all immunoassays

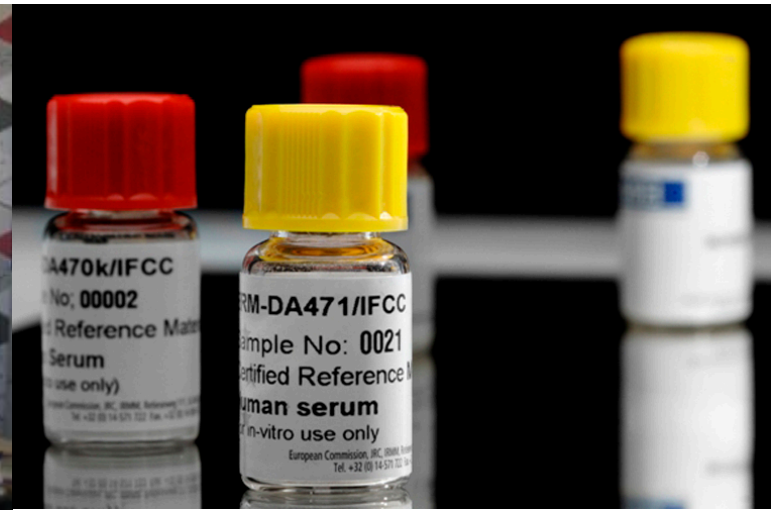


The lyophilised material is still commutable

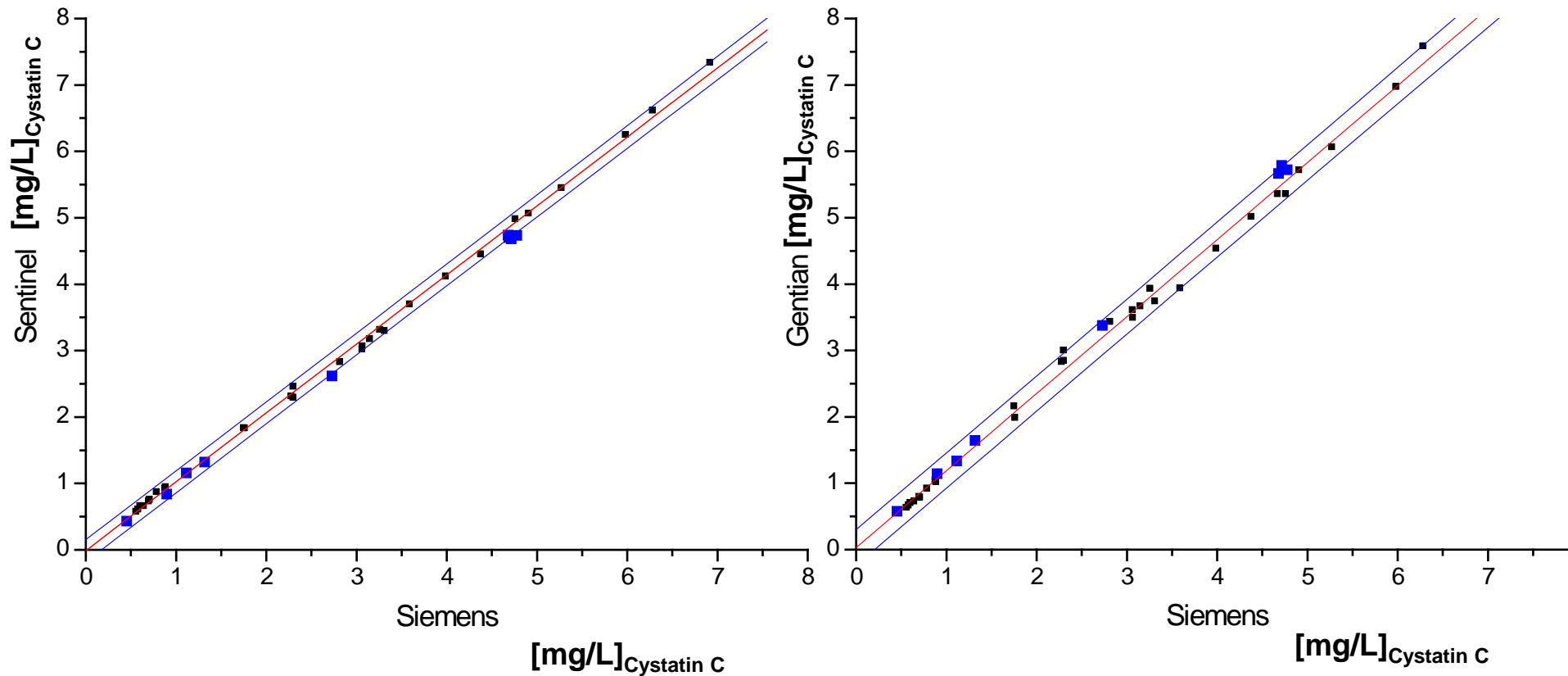
Liquid frozen RM is both commutable and giving unbiased results



## Marker for kidney functioning Produced in collaboration with the IFCC Working Group for cystatin C

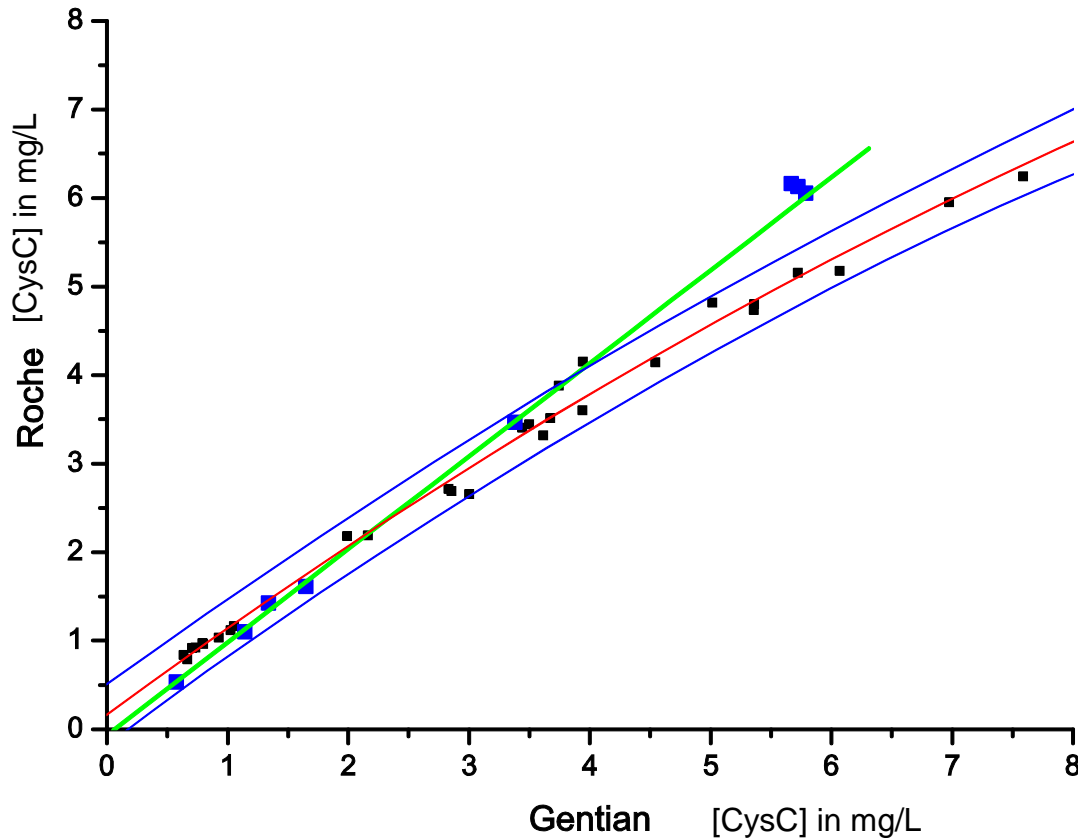


## ERM-DA471/IFCC is commutable for combinations of 11 methods



- Clinical samples
  - ERM-DA470/IFCC or dilutions there-of in saline
- results shown were obtained without use of a common calibrator





- patient samples (black)  
- dilutions of ERM-DA471/IFCC (blue)

The green line is the linear regression for the ERM-DA471/IFCC and dilutions thereof.

The red line is the result of the polynomial fit of patient samples results

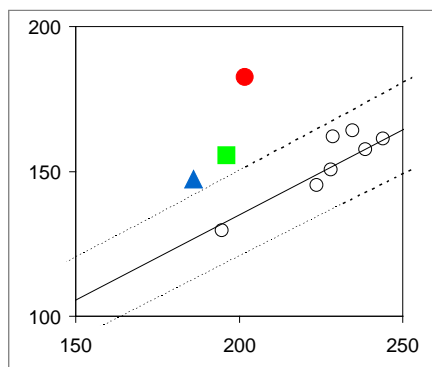
The blue lines correspond to the 95 % prediction bands of the polynomial regression.

- Relationship between results not equivalent: linear vs curved
- Commutability for some patient groups and not for others

| center | 1 | 3 | 4 | 6 | 7 | 8 | 9 | 10 | 11 | 16 | 5 | 12 | 15 | 13 | 14 | 2 |
|--------|---|---|---|---|---|---|---|----|----|----|---|----|----|----|----|---|
| 1      | . | y | y | y | y | y | y | y  | y  | y  | y | n  | n  | n  | n  | n |
| 3      | y | . | y | y | y | y | y | y  | y  | y  | y | n  | n  | n  | n  | n |
| 4      | y | y | . | y | y | y | y | y  | y  | y  | y | n  | n  | n  | n  | n |
| 6      | y | y | y | . | y | y | y | y  | y  | y  | y | n  | n  | n  | n  | n |
| 7      | y | y | y | y | . | y | y | y  | y  | n  | y | n  | n  | n  | n  | n |
| 8      | y | y | y | y | y | . | y | y  | y  | y  | y | n  | n  | n  | n  | n |
| 9      | y | y | y | y | y | y | . | y  | y  | y  | y | n  | n  | n  | n  | n |
| 10     | y | y | y | y | y | y | y | .  | y  | y  | y | n  | n  | n  | n  | n |
| 11     | y | y | y | y | y | y | y | y  | .  | y  | y | n  | n  | n  | n  | n |
| 16     | y | y | y | y | n | y | y | y  | y  | .  | n | n  | n  | n  | n  | n |
| 5      | y | y | y | y | y | y | y | y  | y  | n  | . | y  | y  | n  | n  | n |
| 12     | n | n | n | n | n | n | n | n  | n  | n  | y | .  | y  | y  | n  | n |
| 15     | n | n | n | n | n | n | n | n  | n  | n  | y | y  | .  | y  | n  | n |
| 13     | n | n | n | n | n | n | n | n  | n  | n  | n | y  | y  | .  | y  | n |
| 14     | n | n | n | n | n | n | n | n  | n  | n  | n | n  | n  | y  | .  | n |
| 2      | n | n | n | n | n | n | n | n  | n  | n  | n | n  | n  | n  | n  | . |

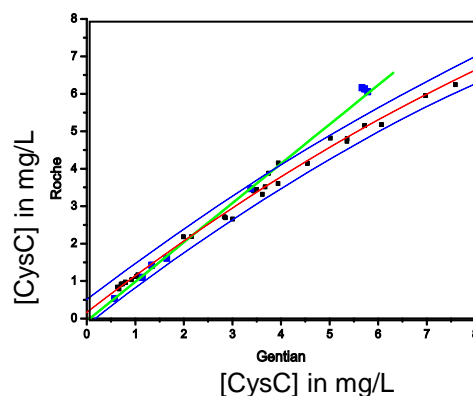
- **ERM-DA471/IFCC is commutable for all major methods**
- **Methods can be organised in blocks for which the material is commutable**
- **Methods 14 and 15 have since the study been modified, now belong to the main block**

## ceruloplasmin



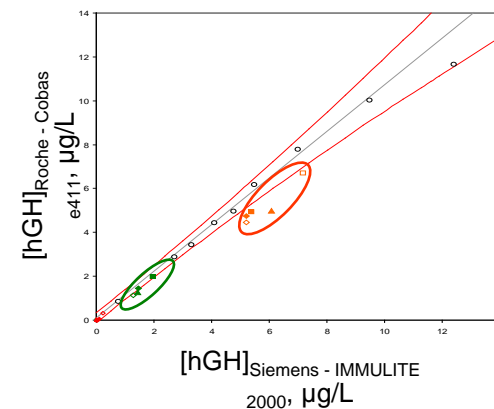
Modifications of the protein (ageing)

## cystatin C



Matrix effects

## hGH



Protein isoform and matrix effects

Possible causes:

- Analyte: isoform composition, (partial) denaturation, glycosylation, oligomeric form
- Matrix: interfering substances, turbidity, absence of trace elements, ...
- Interactions between matrix and analyte (e.g. ligands, complexes, effect of pH)

- Ceruloplasmin:
  - Use of a common calibrator is not a sufficient condition for equivalence of results
  - A RM may be commutable for subgroups of methods
- Cystatin C
  - A RM may not be commutable for samples from all patient groups
  - Commutability is a measure of the degree of equivalence that can be achieved
- hGH
  - Traceability is a multi-parametric issue: quantity values need to be combined with information on the identity of measurand
- CRP
  - Commutability does not mean lack of bias

# Thanks!

