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)
Traceability chain

Aim of traceability is to ensure comparability of results

Definition of the SI unit
- "Pure" protein
- CRM matrix material
- Manufacturer’s working calibrator
- Manufacturer’s product calibrator
- Routine sample

Value transfer 1:
- Reference procedure, immunoassay ring trial
- Value transfer 2:
- Immunoassay

Value transfer 3:
- e.g. AAA, refractive index

Manufacturer’s working calibrator

Immuonassay

Traceability chain
Planning

Material selection

Feasibility study (collection, processing, characterisation, commutability)

Homogeneity study

Storage

Processing

Interim transport and storage

Material collection

Short-term stability study

Long-term stability study

Value assignment

Commutability study

Follow-up stability monitoring of CRM

Assessment by experts

Documentation

CRM Distribution and Sales
## Commutability studies

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Issues</th>
<th>Commutability*</th>
</tr>
</thead>
<tbody>
<tr>
<td>β 2-microglobulin (B2M)</td>
<td>recombinant</td>
<td>yes</td>
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<tr>
<td>C4</td>
<td></td>
<td>yes</td>
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<tr>
<td>ceruloplasmin</td>
<td>sample ageing</td>
<td>±</td>
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<tr>
<td>myoglobin</td>
<td>recombinant, $^{15}$N labelled</td>
<td>±</td>
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<tr>
<td>HbA2 $^1$</td>
<td>processing</td>
<td>yes</td>
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<tr>
<td>HbA1c $^1$</td>
<td>processing</td>
<td>yes</td>
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<tr>
<td>albumin $^2$</td>
<td>urine/serum analysis ongoing</td>
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<tr>
<td>C-reactive protein</td>
<td>oligomerisation, matrix</td>
<td>yes</td>
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<tr>
<td>cystatin C $^1$</td>
<td>non-linear method correlation</td>
<td>±</td>
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<tr>
<td>Aβ 42 $^1$</td>
<td>spiking, matrix</td>
<td>±</td>
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<tr>
<td>IgG anti-MPO, anti-PR3 $^1$</td>
<td>method correlation</td>
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<tr>
<td>IgG anti B2GP</td>
<td>monoclonal</td>
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<tr>
<td>enzymes (LD, CK, ALT) $^1$</td>
<td>recombinant, isoform, matrix</td>
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<tr>
<td>human growth hormone</td>
<td>recombinant, matrix</td>
<td>no</td>
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$^1$ in collaboration with IFCC, $^2$ 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

Anti-B2GPI IgG

95% prediction interval

Clinical samples

Immunooassay B (arbitrary units)

Immunooassay A (arbitrary units)
### Evaluation of routine methods – Comparison for IgG anti MPO

<table>
<thead>
<tr>
<th>Method 1</th>
<th>Method 2</th>
<th>Method 3</th>
<th>Method 4</th>
<th>Method 5</th>
<th>Method 6</th>
<th>Method 7</th>
<th>Method 8</th>
<th>Method 9</th>
<th>Method 10</th>
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#### Methods claiming to measure the same analyte may in fact have different selectivities

→ clinical significance of different analytes?

→ selection of analyte to standardise
**RM format evaluation:** e.g. Alzheimer marker Aβ42, results obtained without use of common calibrator
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

- Serum samples
- Dilutions
- ERM-DA470
- ERM-DA470k
Commutability of ERM-DA470 for ceruloplasmin

- 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

CRM used to calibrate both methods
C-reactive protein

- 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
Cystatin C

ERM-DA471/IFCC

- 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
- 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
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)
Summary

• 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!