The IRMM approach to selection and characterisation of enzyme Reference Materials

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Content

1. Introduction
2. Certified Reference Materials (CRMs)
3. Clinical Enzymology
4. Example of characterisation of a CRM for enzyme
5. Commutability
6. Use of CRMs for enzymes
7. Conclusions
The issues

- Measurement results are often not comparable, neither in scientific investigative work nor in clinical analysis.
- In vitro diagnostics materials have to fulfil requirements of the IVD Directive (Directive 98/79/EC).

‘...the traceability of values assigned to calibrators and control materials for in vitro diagnostics must be assured through available reference measurement procedures and/or materials of higher order.’

Metrological traceability

Definition

« property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty »

International Vocabulary of Basic and General Terms in Metrology, VIM, 3rd edition 2007
Traceability

- EC mandated standard related to the IVD directive: ISO 17511: In vitro diagnostic medical devices - Metrological traceability of values assigned to calibrators and control materials

- Underlying assumption: Being traceable to a common standard or stated reference should ensure that independently obtained measurement results will overlap within their stated uncertainties and at a certain level of confidence with the true value and consequently with each other
  - provided measurement procedures applied in the traceability chain determine the same measurand
  - if the comparison measurements do not introduce unrecognised bias (e.g. matrix effects, differential extraction etc.)
  - if all relevant uncertainty components are included in the estimate of the combined uncertainties

Metrological traceability chain (ISO 17511)
Reference System for enzyme measurement

primary reference material

certify

reference measurement procedure (IFCC)

verify

certify

secondary ("matrix") reference material

calibrate

manufacturers standing measurement procedure

manufacturer's product calibrator

routine sample

end user's routine measurement procedure

result

CCLM 39 (2001) 795-800, Fig. 1

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Analytical Quality Assurance

AQA

- Reference Meas. Procedures
- Reference Materials
- Reference Lab.

- Validated methods fit for purpose
- Suitable for selected measurand
- Successful proficiency testing

Materials for QA

Quality assurance

Sampling, Processing

Conservation

Sample preparation

Analyte identification & Quantitative measurement

Evaluation

Assessment

Matrix CRMs

→ Quality control

RM for qualitative analysis

- chemical identity
- as close as possible to real sample

- often pure analyte
The “RM Family”

characteristics:
• homogeneity (fit for intended use)
• stability (fit for intended use)

additional features:
• certificate
• certified value with uncertainty
• stated traceability

CRMs with uncertainties of property values fit for calibration

additional features:
• property value with uncertainty
• traceability

QCMs
CALs

Reference Material Programmes at IRMM

Expert advice
to standardization & metrology bodies

Network in Health
e.g. IFCC, JCTLM, CLSI, ISO, EC, CCQM, IVD manufacturers

User support
e.g. training workshops

Searchable on-line catalogue: www.irmm.jrc.be

General Procedure for CRM Production

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Clinical enzymology

Challenge:

The catalytic activity of an enzyme is a property measured by the catalyzed rate of a chemical reaction in specific experimental conditions

→ the measurement result is procedure-dependent
(influence quantities: temperature, pH, substrate nature and concentration, inhibitors)

Clinical enzymology

1. IFCC Primary Reference Measurement Procedure for the measurement of the catalytic activity concentration of AST at 30 °C (1986).

→ unique routine procedure

2. Calibration of routine procedures using validated calibrators, traceable to a reference measurement procedure.
Clinical enzymology

IFCC Reference Procedures for the measurement of catalytic concentrations of enzymes at 37 °C (alanine aminotransferase, creatine kinase, lactate dehydrogenase, γ-glutamyltransferase, α-amylase and AST)

IRMM CRM for enzymes to check to the performances of the IFCC Reference Procedure

Clinical enzymology

- Gamma-glutamyltransferase ERM-AD452
- Lactate dehydrogenase 1 ERM-AD453
- Alanine aminotransferase ERM-AD454
- Creatine kinase-2 (CK-MB) ERM-AD455
- Creatine kinase-1 (CK-BB) BCR 299
- Pancreatic alpha-amylase IRMM/IFCC-456
- Aspartate transaminase ERM-AD457/IFCC
- Prostatic acid phosphatase BCR 410
- Adenosine deaminase BCR 647
- Pancreatic lipase BCR 693
- Recombinant lipase BCR 694
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ERM-AD457/IFCC (AST)

Recombinant AST (E. Coli) in buffer
→ Processing (FD) & reconstitution
→ Homogeneity
→ Short-term stability
→ Characterisation (routine procedure)

Final batch
→ Homogeneity
→ Short- & long-term stability
→ Stability on reconstituted CRM
→ Characterisation (IFCC Ref. Proc.)
  • Feasibility study
  • Value assignment

Test batch

Released CRM
→ Stability monitoring
Characterisation

Target is the material property/quantity – not the analytical method!

Strategies:

• multi-method approaches in interlaboratory study
  *(with truly independent methods, “expert” labs)*

• multi-method approach in one laboratory
  *(including a “primary” method)*

• single method in interlaboratory study
  *(for method-defined measurands)*

• single method in one laboratory
  *(not for certification)*

Method-defined measurand

**Catalytic activity concentration** determined by:
Single-method in interlaboratory study (12 expert labs)
- IFCC reference procedure at 37 °C
- calibrated balances, pipettes; linear response of spectrophotometer

Assigned value of AST in ERM-AD457/IFCC traceable to the SI applying the IFCC Reference Procedure
Table: ERM-AD457/IFCC (AST)

<table>
<thead>
<tr>
<th>Uncertainty budget</th>
<th>ERM-AD457/IFCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative ( u_{bb} ) [%]</td>
<td>0.72</td>
</tr>
<tr>
<td>Relative ( u_{lts} ) [%]</td>
<td>0.39</td>
</tr>
<tr>
<td>Relative ( u_{char} ) [%]</td>
<td>0.84</td>
</tr>
</tbody>
</table>

Certified value: 1.74 µkat/L
Expanded uncertainty of the certified value \( U_{CRM} (k=2) \): 0.04 µkat/L

1 µkat/L = 60 U/L
1 U = 10^{-6} mol/60 s = 16.7 x 10^{-9} mol/s

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Commutability

Wide spread belief:

- Use of a common standard (eventually with an arbitrarily assigned unit) to calibrate different methods will improve comparability of measurement results

- Only true under the condition that
  - the methods to be compared measure the same analyte or different analytes but in a constant relationship in the samples to be analysed
  - the common standard is commutable

Non commutable / commutable calibrator
Different analyte / commutable calibrator

Limitations in standardisation

• In case of commutable calibrators harmonisation (conventional standards) or standardisation (SI traceable standards) is possible.

• If analytical specificity of assays is different (large scatter in commutability plots) tighter definition of the measurand and eventually reformulation of assays is necessary, otherwise differences on individual samples will remain even with a commutable standard (only means of sample populations will agree).
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**Method Validation / Verification**

Comparison of result and CRM data

1. Determine difference ($\Delta_m$) measured vs. certified value

   \[ \Delta_m = |C_m - C_{CRM}| \]

2. Determine uncertainty of the cert. value ($u_{CRM}$): $u_{CRM}/k$

3. Determine uncertainty of the measurements ($u_m$):
   - reproducibility standard deviation = rough estimate

4. Combine $u_{CRM}$ and $u_m$ to $u_\Delta$:

   \[ u_\Delta = \sqrt{u_m^2 + u_{CRM}^2} \]

5. Compare $\Delta_m$ with $2 \times u_\Delta$:
   - if $\Delta_m < 2 \times u_\Delta$: no difference
Control charts

Added value using a CRM for QC Charts:
- guaranteed homogeneity
- usually better stability
- immediate trueness check

Control limits should reflect uncertainty of certified value and variation of results

\[ u_c = \sqrt{u_{CRM}^2 + S^2} \]

Conclusion

- The catalytic activity of an enzyme is a property, not an amount of substance
- The result is method-dependent
- CRMs are a key component of the Reference Measurement System for enzymes, ensuring the traceability of the measurement result to the reference procedure.
- ..under the condition that the reference procedure and the routine procedures have the same specificities for the enzyme