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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
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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.'

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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

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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

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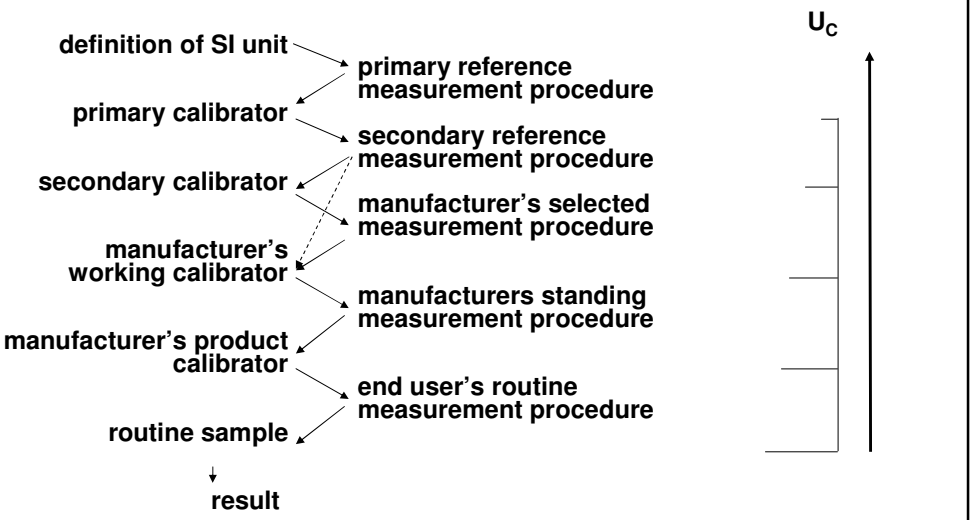
Metrological traceability chain (ISO 17511)



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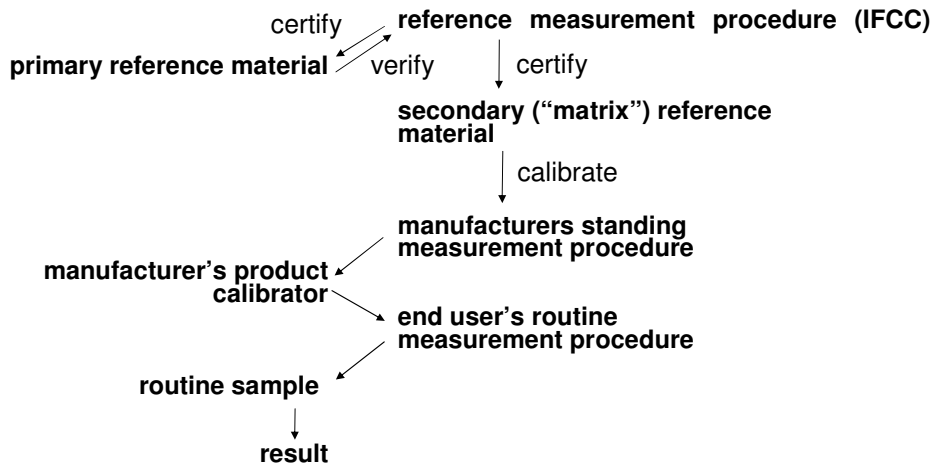
Reference System for enzyme measurement



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

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Analytical Quality Assurance

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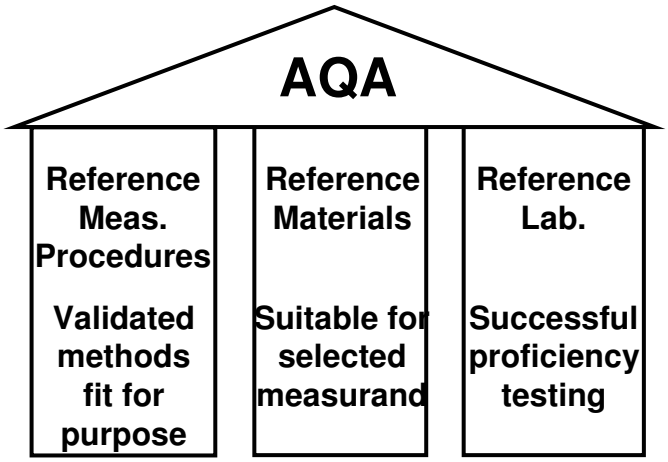

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Materials for QA

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Quality assurance

Sampling, Processing

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Conservation

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Sample preparation

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Analyte identification

&

Quantitative measurement

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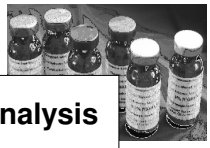
Evaluation

↓

Assessment

Matrix CRMs
→ Quality control



RM for qualitative analysis

• chemical identity

• often pure analyte

• as close as possible to real sample

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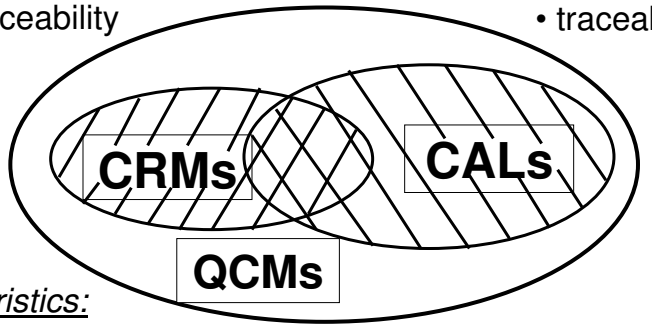
The "RM Family"

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



characteristics:
- *homogeneity (fit for intended use)*
- *stability (fit for intended use)*

H. Emons: Accred. Qual. Assur. 10 (2006) 690

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Reference Material Programmes at IRMM

Expert advice

to standardization & metrology bodies

Network in Health

e.g. IFCC, JCTLM, CLSI, ISO, EC, CCQM, IVD manu-facturers

TSE QCMs

Food CRMs

Clinical CRMs

Microbiol. CRMs

GMO CRMs

User support

e.g. training workshops

Soil/ Sediment CRMs

Water CRMs

Engin. Mat. CRMs

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

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General Procedure for CRM Production

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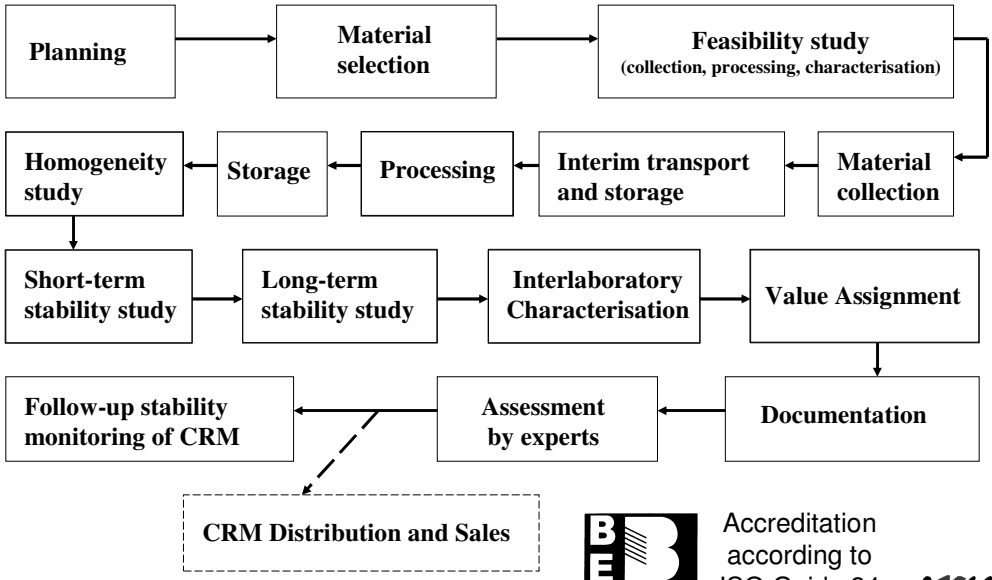

Accreditation according to ISO Guide 34 & ISO 17025

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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)

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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.

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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 CRMs for enzymes to check to the performances of the IFCC Reference Procedure**

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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

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Test batch ⟶
- ➢ 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

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Released CRM ⟶ Stability monitoring

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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)***

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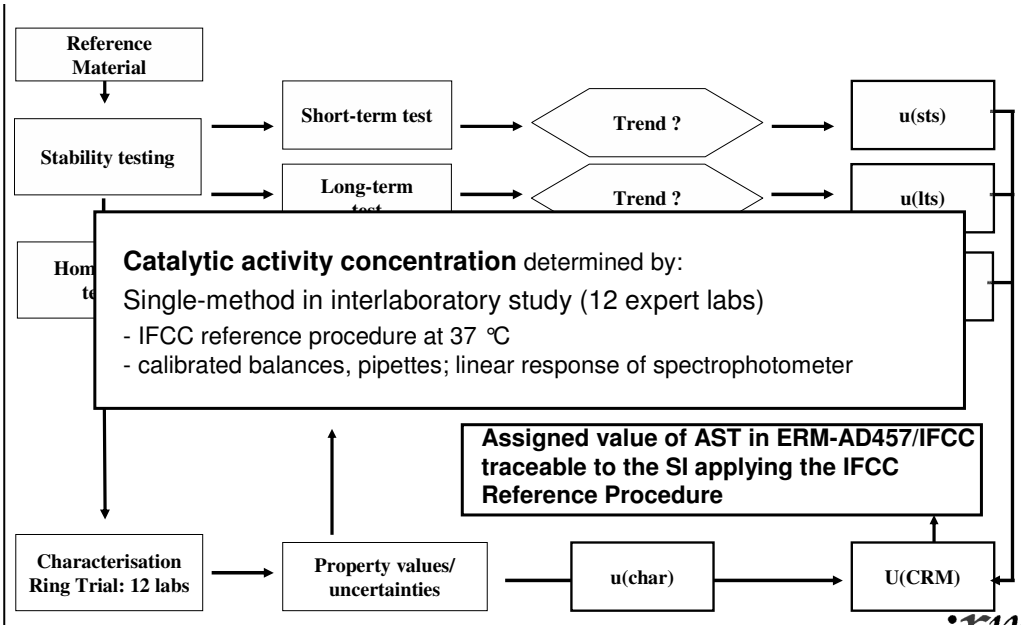
Method-defined measurand



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ERM-AD457/IFCC (AST)

Uncertainty budget	ERM-AD457/IFCC
Relative u_{bb} [%]	0.72
Relative u_{lts} [%]	0.39
Relative u_{char} [%]	0.84

Certified value	1.74 µkat/L 104.7 U/L
Expanded uncertainty of the certified value U_{CRM} (k=2)	0.04 µkat/L 2.5 U/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

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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

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Non commutable / commutable calibrator

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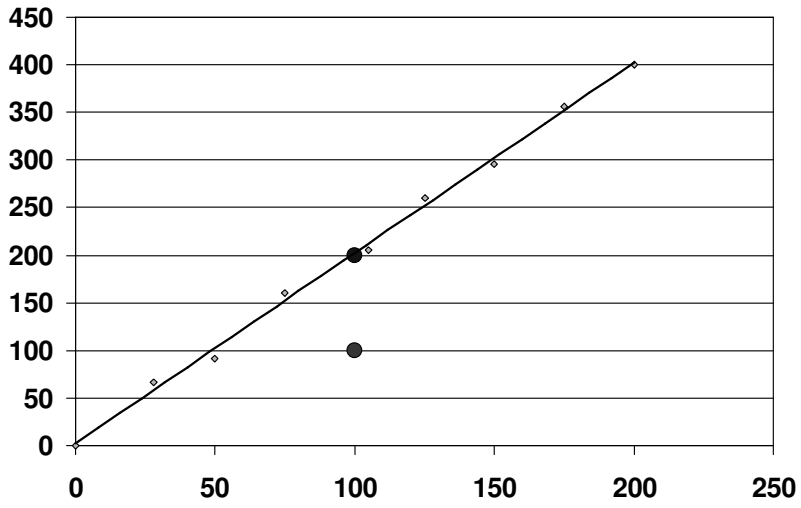

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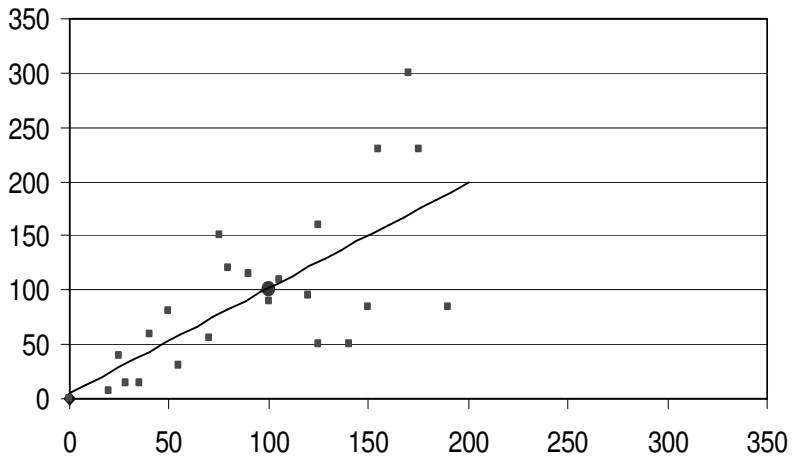
Different analyte / commutable calibrator



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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 (Δ_m) measured vs. certified value

$$\Delta_m = \left| c_m - c_{CRM} \right|$$

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
3. Combine u_{CRM} and u_m to u_Δ: $u_\Delta = \sqrt{u_m^2 + u_{CRM}^2}$
4. Compare Δ_m with 2 * u_Δ

   if $\Delta_m < 2 * u_\Delta$: no difference

www.erm-crm.org

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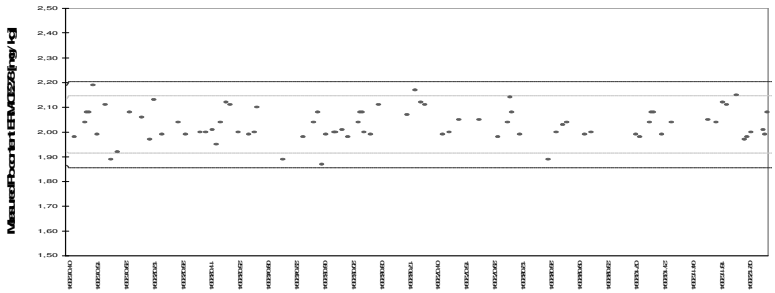
Control charts



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

$$u_c = \sqrt{u_{CRM}^2 + s^2}$$

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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**

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