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Uncertainty sources of reference measurement procedures for enzymes

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Officially accredited
Calibration laboratory
(Reference laboratory)

DKD-K-20602
DKD-K-20603



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Measurement uncertainty (MU) is inherent in measurements obtained in clinical laboratories.

Measurement uncertainty is a range around the given value.

Parameter characterizing the dispersion of the quantity values being attributed to a measurand, based on the information used.
Vocabulary of Metrology (VIM)

MU_{ref. measurement proc.} << MU_{routine proc.} (for the same measurand)

The investigation of MU components is a fundamental (never ending) task of reference methodology.

What is different with the measurand “enzyme” compared to potassium, glucose, creatinine?

**A property of the enzyme is measured
(not the amount or the mass of the enzyme)**



**The catalytic concentration
(= catalyzed reaction rate)
of the enzyme is measured.**

What is different with the measurand “enzyme” compared to potassium, glucose, creatinine?

- ◆ There is no procedure for a direct measurement of the catalytic concentration.
- ◆ The determination of the catalytic concentration is performed by spectrometrical monitoring the reaction rate.
- ◆ The reaction conditions are defined in a primary reference procedure (IFCC).
- ◆ The catalytic concentration **depends strongly** on the reaction conditions of the measurement procedure.

Two types of uncertainty components

- **Uncertainty components influencing the catalytic concentration in the final complete reaction mixture directly:**

Components (such as the temperature in the cuvette) can effect the reaction rate of the employed substrate directly.

- **Components influencing the signal of kinetic spectrometry:**

Such components have no influence on the reaction rate.

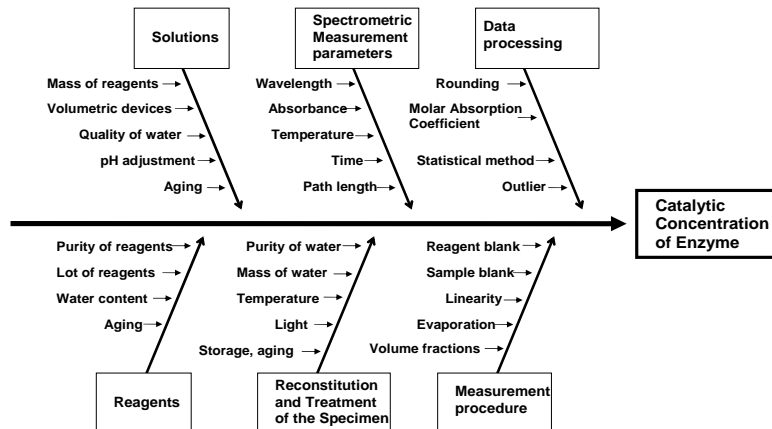
Sources of MU with direct influence on the substrate rate catalyzed by the enzyme

- **Measurement temperature**
- **pH**
- **Volume fraction of sample**
- **Final concentration of the reagents in the reaction mixture**
- **Linearity of the reaction rate**
- **Evaporation in the cuvette**
- **Aging of the specimen and aging of the reagent solutions**
- **Lot of the reagents (minor impurities!)**
- **Reconstitution of lyophilized materials (e.g. light, temperature)**

Sources of MU influencing the kinetic spectrometry

- ◆ **Measurement wavelength and band width**
- ◆ **Measurement of absorbance**
- ◆ **Path length of the cuvette**
- ◆ **Time measurement of the kinetic spectrometric signal**
- ◆ **Effects of the matrix of the sample (sample blank)**
- ◆ **Spectrometric stray light and noise,
linearity,
stability of the baseline**
- ◆ **Reagent blank rate**

Overview of relevant uncertainty components



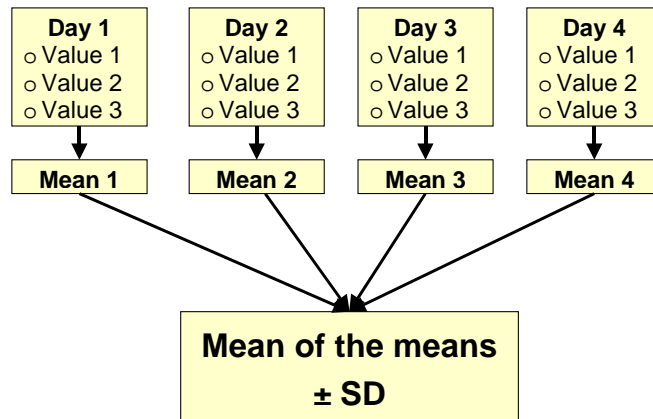
Different ways estimating MU components

❖ **Type A:**
 Random components estimated statistically as standards deviation. → (mean of means)
 ("standard uncertainty")

❖ **Type B:**
 Components estimated from specific information and additional investigations of the reference laboratory:
Calibration procedures for spectrometry, gravimetry, volumetry, potentiometry, thermometry.

(Type B uncertainty compounds are constant for all measurement values)

Measurement design of the reference procedure (example)



SD = standard uncertainty of the mean of the means

MU components of the standard uncertainty of the mean of the means

Uncertainty component	Explanation/Comment
path length of the cuvette	use of different cuvettes
measurement of absorbance	repeated measurements on 4 days
uncertainty of temperature adjustment	continuous temperature control
volume for reconstitution of lyophilized materials	a new specimen is reconstituted on each of 4 days
volume fraction of sample	the sample is independently pipetted 12 times
uncertainty of wavelength adjustment	the wavelength adjustment is performed for each measurement series separately

Correction of systematic effects

Postulate of GUM:

“The result of a measurement should be corrected for all recognized significant systematic effects.”

This condition is fulfilled by the implementation of correction factors in the model function for the calculation of the reference method value.

$$RMV = \text{mean}(\text{means}) \cdot c_{\text{wavelength}} \cdot c_{\text{absorb}} \cdot c_{\text{pH}} \cdot c_{\text{temp}} \cdot c_{\text{conc}} \cdot c_{\text{charge}} \cdot c_{\text{vol. fract.}} \cdot c_{\text{time}} \cdot c_{\text{evapor}} \cdot c_{\text{aging}} \cdot c_{\text{lin}}$$

Provided all $c = 1$: $RMV = \text{mean of means} + (\text{uncertainty components})$

Uncertainty components due to calibration

- The measurement conditions of the reference measurement procedure are controlled by calibration procedures and are corrected to the target value by adjustment.
- Consequently all correction factors in the model function were set to the value 1
- All correction factors have a standard uncertainty, resulting from the uncertainty of the calibration procedure and the uncertainty of the adjustment or is deduced from the experience of the reference laboratory (e.g. c_{charge})

Type B - Standard uncertainties and sensitivity coefficients

- Step 1:
Estimation of the standard uncertainty of each parameter that is considered by a correction factor ($c=1$) in the model function.
- Step 2:
Experimental determination of the sensitivity coefficients
(= the rate of change in the final result with the changes of the parameter)

Example of the determination of standard uncertainties

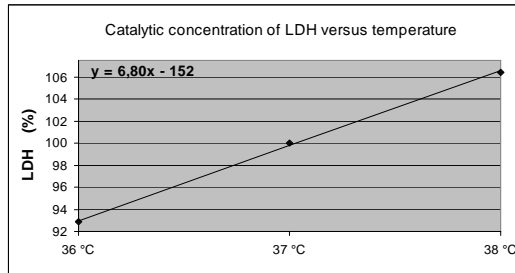
- The IFCC procedure prescribes maximum uncertainties. There is no information about the distribution of the uncertainties. Therefore, a rectangular distribution is assumed.
- The calibration procedure of the reference laboratory must assure, that these uncertainties are not exceeded.
- Example LDH

Parameter	Uncertainty (IFCC)	Type	Distribution	Standard uncertainty
pH	0,05	B	rectangular	0,03
Temperature	0,1 °C	B	rectangular	0,06 °C
Wavelength	1 nm	B	rectangular	0,6 nm

Example for the determination of sensitivity coefficients

LDH: measurement temperature

The catalytic concentration of LDH was determined at 36 °C, 37 °C and 38 °C



The sensitivity coefficient for the correction factor c_{temp} is 6,8% per 1 °C

Comparison of sensitivity coefficients for different enzymes

Temperature

Enzyme	$\Delta b_{rel}/\Delta T$
ALT	5,05 %/K
AMY	4,62 %/K
AST	5,30 %/K
CK	5,28 %/K
GGT	3,20 %/K
LDH	6,80 %/K

Wavelength

Enzyme	$\Delta b_{rel}/\Delta \lambda$
ALT	0,13 %/1 nm
AMY	0,40 %/1 nm
AST	0,20 %/1 nm
CK	0,28 %/1 nm
GGT	3,25 %/1 nm
LDH	0,08 %/1 nm

pH

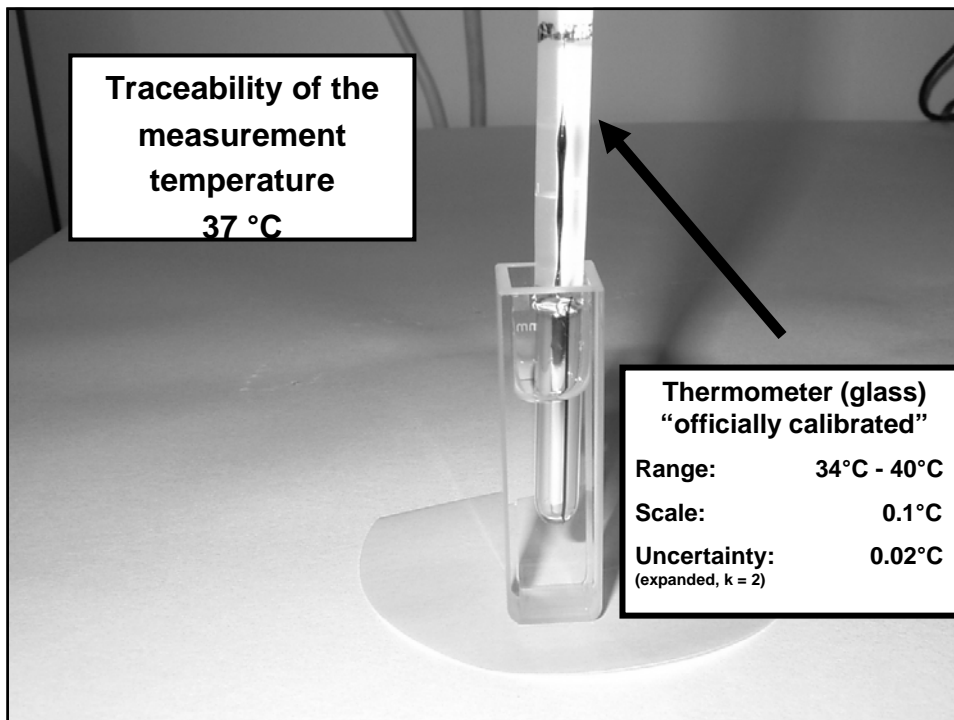
Enzyme	$\Delta b_{rel}/\Delta pH$
ALT	0,23 %/ ΔpH 0,05
AMY	2,47 %/ ΔpH 0,03
AST	0,25 %/ ΔpH 0,05
CK	0,90 %/ ΔpH 0,05
GGT	0,22 %/ ΔpH 0,05
LDH	0,09 %/ ΔpH 0,05

Uncertainty contribution of a parameter to the combined uncertainty of the result

(Example LDH)

standard uncertainty of the component	x	sensitivity coefficient	=	standard uncertainty component of the result
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0,06 °C	x	6,8 % per °C	=	0,4 %
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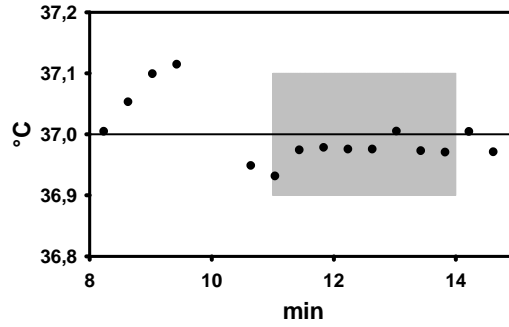




Traceability chain:

spectrometer → digitale probe → glass thermometer

↑
Temperature in the cuvette and in the measurement window!



Temperature programming using a pH-sensitive dye .

(Koedam & Koedam, Intern. Lab News 2005)

Example of an uncertainty budget for LDH

Quantity x_i	Value	Parameter	Uncertainty y	Source	Distribution	Type	Standard uncertainty of the	Coefficient of sensitivity	Standard uncertainty y
C_{wl}	1	Wavelength	1,0 nm	FCC-Dokument	rectangular	B	0,58 %	0,08 % per nm	0,05 %
C_{absorb}	1	Absorbance	0,30 %	Manufacturers specification	rectangular	B	0,17 %	1,00 % per %	0,17 %
C_{ph}	1	pH	0,05	FCC-Dokument	rectangular	B	0,03	0,09 % per 0,05	0,05 %
C_{temp}	1	Temperature	0,1 °C	FCC-Dokument	rectangular	B	0,06 °C	6,80 % per 1°C	0,39 % ②
C_{conc}	1	Concentration of reagents	1,5 %	FCC-Dokument	rectangular	B	0,87 %	0,17 % per 1 %	0,15 %
C_{charge}	1	Lot of reagents	1,5 %	Experiment	rectangular	B	0,87 %	1,00 % per 1 %	0,87 % ①
$C_{volfrac}$	1	Volume fraction of sample	0,4 %	Data basis of calibrations	rectangular	B	0,22 %	1,00 % per 1 %	0,22 %
C_{time}	1	Time	0,03 %	Experiment	rectangular	B	0,02 %	1,00 % per 1 %	0,02 %
C_{evapor}	1	Evaporation	0,10 %	Experiment	rectangular	B	0,06 %	1,00 % per 1 %	0,06 %
C_{aging}	1	Aging	0,50 %	Experiment	rectangular	B	0,29 %	1,00 % per 1 %	0,29 %
C_{lin}	1	Linearity	0,60 %	Experiment	Normal	B	0,30 %	1,00 % per 1 %	0,30 % ③
<i>mean of means</i>	124,5 UI	Mean of the means	0,40 %	Measurements	Normal	A	0,20 %	1,00 % per 1 %	0,20 %

Combined uncertainty (example for LDH)

Combined (standard) uncertainty
 =
 (positive) square root
 of the sum of the variances
 (calculated from the standard uncertainty components)

Example LDH		
Quantity x_j	Standard uncertainty y	Variance
C_{wt}	0,05 %	0,002
C_{absorb}	0,17 %	0,030
C_{ph}	0,05 %	0,003
C_{temp}	0,39 %	0,154
C_{conc}	0,15 %	0,022
C_{charge}	0,87 %	0,750
$C_{volfrac}$	0,22 %	0,048
C_{time}	0,02 %	0,000
C_{evapor}	0,06 %	0,003
C_{aging}	0,29 %	0,083
C_{lin}	0,30 %	0,090
<i>mean of means</i>	0,20 %	0,040
sum		1,23
square root		1,1 %
combined uncertainty		

Expanded uncertainty

- The expanded uncertainty describes an interval, which includes the true value with a defined probability.
- The expanded uncertainty is calculated from the combined uncertainty by multiplying with the coverage factor k .
- The value of the k -factor for a defined probability depends on the degrees of freedom of the combined uncertainty.

Determination of the degrees of freedom

Formula from Welch-Satterthwaite

$$v_{eff} = \frac{u^4(\mathbf{y})}{\sum_{i=1}^N \frac{u_i^4(\mathbf{y})}{v_i}}$$

u combined standard uncertainty of the result (reference method value)

u_i standard uncertainty of each uncertainty component

The uncertainty components of type B (all correction factors): $v_{eff} = \infty$

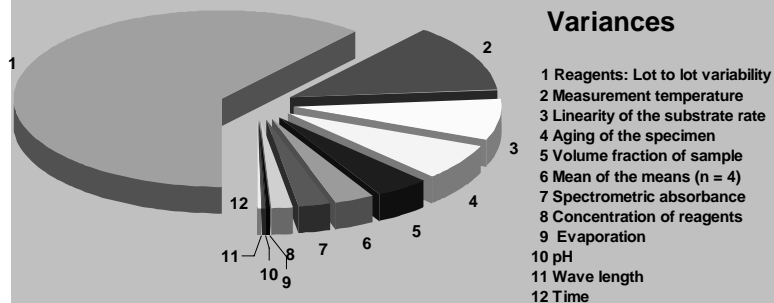
(DKD-3, Anhang E, Abschnitt E2, Punkt b).

The number of the degrees of freedom of the mean of the mean is 3 ($n - 1$)

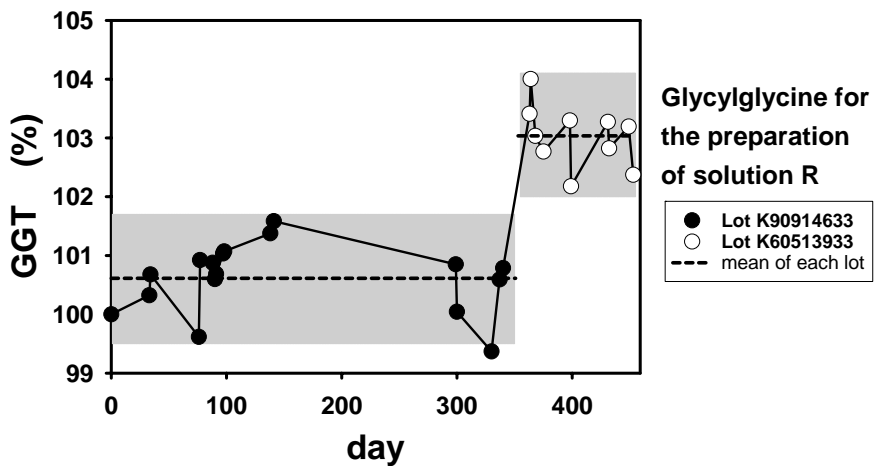
In the sum in the denominator in the formula from Welch-Satterthwaite all summands become 0, with the exception of the standard uncertainty of the mean of the means.

Thus the number of degrees of freedom only depends on the number of measurement days and the standard uncertainty of the mean.

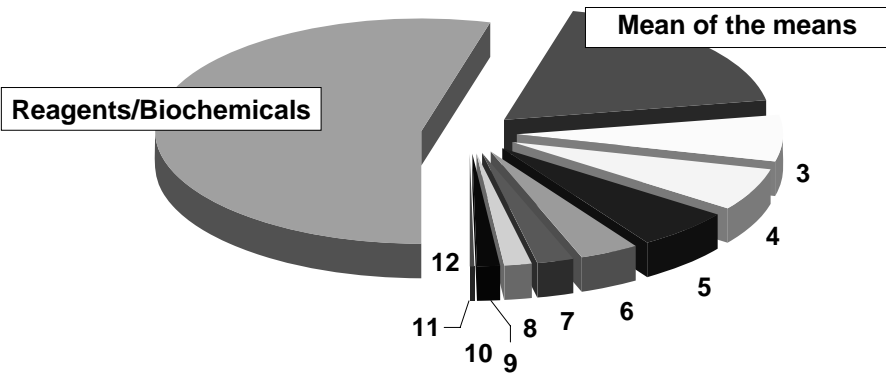
Uncertainty Components of a Reference Method Value for the Catalytic Concentration of LDH



MU deriving from lot to lot variation





MU components for ALT



- (3) Linearity (signal / rate of substrate)
- (5) Aging of the reconstituted specimen

(4) Temperature (37° C)

<p style="text-align: center;">Deutscher Kalibrierdienst (DKD) Accreditation Body <small>represented in</small></p> <p style="text-align: center;">Deutscher Akkreditierungsrat</p> <p style="text-align: center;"></p> <p style="text-align: center;">Accreditation</p> <p style="text-align: center;"><small>The Accreditation Body of Deutscher Kalibrierdienst (DKD) hereby accredits Deutsche Vereinigte Gesellschaft für Klinische Chemie und Laboratoriumsmedizin e.V. Referenzinstitut für Bioanalytik Kalibrierlaboratorium III Carl-Neuberg-Straße 1 30625 Hannover</small></p> <p style="text-align: center;"><small>according to DIN EN ISO/IEC 17025: 2005 for calibrations in the field / fields: chemical quantities</small></p> <p style="text-align: center;"><small>Part of the certificate is: Annex 03 (1 page), 2007-05-25</small></p> <p style="text-align: center;"><small>DAR registration number: DKD-K-20602 DKD accredited since: 2004-11-26 Braunschweig, 2007-05-25</small></p> <p style="text-align: center;"><small>Dr.-Ing. Wolfgang Bischof Head of DKD Accreditation Body</small></p> <p style="text-align: center;"></p>	<p style="text-align: center;">Best measurement capability = Combined expanded MU (coverage probability of 95%)</p> <table border="0"> <tr> <td>Glucose</td> <td style="text-align: right;">1,0 %</td> </tr> <tr> <td>ALT</td> <td style="text-align: right;">2,2 %</td> </tr> <tr> <td>AST</td> <td style="text-align: right;">2,2 %</td> </tr> <tr> <td>α-Amylase</td> <td style="text-align: right;">2,7 %</td> </tr> <tr> <td>CK</td> <td style="text-align: right;">2,4 %</td> </tr> <tr> <td>γ-GT</td> <td style="text-align: right;">2,2 %</td> </tr> <tr> <td>LDH</td> <td style="text-align: right;">2,2 %</td> </tr> </table>	Glucose	1,0 %	ALT	2,2 %	AST	2,2 %	α-Amylase	2,7 %	CK	2,4 %	γ-GT	2,2 %	LDH	2,2 %
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Summary

- ✓ **MU is more than standard deviation and standard uncertainty of the contributing values.**
- ✓ **Each reference laboratory has to establish its own control procedures for type B uncertainty components.**
- ✓ **Each reference laboratory has to establish its individual MU budget.**
- ✓ **Critical review of the MU components and budget from time to time is required.**