

Reference systems and international standardisation of measurements for clinically important enzymes

**VIIth Czech National Congress of Clinical Biochemistry
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Objectives of this presentation

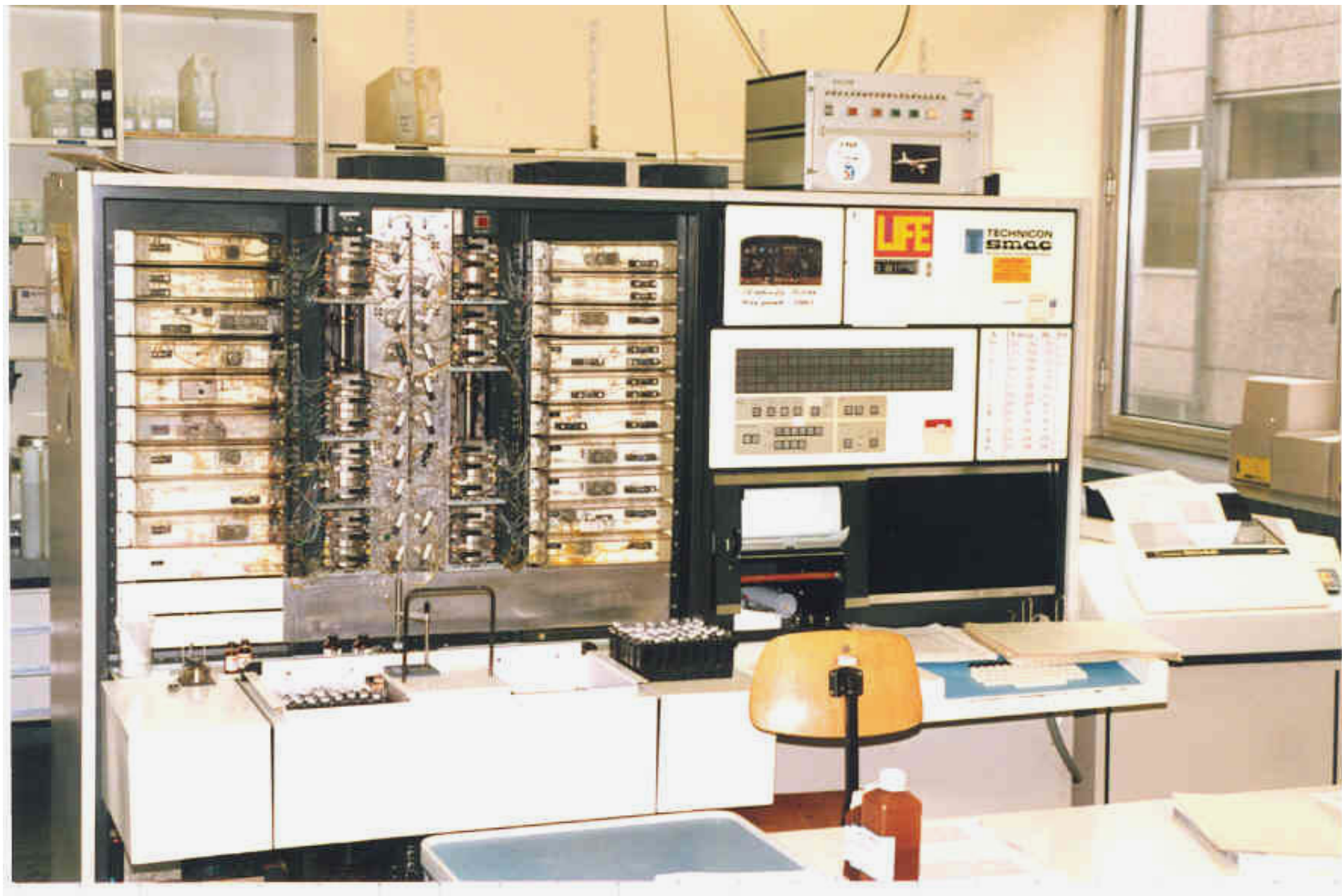
- ◆ The **components of a reference system** for “catalytic concentration measurements of enzymes”
(In short form: “enzyme measurements” or “enzymes”)
- ◆ **Measurement uncertainty** of reference procedures for enzymes
- ◆ **Metrological traceability** of measurements for enzymes from routine procedures to the primary reference measurement procedure
- ◆ **Control of the standardization** of routine measurements for enzymes in the medical laboratory
- ◆ **Outcome of the standardization** of measurements for enzymes

Manual filterphotometer for enzyme measurements

(1980; emergency laboratory of Medizinische Hochschule Hannover)



Continuous Flow Autoanalyzer SMAC (Technicon)



Development of standardisation during three decades

- ◆ **From manual procedures to automation**
(Every three to five years a new generation of instruments and technology for diagnostics)
- ◆ **National standardisation**
- ◆ **International standardisation: 30 °C und 37 °C**
- ◆ **Quality management (certification / accreditation)**
- ◆ **Rapid development of the European Union, nationale barriers for trade were abolished.**

The European In Vitro Diagnostics Medical Devices Directive (98/79/EC)

“**IVD Directive**”

Purpose

- Facilitate a single market with minimal technical barriers to trade
- Harmonize the rules on safety and quality

Essential requirements

- Ensure that medical products do not compromise the health and the safety of patients and users.

Time frame

Dec. 1998: Directive published

Dec. 2003: End of transition period

Dec. 2005: End of transition for putting into service

International standards

In vitro medical devices – Measurement of quantities in biological samples –

Presentation of reference measurement procedures **ISO 15193**

Description of reference materials **ISO 15194**

Requirements for reference measurement laboratories **ISO 15195**

Metrological traceability of values assigned to
calibrators and control materials **ISO 17511**

**Metrological traceability of values for catalytic
concentration of enzymes assigned to
calibrators and control materials **ISO 18153****

International standards

In vitro medical devices – Measurement of quantities in biological samples –

**Metrological traceability of values for
catalytic concentration of enzymes
assigned to calibrators and control materials**

ISO 18153

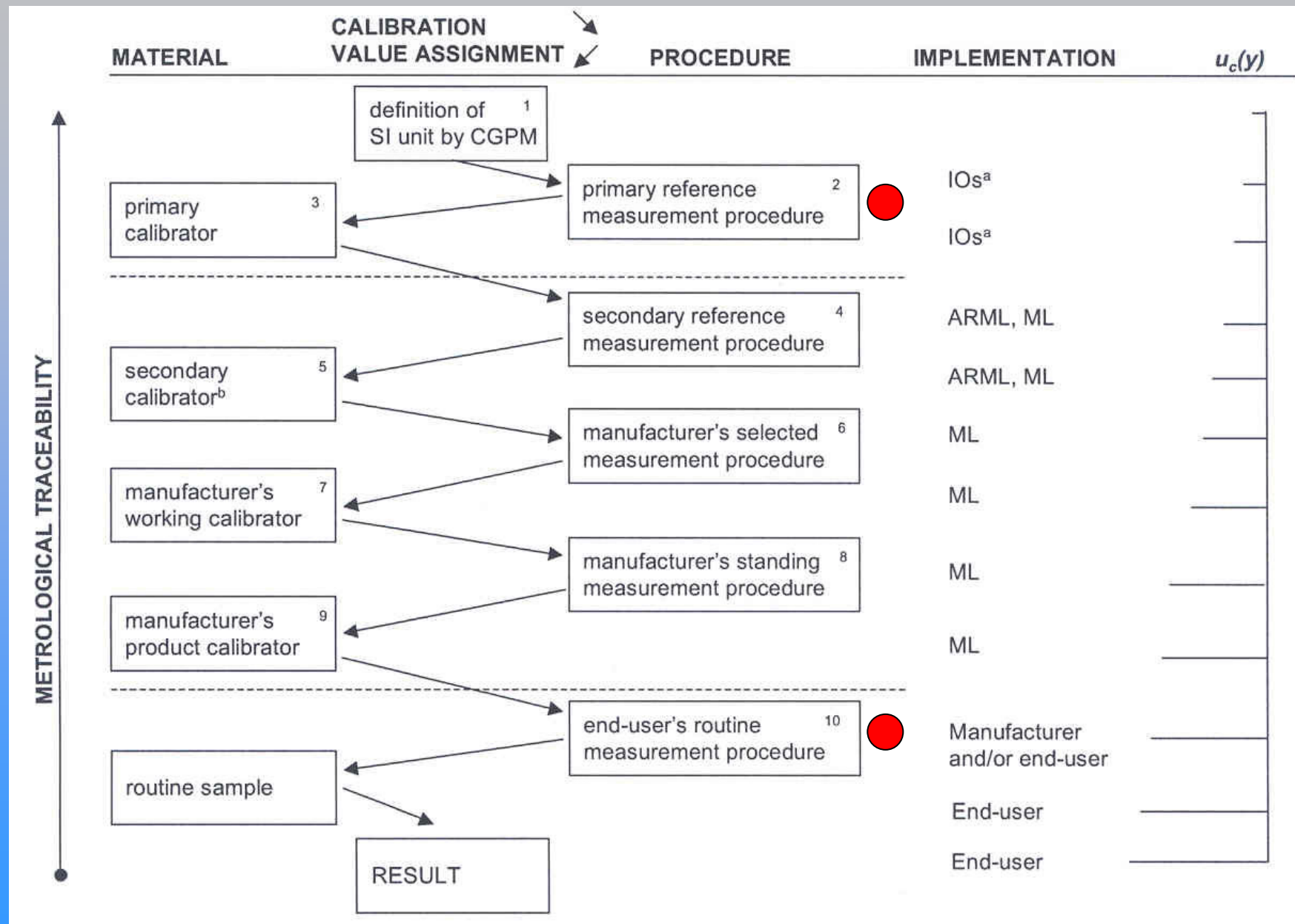
Primary reference measurement procedure

The enzyme measurand is described by the specified measurement procedure

- kind of substrate and its concentration,
- activators and their concentrations,
- direction of catalyzed reaction,
- indicator component,
- buffer system and pH,
- measurement temperature,
- pre-incubation time,
- material used for starting the reaction,
- lag time (waiting time)
- reaction time (measurement window).

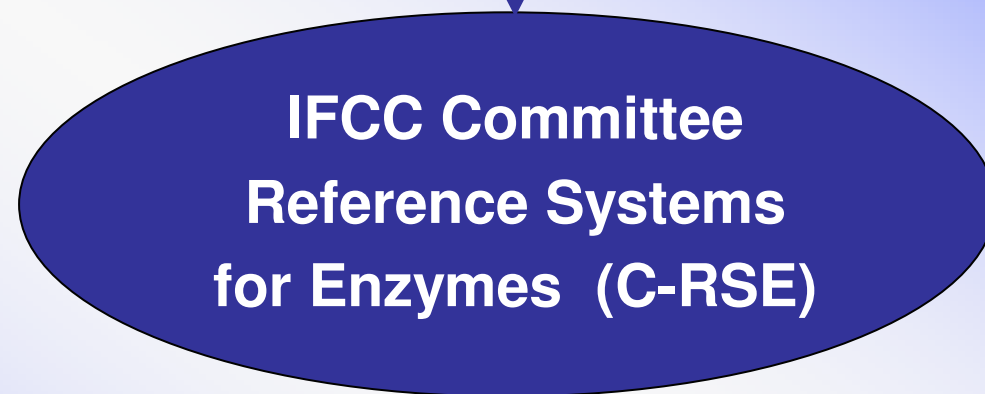
Hierarchy of Procedures and Calibrators

(Figure originally taken from the document ISO 18153)





Internationally accepted standards



Components of a Reference System for Enzymes

- **Reference measurement procedure**
(IFCC-CRSE)
- **Certified reference material**
(IRMM / IFCC)
- **Network of reference laboratories**
(IFCC-CRSE)

The analytical design for the determination of reference method values for enzymes

- ❖ **Standard operating procedure based on the IFCC approved and published primary reference procedure**
- ❖ **Calibration and adjustment of instrument parameters and equipment to the respective target values**
(photometer, pH-meter, thermometer, volumetric devices, etc.)
- ❖ **Control of the entire reference procedures**
(certified reference material, in-house prepared pooled serum with RMV)
- ❖ **Three replicate measurements on four measurement days**
(an additional measurement day if necessary)

Present status of enzyme standardisation

ALT, CK, γ GT, LDH, **AST**

α -Amylase, ALP

>> Lipase <<

CHE, GIDH, α -Amylase_(pancreas-spec.), CK-MB



CERTIFIED REFERENCE MATERIAL
IRMM/IFCC-454 N° 12
CERTIFICATE OF ANALYSIS

ALANINE AMINOTRANSFERASE AS DETERMINED BY THE IFCC METHOD AT 37 °C			
	Certified value ²⁾	Uncertainty ³⁾	Accepted data sets of results (p)
Catalytic concentration of alanine aminotransferase in reconstituted material ¹⁾ as determined by the IFCC method at 37 °C	186 U/L 3.09 µkat/L	4 U/L 0.07 µkat/L	12

The CRM was produced and certified in close co-operation between IRMM and IFCC in the frame of the Working Group for Calibrators in Clinical Enzymology (WG-CCE).

1) The material must be reconstituted according to the specified procedure (see below). Values were converted from U/L into µkat/L by multiplication with 0.01667.
 2) The catalytic concentration was determined by the reference method published by the International Federation of Clinical Chemistry (IFCC). The certified value is the unweighted mean of p unweighted mean values, independently obtained by p laboratories.
 3) Expanded uncertainty according to the Guide to the Expression of Uncertainty in Measurement.

DESCRIPTION OF THE SAMPLES

Each sample is in lyophilised form and equivalent to about 1 ml of a solution of purified lactate dehydrogenase from pig heart. The preparation has been stabilised by incorporation in a matrix consisting of human serum albumin at a mass concentration of 30 g/L. No contamination, as assessed by measurement of their catalytic concentrations, has been detected for the following enzymes: gamma-glutamyltransferase, alkaline phosphatase, acid phosphatase and lactate dehydrogenase. Creatine Kinase and aspartate aminotransferase catalytic concentrations are 0.01 and 0.001 respectively of the alanine aminotransferase catalytic concentration. The material is kept under dry nitrogen in sealed glass ampoules. The residual water mass fraction was found to be below 0.0003.

RECONSTITUTION

1. Take the ampoule out of the freezer and allow reaching room temperature.
2. Tap the vertically positioned ampoule gently to ensure that the lyophilised material is at the bottom of the ampoule.
3. Score the ampoule at the constriction with a sharp file and open, by applying a red-hot glass rod to the score for about 1 s, while holding the ampoule almost horizontally to prevent glass from entering the ampoule.
4. Weigh the ampoule with its contents to the nearest 0.1 mg.
5. Reconstitute by slow addition to the sides of the ampoule of (1.00 ± 0.01) ml distilled water (20-22 °C) with calibrated volumetric equipment. Note the temperature.
6. Weigh the ampoule after adding the water.
7. Seal the ampoule with an inert plastic film, invert several times and mix contents by gentle swirling. Allow to stand at room temperature for 1 hour. During this time, swirl ampoule every 20 minutes.
8. Calculate the volume of water at 20 °C from the weight of the volume taking into account the temperature-dependent density.
9. The catalytic concentration of ALAT must be measured within 4 hours following the reconstitution.

J. Pauwels
 Head of the IRMM Unit
 for Reference Materials

B-2440 Geel
 March 2000
 revised February 2001

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Section of the certificate for ALT



EUROPEAN COMMISSION
DIRECTORATE GENERAL JRC
JOINT RESEARCH CENTRE
IRMM
Institute for Reference Materials and Measurements

CERTIFIED REFERENCE MATERIAL IRMM/IFCC-454 N° . 12 CERTIFICATE OF ANALYSIS

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Metrological uncertainty of measurement ...

- ... is not an analytical error.
- ... is combined with the analytical result.
(like peel and fruit)
- ... normally consists of several components, which have to be identified and investigated.
- ... has to be summarized in an uncertainty budget.
(this is mandatory in reference methodology)
- ... is to quantify according to the *Guide to the Expression of Uncertainty in Measurements* (GUM).

Calculation of the Reference Method Value (RMV)

All correction factors in a model function are set to the value $1 \pm$ uncertainty

Correction factors have a standard uncertainty ...

... resulting from the uncertainty of the calibration procedure and from the uncertainty of the adjustment

or

they are deduced from the experience of the reference laboratory (e.g. c_{lot})

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

The measurement budget contains:

- Mean value and the standard uncertainty of the mean
- **If $c_{xyz} = 1$, in addition only the uncertainties of the correction factors**

Example for the calculation of the measurement uncertainty: ALT

Variable x_i	Value	Parameter	Uncertainty	Reference	Distribution	Standard uncertainty (Parameter)	Sensitivity coefficient	Relative standard uncertainty (x_i)
C_{wl}	1	Wavelength	1 nm	IFCC docu.	Rectangle	0,50 nm	0,11 % per 1 nm	0,05 %
C_{absorb}	1	Absorbance	0,30 %	Specification	Rectangle	0,17 %	1,00 % per 1 %	0,17 %
C_{pH}	1	pH	0,05	IFCC docu.	Rectangle	0,03	0,23 % pro 0.05	0,13 %
C_{temp}	1	Temperature	0,1 °C	IFCC docu.	Rectangle	0,06 °C	5,05 % per 1 °C	0,29 %
C_{konz}	1	Reag. Conc.	1,5 %	IFCC docu.	Rectangle	0,87 %	0,18 % per 1 %	0,15 %
C_{charge}	1	Reag. Lot	--	Experiment	--	1,00 %	1,00 % per 1 %	1,00 %
$C_{vol\ fract}$	1	Vol. fract.	--	Experiment	Normal	0,22 %	1,00 % per 1 %	0,22 %
C_{time}	1	Time	--	Experiment	Rectangle	0,02 %	1,00 % per 1 %	0,02 %
C_{evapor}	1	Evaporation	0,10 %	Experiment	Rectangle	0,06 %	1,00 % per 1 %	0,06 %
C_{aging}	1	Aging (sample)	0,50 %	Experiment	Rectangle	0,29 %	1,00 % per 1 %	0,29 %
C_{lin}	1	Linearity	--	Experiment	Normal	0,30 %	1,00 % per 1 %	0,30 %
$mean^*$	93,6 U/l	Mean of all results	--	Std. error	Normal	0,50 %	1,00 % per 1 %	0,50 %

Relative combined expanded (k = 3.31) measurement uncertainty

4,2 %

Absolute combined expanded (k = 3.31) measurement uncertainty

3.90 U/l

IFCC Primary Reference Procedures for the Measurement of Catalytic Activity Concentrations of Enzymes at 37 °C

IFCC 2002/3

Table 2: Conditions for the measurement of CK

Temperature 37.0 °C ± 0.1 °C

Wavelength 339 nm ± 1 nm

Band width = 2 nm

Light path 10.00 mm ± 0.01* mm

Incubation time 180 s

Delay time 120 s

Measurement interval 120 s

Readings (measurement points) = 6

Change of the temperature of 0,1 °C (37 °C) means a change of the enzyme activity of about 0,5 %

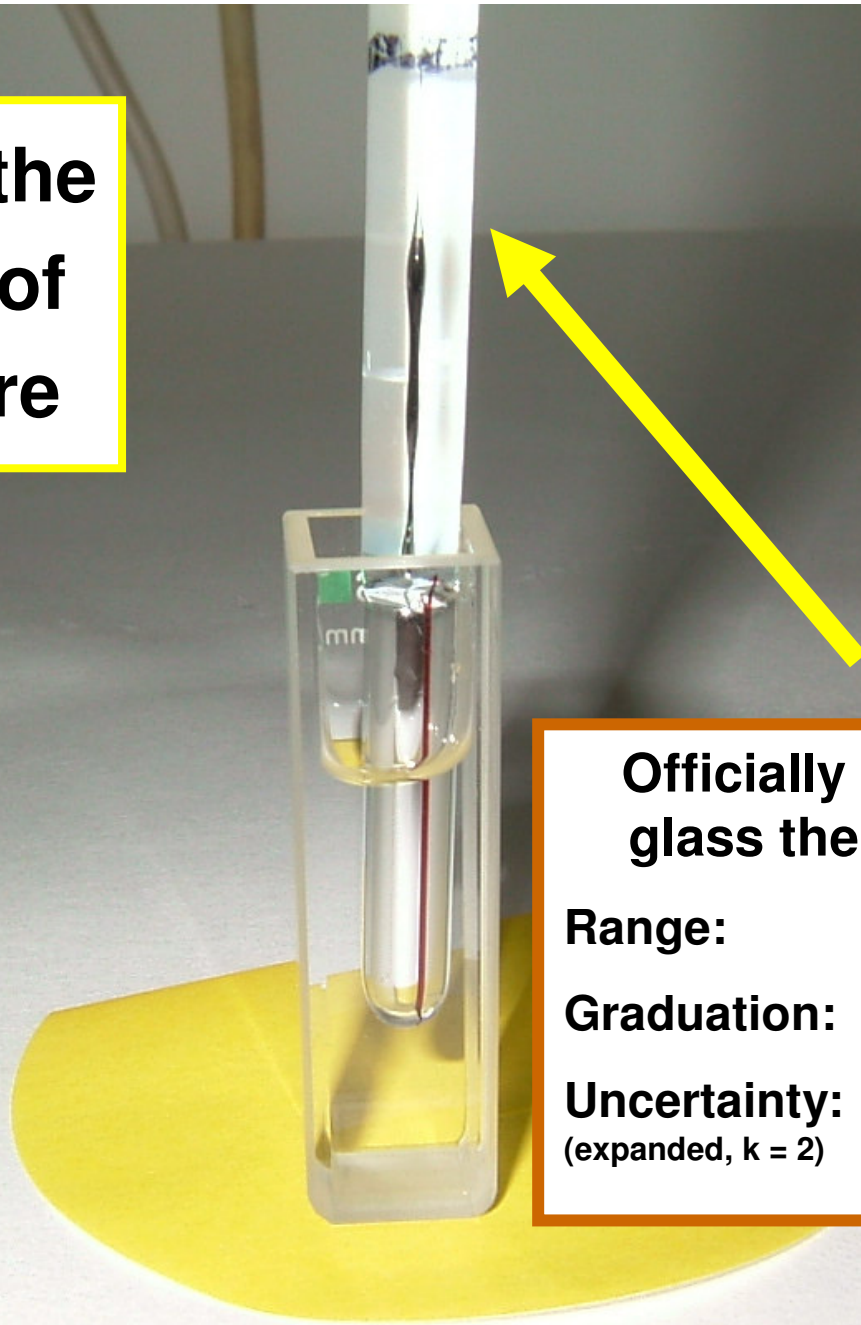
**Traceability of the
measurement of
the temperature**

**Officially calibrated
glass thermometer**

Range: 34 °C - 40 °C

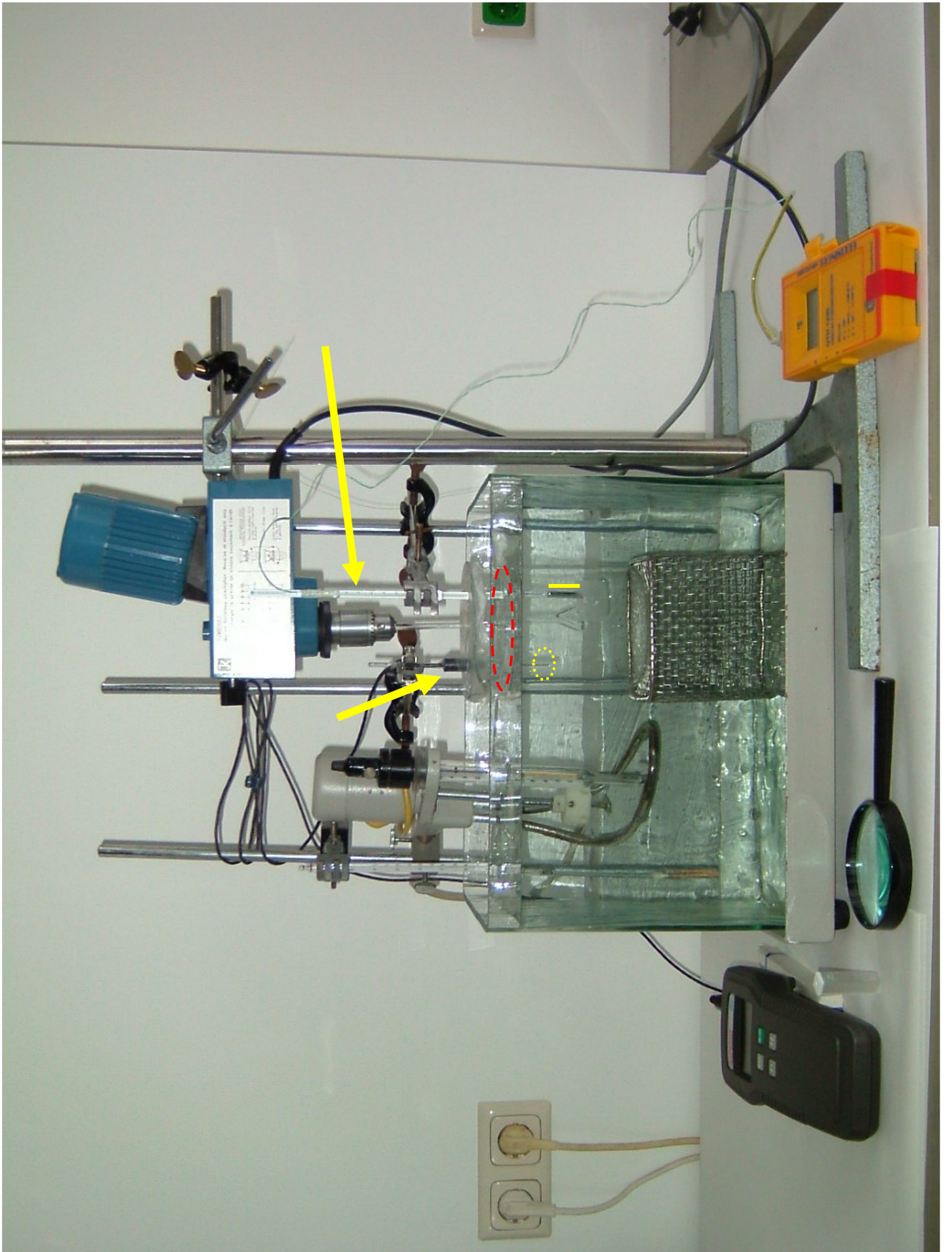
Graduation: 0.1 °C

Uncertainty: 0.02 °C
(expanded, $k = 2$)

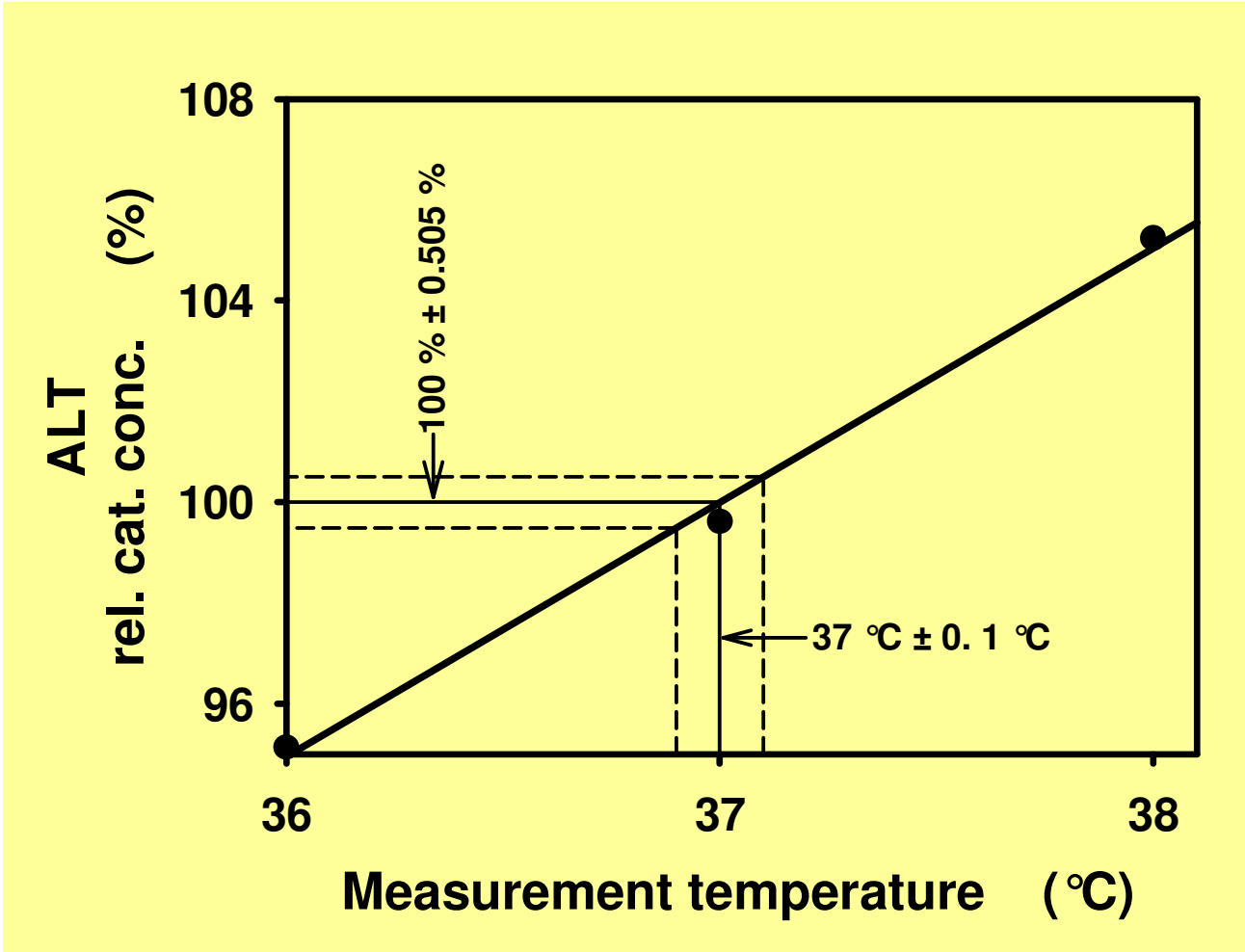




Probe for the digital recording of the temperature in a capped cuvette



Variable x_i	Value	Parameter	Uncertainty	Reference	Distribution	Standard uncertainty (Parameter)	Sensitivity coefficient	Relative standard uncertainty (x_i)
C_{temp}	1	Temperature	0.1 °C	IFCC docu.	Rectangle	0.06 °C	5.05 % per 1 °C \cong 0.505 % per 0.1 °C	0.29 %



The equation for the calculation of the measurement uncertainty

Variable x_i	Value	Parameter	Uncertainty	Reference	Distribution	Standard uncertainty (Parameter)	Sensitivity coefficient	Relative standard uncertainty (x_i)
C_{wl}	1	Wavelength	1 nm	IFCC docu.	Rectangle	0,50 nm	0,11 % per 1 nm	0,05 %
C_{absorb}	1	Absorbance	0,30 %	Specification	Rectangle	0,17 %	1,00 % per 1 %	0,17 %

$$W = \sqrt{\left(3.2 \cdot \sqrt{\underbrace{(w_{wavelength}^2 + w_{absorb}^2 + w_{pH}^2 + w_{temp}^2 + w_{conc}^2 + w_{lot}^2 + w_{time}^2 + w_{evapor}^2 + w_{aging}^2 + w_{lin}^2 + w_{mean}^2)}_{\text{random}}}\right)^2 + \underbrace{w_{vol,fract}^2}_{\text{systematic}}}$$

W: combined expanded uncertainty

w: uncertainties of the respective parameters in the equation for the calculation of the reference method value

C_{aging}	1	Aging (sample)	0,50 %	Experiment	Rectangle	0,29 %	1,00 % per 1 %	0,29 %
C_{lin}	1	Linearity	--	Experiment	Normal	0,30 %	1,00 % per 1 %	0,30 %
$mean^*$	93,6 U/I	Mean of all results	--	Std. error	Normal	0,50 %	1,00 % per 1 %	0,50 %

Relative combined expanded (k = 3.31) measurement uncertainty	4,2 %
Absolute combined expanded (k = 3.31) measurement uncertainty	3.90 U/I

Magnitude of uncertainty components

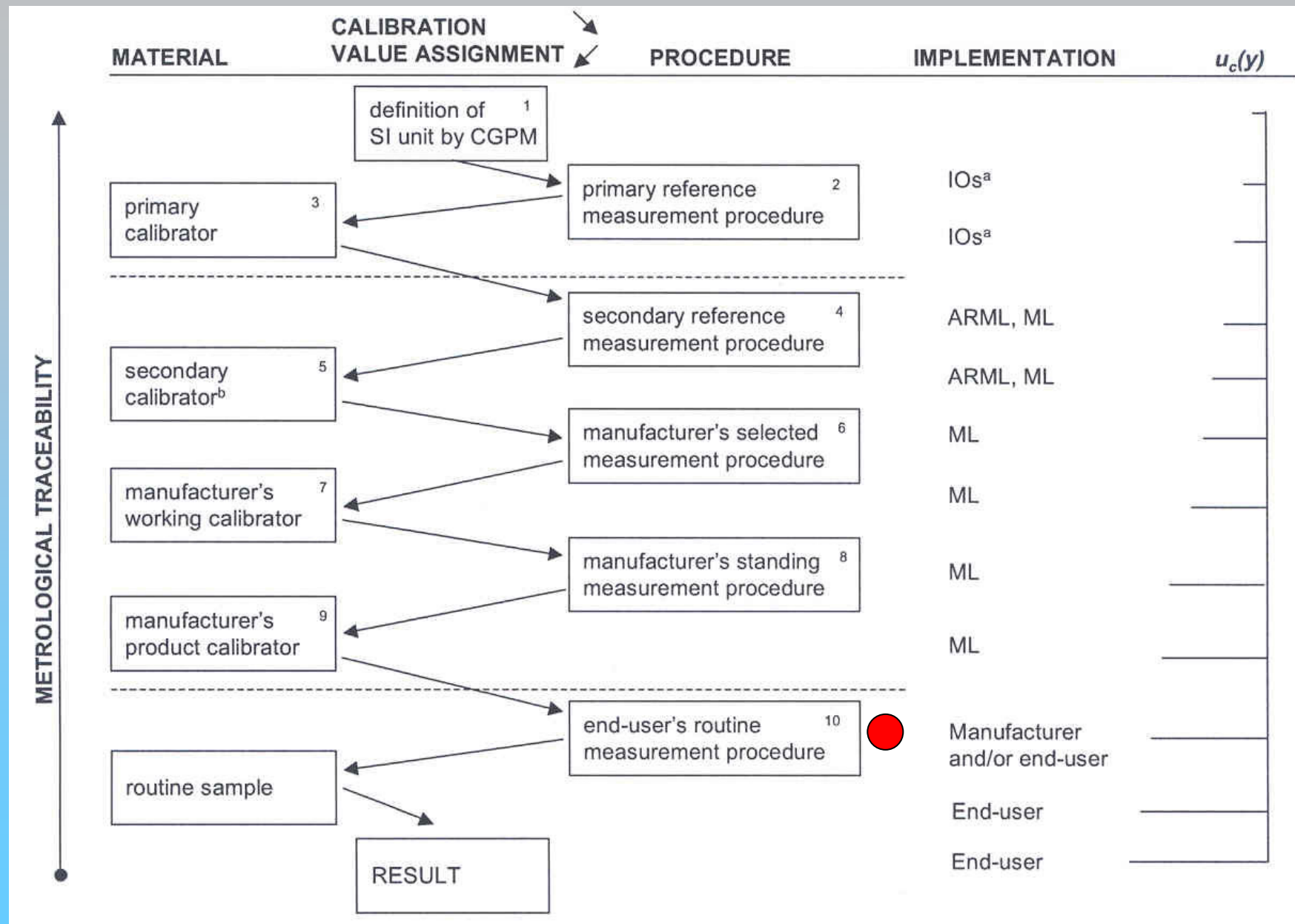


Variiances of the components of uncertainty

Components of uncertainty
(in brackets: rel. stand. uncert.)

Hierarchy of Procedures and Calibrators

(Figure originally taken from the document ISO 18153)



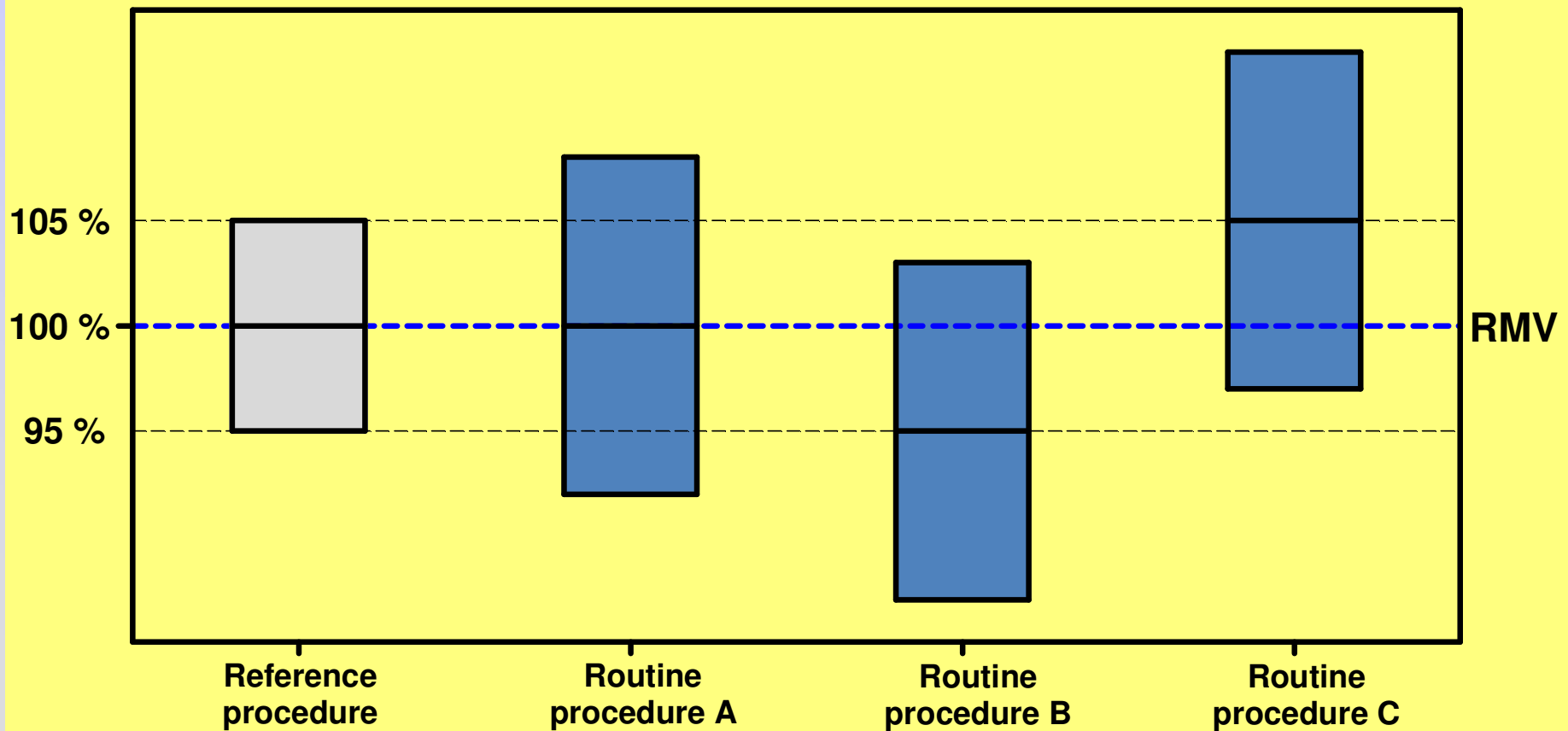
Expectation of the user of a routine procedure for enzyme measurements:

According to the IVD 98/79/EC the following requirements should be fulfill :

The combination of **the parameter setting for the instrument,**
the diagnostics,
the calibrator ...

... shall guarantee adequate traceability to the primary reference procedures.

From routine procedures to the primary reference procedure (Range of accepted and traceable values)



Definition of the term

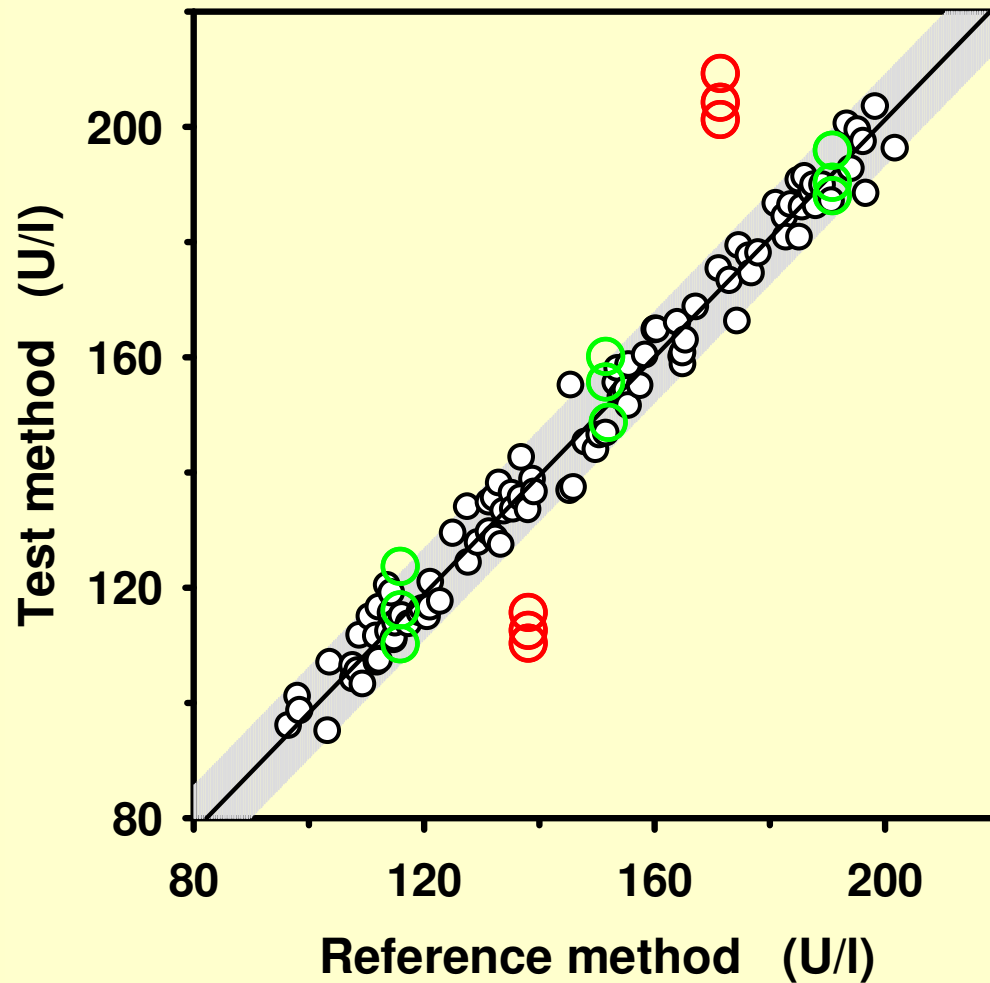
“Commutability of a material“

Closeness of agreement between the mathematical relationship of the measurement results obtained by two measurement procedures for a stated quantity in a given material*, and the mathematical relationship obtained for the quantity in routine samples**.

*) processed material such as a calibrator or a control material

**) serum samples from patients

Commutable or non-commutable?



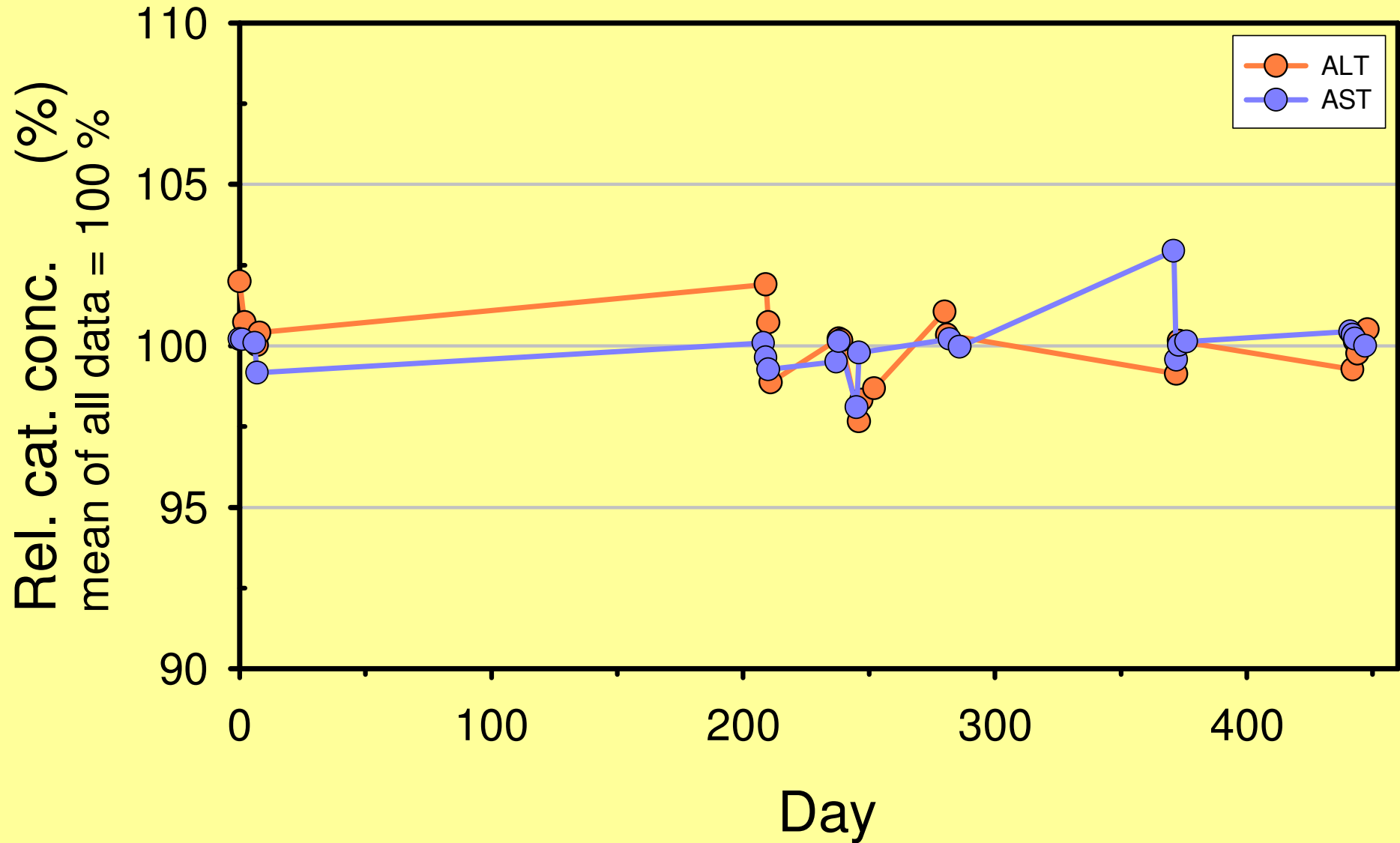
○ Commutable material

○ Non-commutable material

What is a well suited (commutable) sample material for control of the traceability of enzyme measurements in medical routine laboratories?

- ❖ **Pooled human serum stored in aliquots at - 80 °C
(then determination of the reference method value)**
- ❖ **Repeated measurements with the routine procedure together with investigations of the master calibrator.**
- ❖ **Is the master calibrator commutable?
(most of them are not perfectly!)**

Control of the Reference measurement procedure (using pooled human serum, stored at -80 °C)



Control of the commutability of the calibrator C.f.a.s., and control of the traceability

Enzymes: ALT and AST

Calibrator: C.f.a.s. (Calibrator for automated systems)

Roche Diagnostics

Instrument: Modular (P)

Roche Diagnostics

Routine procedure and diagnostics:

Roche Diagnostics

Reference method value

Catalytic concentration of ALT in C.f.a.s.
Lot 168 934-01

Reference laboratory PD Dr. G. Schumann
Manufacturer Roche
Method IFCC primary reference method
Measurement temperature 37 °C
Period of measurements 15.06.05 to 21.06.05

Code no.

Enzyme	Reference method value ¹	Uncertainty of measurement ²	Number of accepted results
ALT	93,6 U/l	3,90 U/l	4 Days 12 Single values
	1,560 µkat/l	0,065 µkat/l	

One specimen was reconstituted on each measurement day.

¹⁾ The reference method value is the mean of the means of the results on each measurement day.

²⁾ The uncertainty of measurement is the combined expanded uncertainty.
The coverage factor is $k = 3,31$
The uncertainty takes into account:
Standard error of the mean of the means
Standard uncertainty of the adjustment of the pH value
Standard uncertainty of the photometric measurement
Standard uncertainty of the volume fraction of sample
Standard uncertainty due to influence of the lot of reagents on the result
Standard uncertainty of the adjustment of the temperature in the cuvette

The estimation of the uncertainty was performed according to the "Guide to the Expression of Uncertainty in Measurement".

Note: The day to day imprecision had a coefficient of variation (CV) of 1,1 %.

Hannover, 2005-06-23



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Klinische Chemie
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Tel. 0511/532-2523

Description of the calibrator C.f.a.s.

Calibrator for automated systems

10759350

LOT 168934

2006-09

COMP	MET	INT/Test-ID	HITACHI Systems	CORAS INTEGRA	Unit
ALT/ALAT/GPT <small>Alanine Aminotransferase Alanin-Aminotransferase Alanina-aminotransferase Alanina-aminotransferasi Alanina-aminotransferase</small>	IFCC	with pyridoxal phosphate mit Pyridoxalphosphat avec pyridoxal-phosphat con piridossalfosfato con piridossal fosfato com piridoxal fosfato	0-595 37°C 97.1 1.62	97.0 1.62	U/L µkat/L
	IFCC	without pyridoxal phosphate ohne Pyridoxalphosphat sans pyridoxal-phosphat sen piridossalfosfato senza piridossal fosfato sem piridoxal fosfato	0-485 37°C 93.7 1.56	93.3 1.56	U/L µkat/L
	opt. (DGKC)		37°C 102 ○ 1.70		U/L µkat/L

ALT: 97.1 U/l

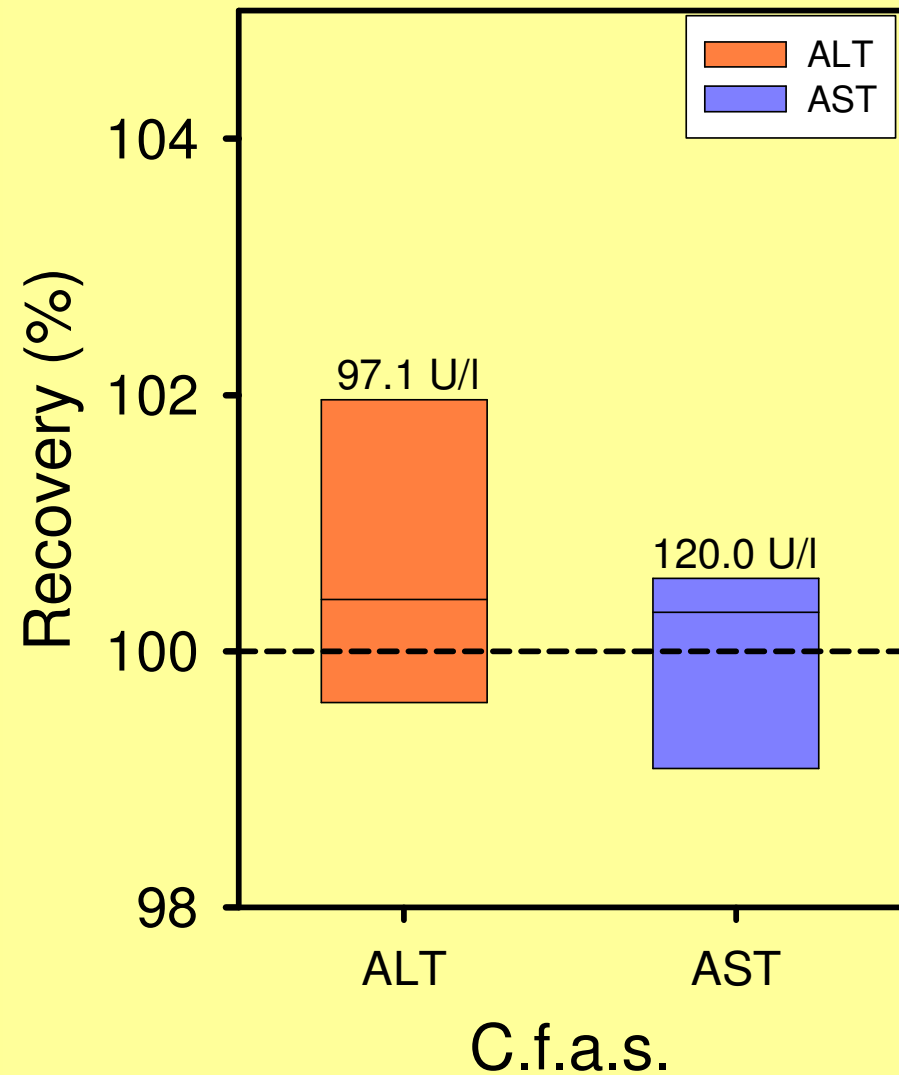
**Calibrator C.f.a.s.
Reference method value *versus* target values**

	ALT	AST
Reference method value	93.6 U/l	114.5 U/l
Target value (Roche Diagnostics)	97.1 U/l	120.0 U/l
Bias	3.7 %	4.8 %

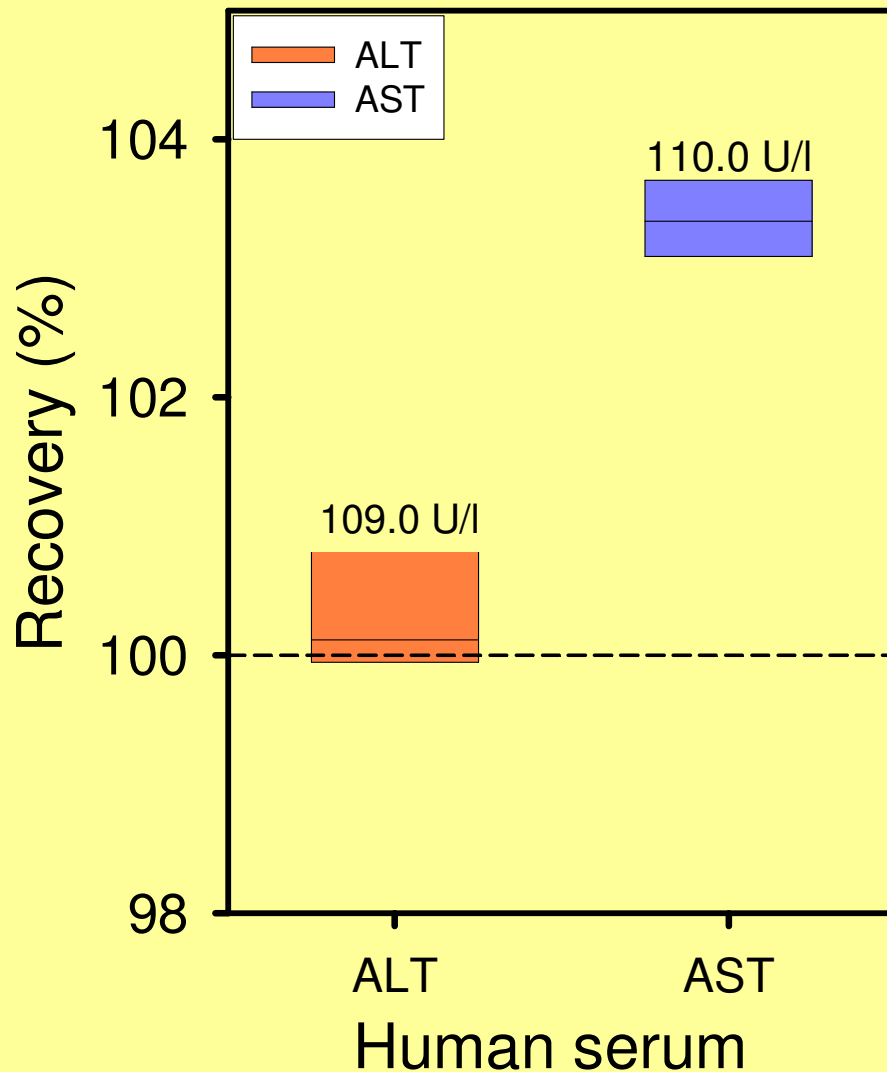
↑ Commutable? ↑

Routine procedure from Roche Diagnostics

Control of the calibration
(100 % recovery as expected)



Routine procedure from Roche Diagnostics

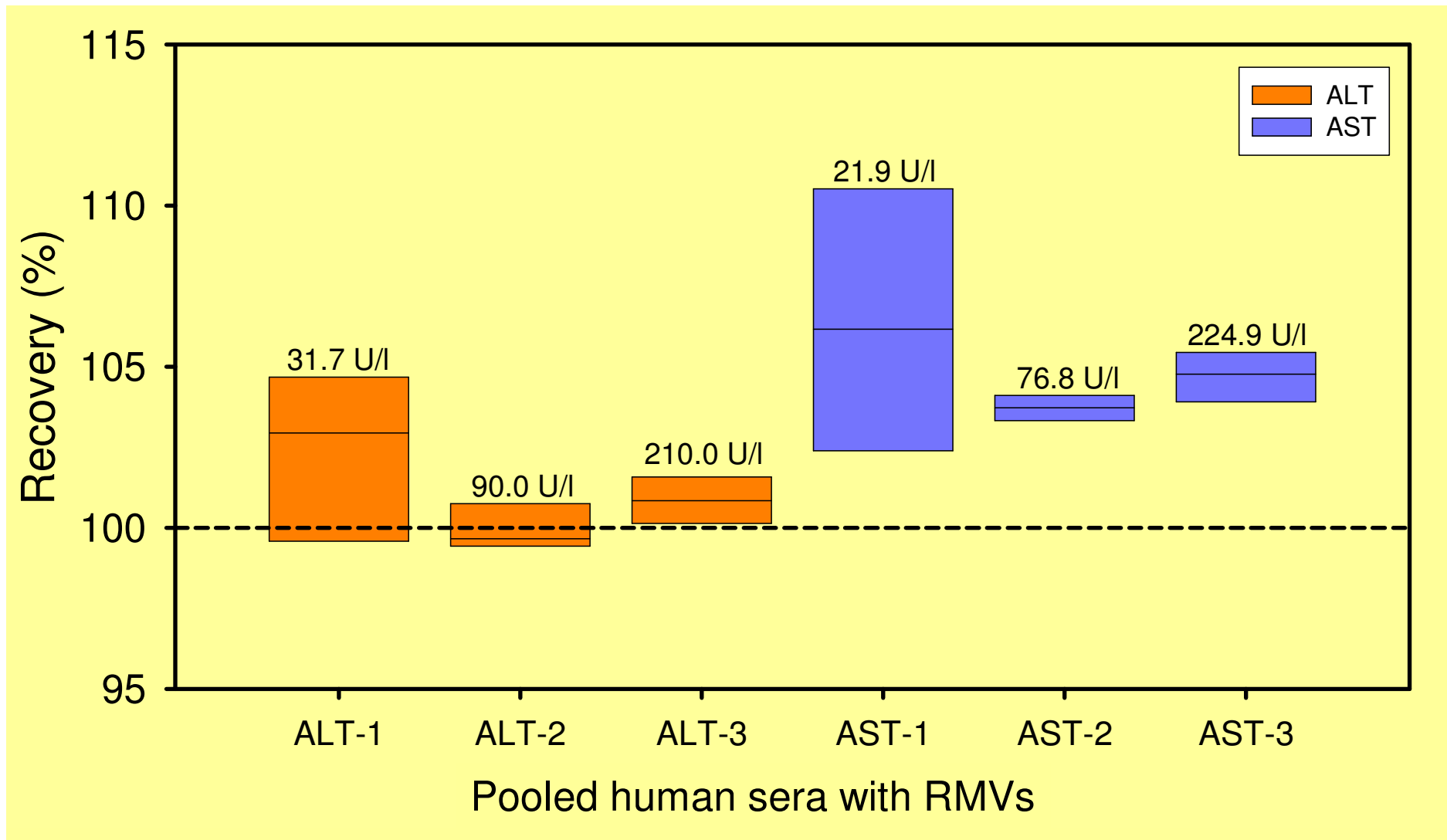


Investigation of pooled human serum, stored at -80 °C.

← 100 % = RMV for ALT and AST

Bias for AST is just acceptable.

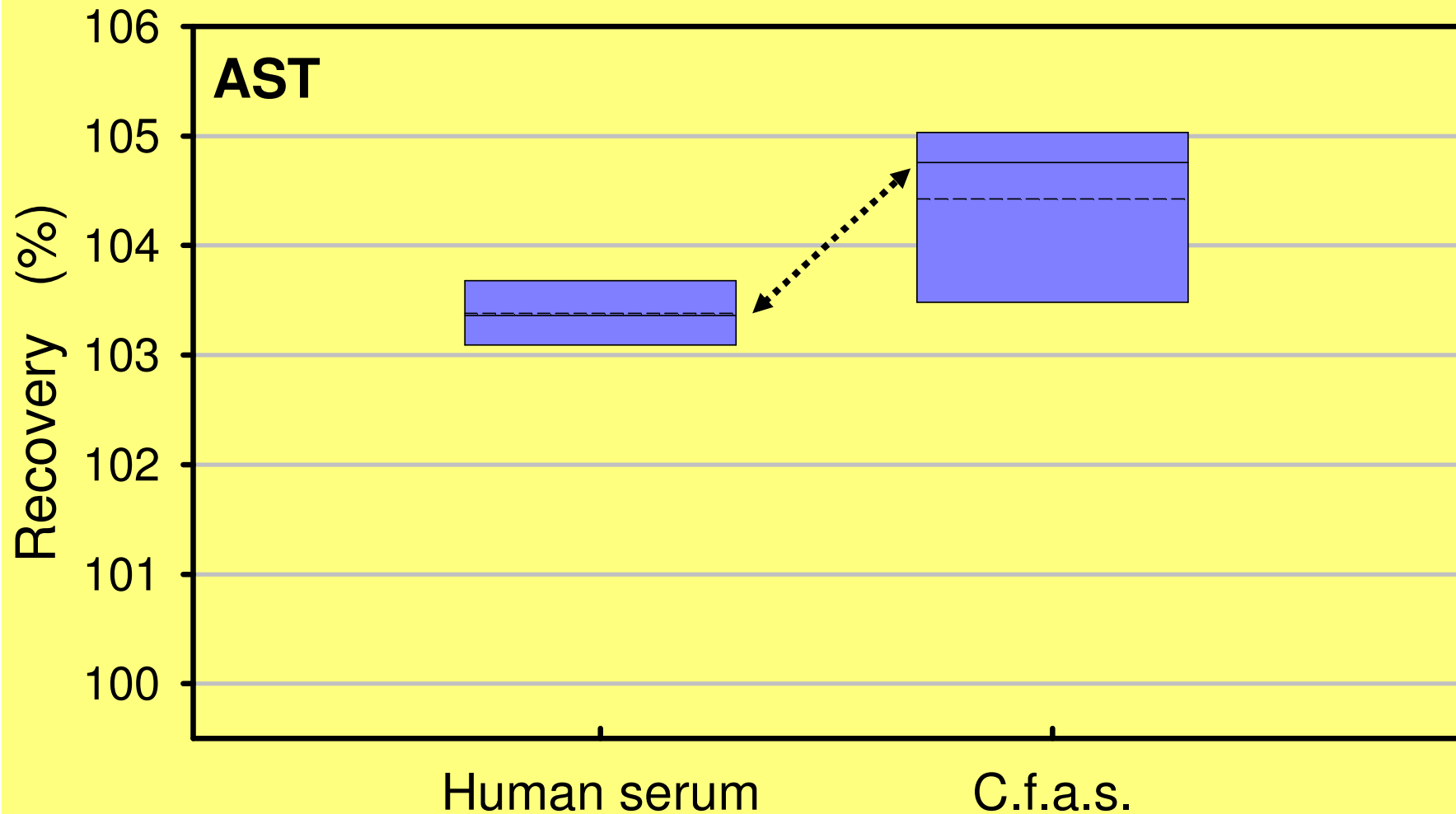
Routine procedures from Roche Diagnostics for ALT and AST



Bias for AST confirmed

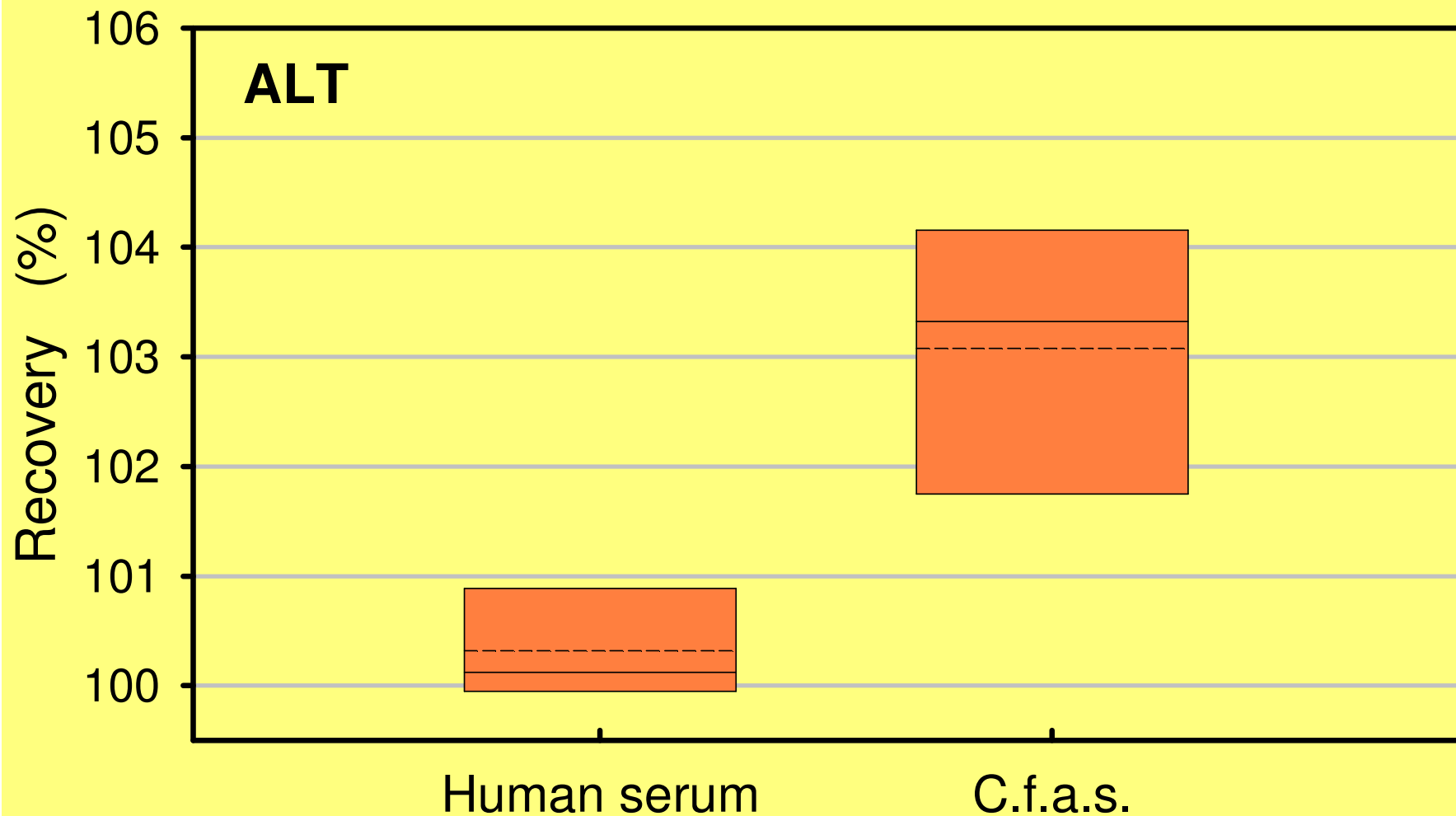
The calibration of the routine procedure from Roche Diagn. should be adjusted.

The commutability of the calibrator C.f.a.s. for AST is very good.



The routine procedure from Roche Diagn. for ALT is very good calibrated.

The commutability of the calibrator C.f.a.s. for ALT is not as good as for AST.



**Calibrator C.f.a.s.
Reference method value *versus* target values**

	ALT	AST
Reference method value	93.6 U/l	114.5 U/l
Target value (Roche Diagnostics)	97.1 U/l	120.0 U/l
Bias	3.7 %	4.8 %

+ Commutability +++

Conclusion of the experiments

- ❖ Many calibrators and control materials for enzyme measurements are not commutable.
- ❖ Traceability to the primary reference procedure is achieved best by use of deep frozen pooled human serum for calibration.
- ❖ The workload for the recognition of calibration problems and the investigations of the commutability is very complex.
- ❖ Cooperation between reference laboratories and manufacturers is required.

Benefit of the standardization

- ❖ **Well agreeing values over time and space**
 - ❖ **Great economic advantages when uniformly manufactured diagnostics are used world wide.**
 - ❖ **Standardization is a valid background to undergo studies for the determination of reference values and decision limits.**
- 