

ESTABLISHING MEASUREMENT TRACEABILITY IN CLINICAL ENZYMOLOGY

Lothar Siekmann

Institute of Clinical Biochemistry, University of Bonn



Scientific Division
Committee on Reference Systemes for Enzymes



Reference Institute of Bioanalysis

In Vitro Diagnostica Directive

European Union

“.... the traceability of values assigned to calibrators and control materials must be assured through available reference measurement procedures and/or reference materials of higher order ...”

Commission of the European Union

mandated standards:

EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

prEN ISO 17511

2000

English version:

In vitro diagnostic medical devices - Measurement of quantities in samples of biological origin - Metrological traceability of values assigned to calibrators and control materials

EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

prEN ISO 18153

2000

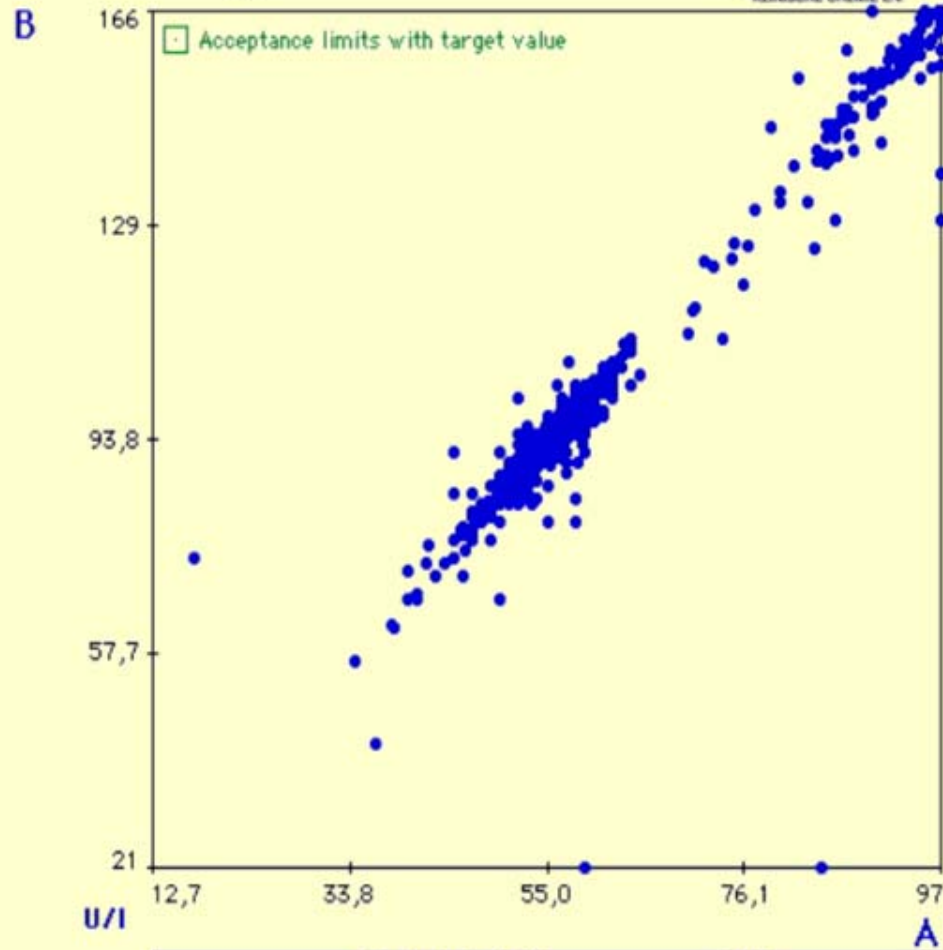
English version:

In vitro diagnostic medical devices - Measurement of quantities in samples of biological origin - Metrological traceability of values for catalytic concentration of enzymes assigned to calibrators and control materials

Gamma-GT

All methods

Kit: All, Split: 1

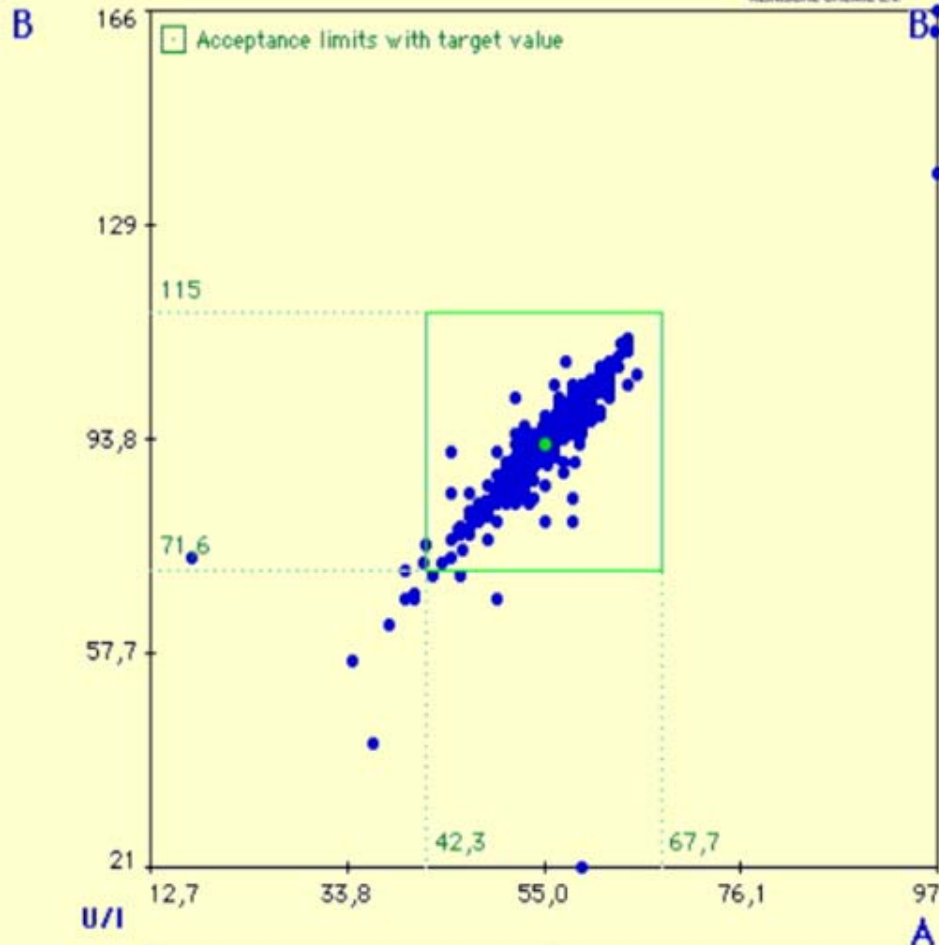


| | | |
|----------------------------|------|------|
| Participants (all) | 706 | |
| Target value (Sv) | | |
| Mean | 59,3 | 100 |
| Standard deviation | 12,9 | 22,0 |
| Coefficient of variation % | 21,8 | 21,9 |

Gamma-GT

Method: 1 - Szasz, 1974 25 C

Kit: All, Split: 1

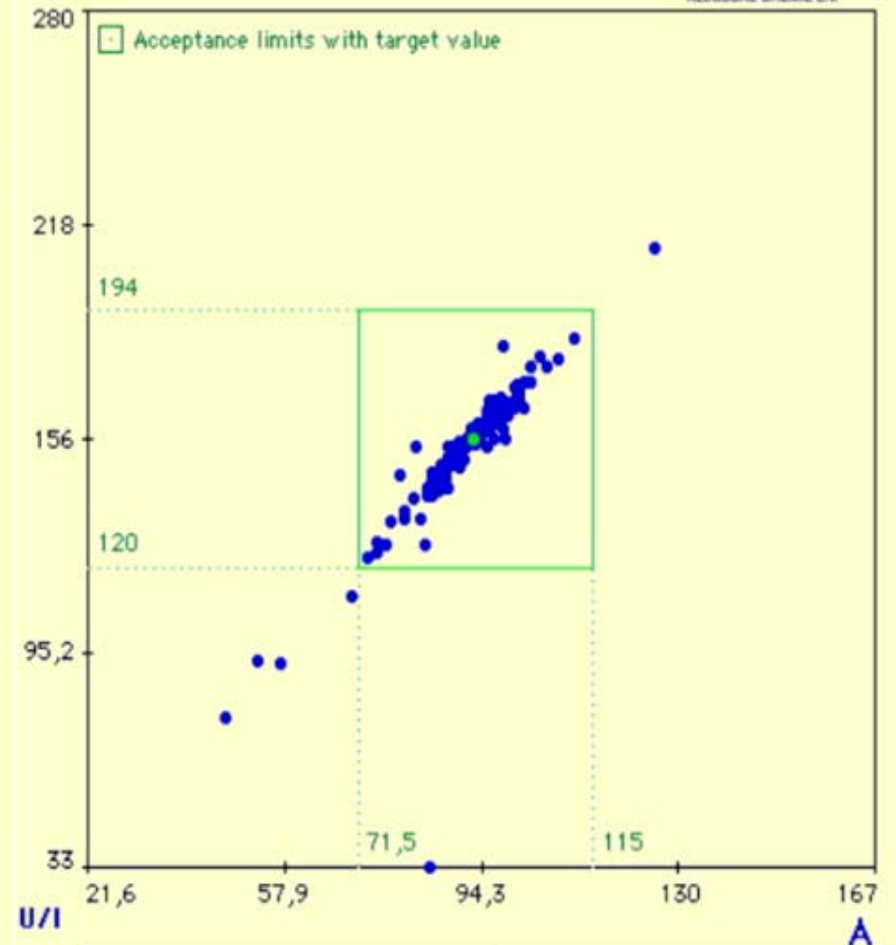


| | | |
|--------------------------------|----------|----------|
| Participants (all) | 548 | |
| Target value (S _Y) | A = 55,0 | B = 93,0 |
| Mean | 54,5 | 92,4 |
| Standard deviation | 4,95 | 8,41 |
| Coefficient of variation % | 9,08 | 9,10 |

Gamma-GT

Method: 2 - Szasz, 1974 37 C

Kit: All, Split: 1

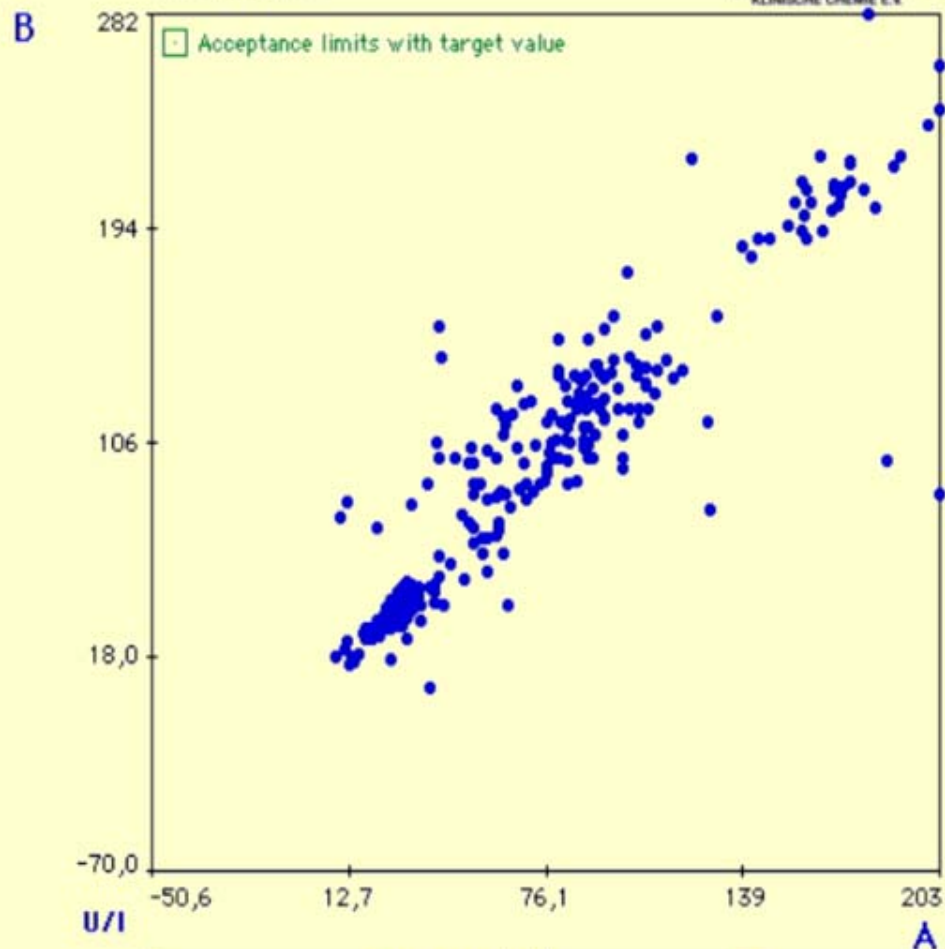


| | | |
|--------------------------------|----------|---------|
| Participants (all) | 124 | |
| Target value (S _Y) | A = 92,9 | B = 157 |
| Mean | 91,3 | 155 |
| Standard deviation | 10,1 | 17,5 |
| Coefficient of variation % | 11,0 | 11,3 |

Lipase

All methods

Kit: All, Split: 1

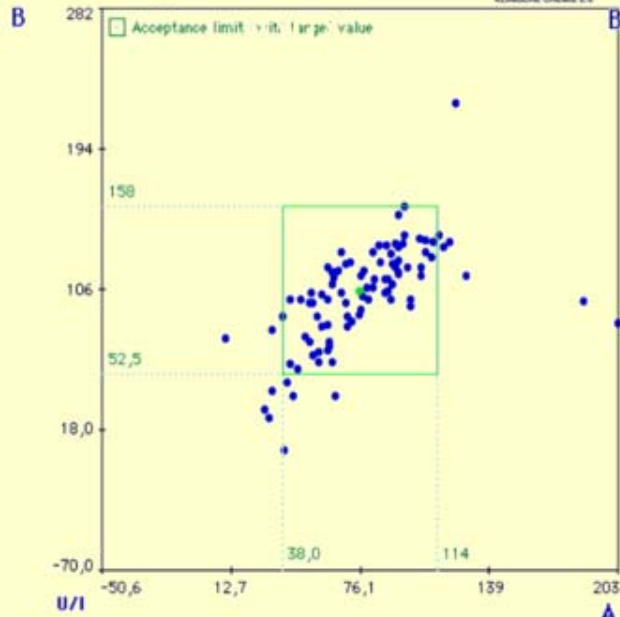


| | | |
|----------------------------|------|------|
| Participants (all) | 535 | |
| Target value (S/W) | | |
| Mean | 49,2 | 65,7 |
| Standard deviation | 38,6 | 48,9 |
| Coefficient of variation % | 78,4 | 74,4 |

KS2/02: Clinical chemical analytes in serum (wet chemistry)

Lipase

Method: 1 - Trübungstest, 25 C
Kit: All, Split: 1

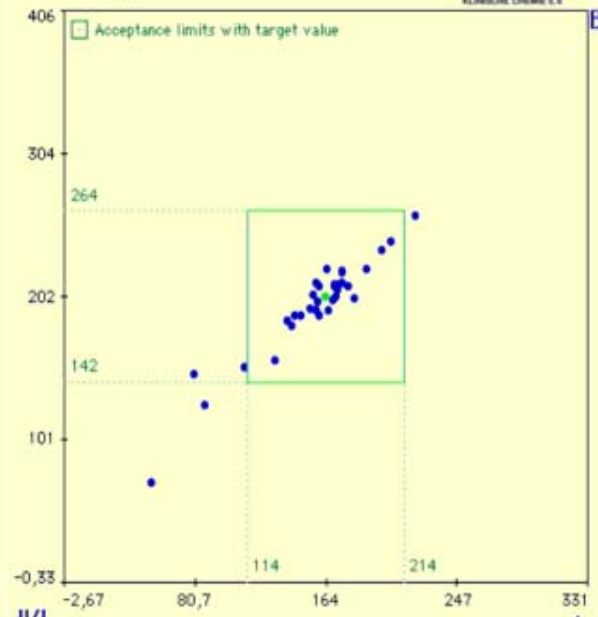


| | | |
|----------------------------|----------|---------|
| Participants (all) | 96 | |
| Target value (Sv) | A = 76,0 | B = 105 |
| Mean | 76,2 | 103 |
| Standard deviation | 27,3 | 32,2 |
| Coefficient of variation % | 35,8 | 31,3 |

KS2/02: Clinical chemical analytes in serum (wet chemistry)

Lipase

Method: 5 - Farbtest, MRE, 37 C
Kit: All, Split: 1

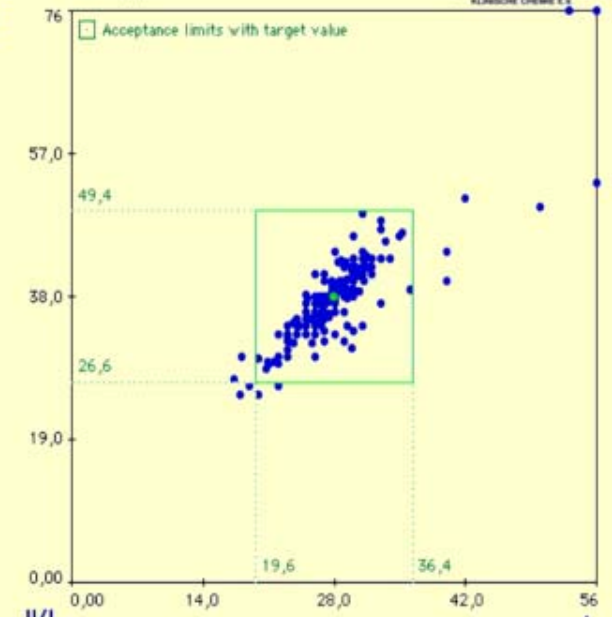


| | | |
|----------------------------|---------|---------|
| Participants (all) | 33 | |
| Target value (Sv) | A = 164 | B = 203 |
| Mean | 157 | 197 |
| Standard deviation | 34,2 | 34,9 |
| Coefficient of variation % | 21,8 | 17,7 |

KS2/02: Clinical chemical analytes in serum (wet chemistry)

Lipase

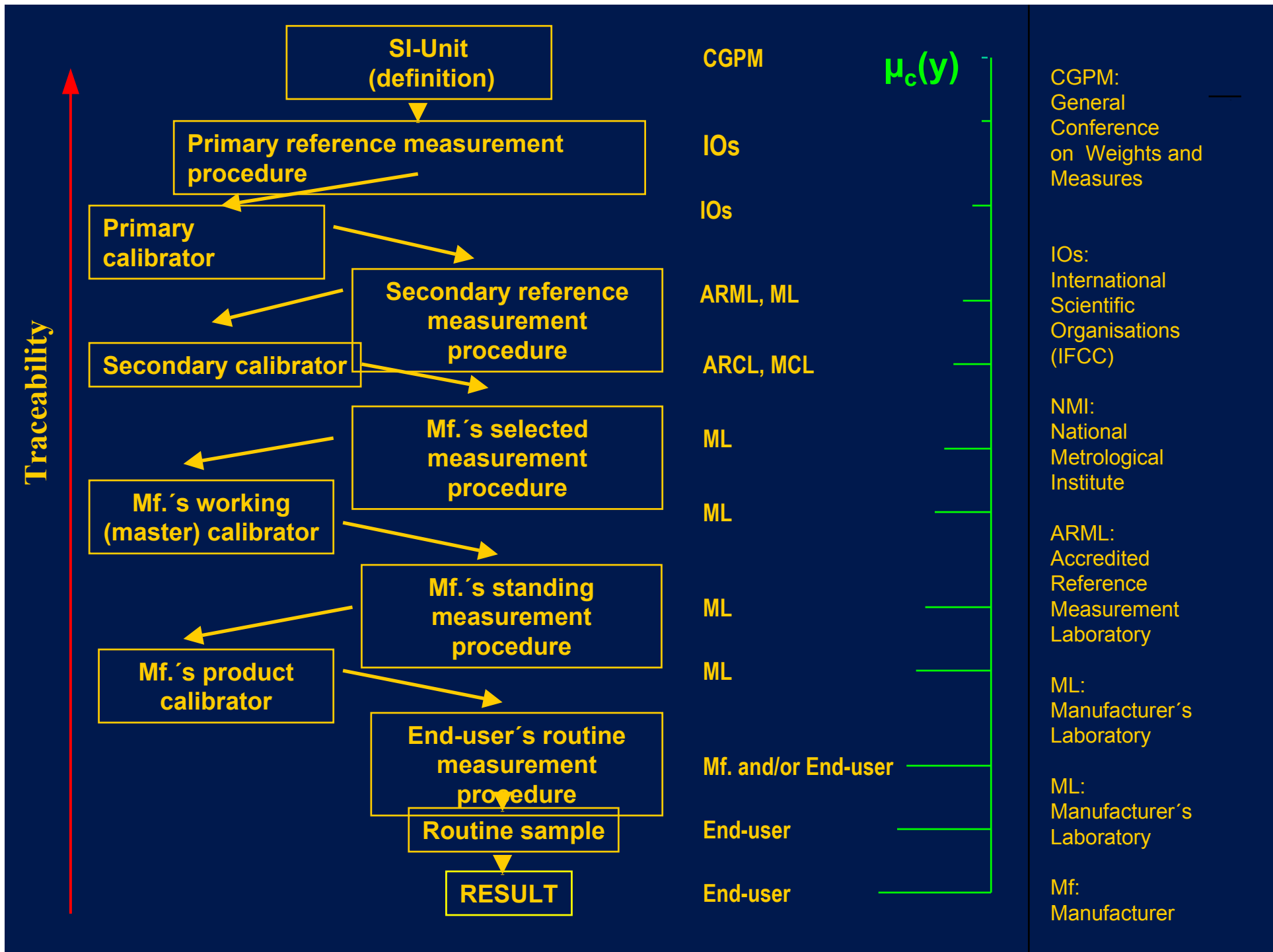
Method: 7 - Farbtest, Colorimetrisch
Kit: All, Split: 1



| | | |
|----------------------------|----------|----------|
| Participants (all) | 303 | |
| Target value (Sv) | A = 28,0 | B = 38,0 |
| Mean | 27,8 | 37,6 |
| Standard deviation | 3,44 | 4,09 |
| Coefficient of variation % | 12,4 | 10,9 |

Measurement Traceability

property of the result of a measurement or the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons all having stated uncertainties



ENZYMES

- **are proteins which catalyze chemical reactions in living cells,**
- **are measured in human body fluids for detection and monitoring of various diseases,**
- **exist in multiple forms (chemical or conformational structure, glycosylation),**
- **exhibit catalytic activities, which strongly depend on the measurement conditions.**

Aspects of Traceability in Clinical Enzymology (1)

- To which level in the traceability chain can results of measurement of catalytic concentrations of enzymes be traceable ?
- to the manufacturer's standing measurement procedure ?
- to a national (secondary) reference procedure ?
- or to the highest level - an internationally accepted primary reference procedure which is a realization of the SI unit "katal" ?

Aspects of Traceability in Clinical Enzymology (2)

- The fact that results depend on the conditions of measurement also applies to other SI-traceable quantities: e.g. the measurement of the length of an iron rod requires at least the knowledge of the temperature.
- The measurand is not the poorly defined protein itself, but one of its well-defined properties: the catalytic activity, which can be expressed as “katal” or “mol/s”.

Definition of Catalytic Activity

Conversion rate of an indicator substance in a specified system according to a given measurement procedure expressed in “katal” which is equivalent to “mol s⁻¹”.

Example:

Rate of conversion of NADH in the IFCC reference procedure for lactate dehydrogenase (LDH).

Reaction:



Aspects of Traceability in Clinical Enzymology (3)

An inevitable precondition for achieving traceability in clinical enzymology is the definition of the measurands by establishing highly optimised, internationally accepted primary reference measurement procedures.

Reference Systems in Clinical Chemistry

- **Reference Measurement Procedures**
- **Reference Laboratories**
- **Reference Materials**

Implementation of a Reference System for Enzymes (1)

- **Decision on primary reference measurement procedures (IFCC committee on enzymes)**
- **Establishing of the reference procedures within a network of reference laboratories according to stringent metrological principles**
- **Selection of commutable reference materials and certification by a network of reference laboratories**

Design of 37°C IFCC Reference Procedures

- IFCC Committee on Enzymes -

- based on existing 30°C IFCC procedures,
- optimised substrate concentration, pH, buffer concentration, lag phase, measuring time interval,
- fixed in exact protocols (standard operating procedures, SOP) prescribing all measurement conditions in detail,
- reporting traceability data (uncertainties) for all relevant steps of the analytical procedures.

37°C IFCC Enzyme Reference Procedures

- IFCC Committee on Reference Systems for Enzymes -

published:

- Alanine aminotransferase (ALT)
- Aspartate amino transferase (AST)
- Creatine kinase (CK)
- Gamma glutamyl transferase (GGT)
- Lactate dehydrogenase (LD)

prepared for publication:

- alpha-Amylase

projected:

- Alkaline phosphatase (ALP)
- Lipase
- Cholinesterase (CHE)

Implementation of a Reference System for Enzymes (2)

- **Decision on primary reference measurement procedures (IFCC committee on enzymes)**
- **Establishing of the reference procedures within a network of reference laboratories according to stringent metrological principles**
- **Selection of commutable reference materials and certification by a network of reference laboratories**

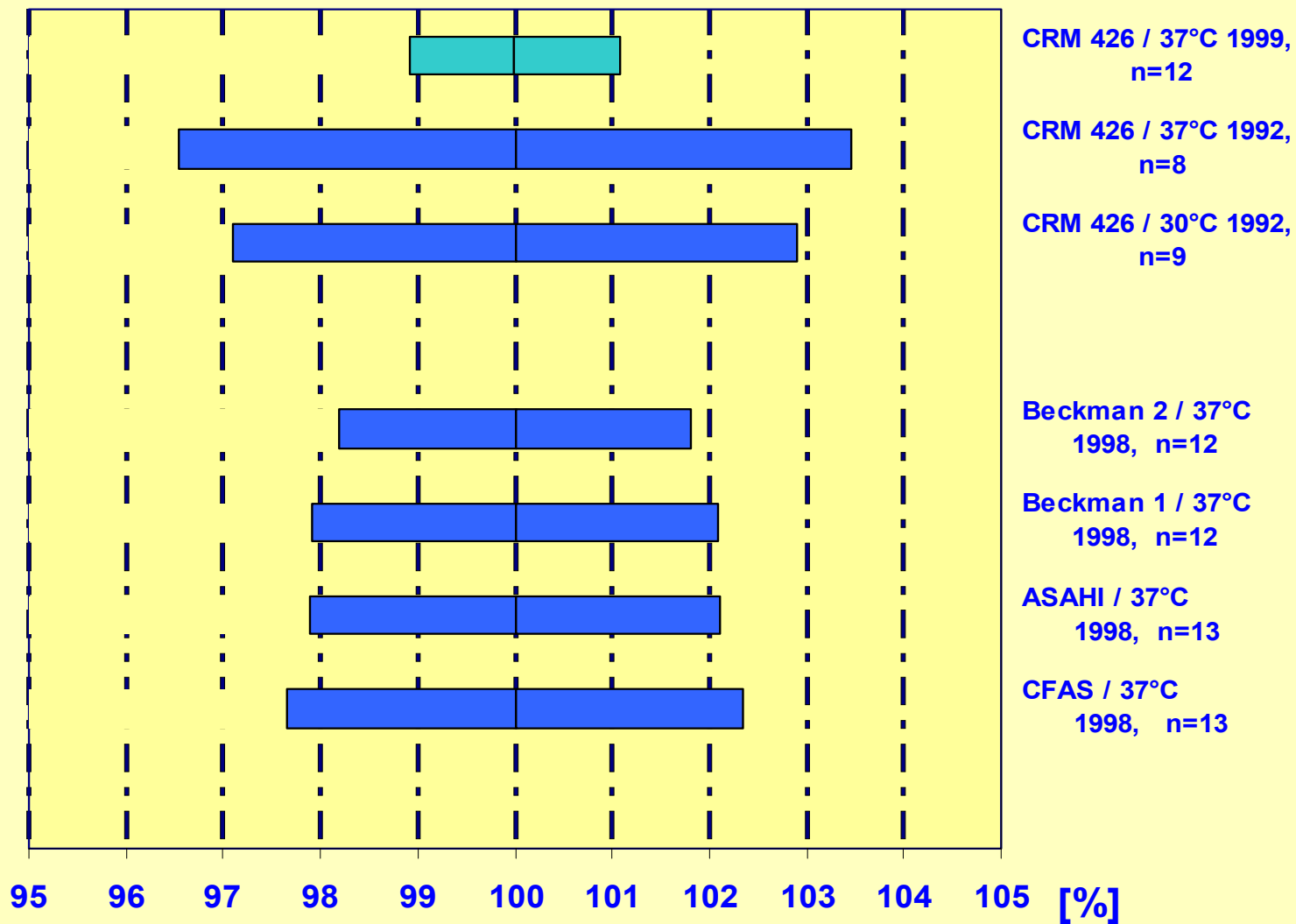
Measurement Conditions in the Network Laboratories

- **Gravimetry controlled by calibrated test weights,**
- **Volumetry controlled by gravimetry,**
- **Temperature controlled by calibrated thermometer,**
- **pH controlled by calibrated equipment,**
- **Photometric wavelength controlled by certified filters or solutions of holmium,**
- **Photometric absorbance checked by test solutions certified by a national metrology institute.**

Implementation of a Reference System for Enzymes (3)

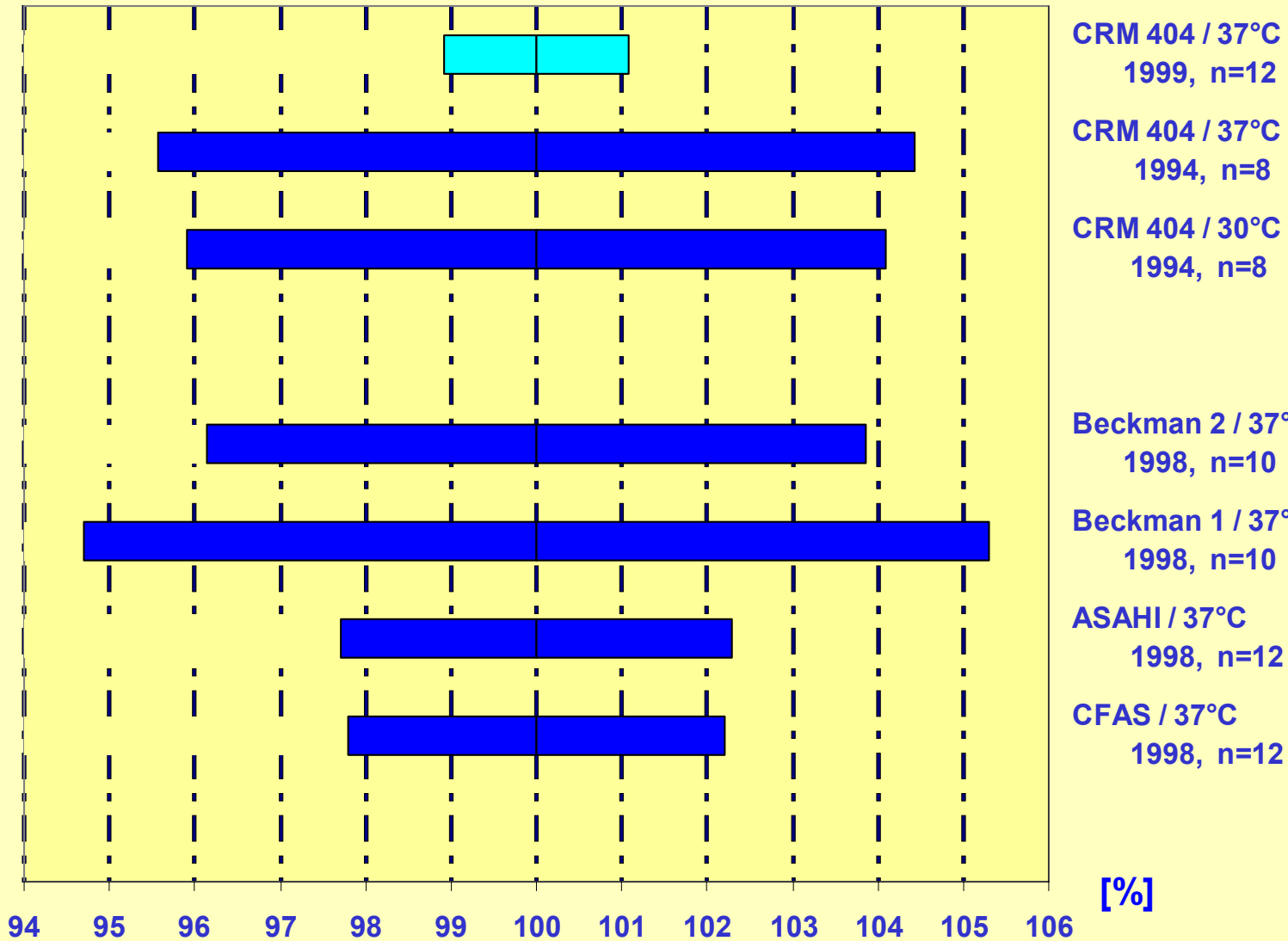
- **Decision on primary reference measurement procedures (IFCC committee on enzymes)**
- **Establishing of the reference procedures within a network of reference laboratories according to stringent metrological principles**
- **Selection and certification of commutable reference materials by a network of reference laboratories**

Alanine aminotransferase (ALT)



Relative Recovery and 95%-Confidence Interval (mean=100%)

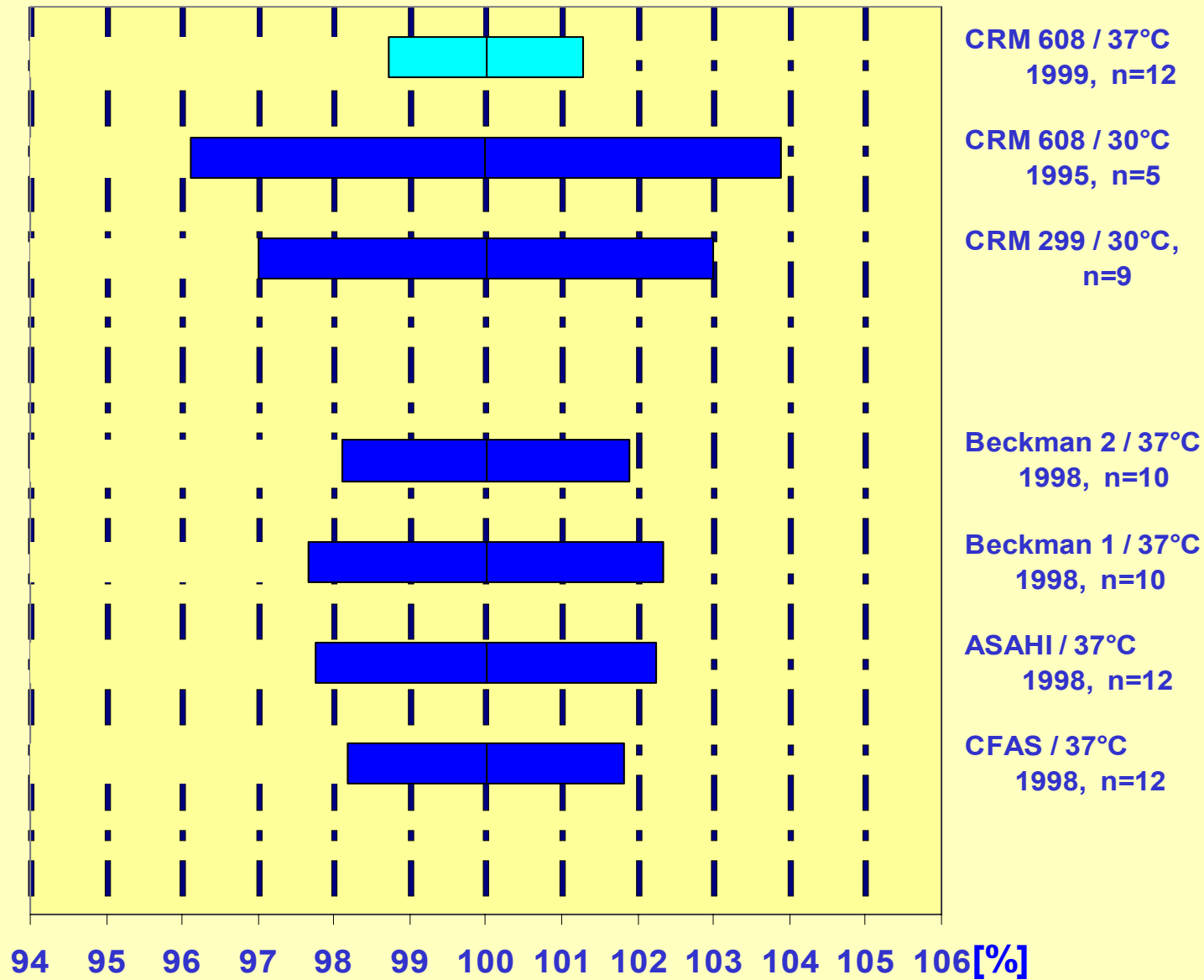
Lactate dehydrogenase (LD)



Relative Recovery and 95%-Confidence Interval (mean=100%)

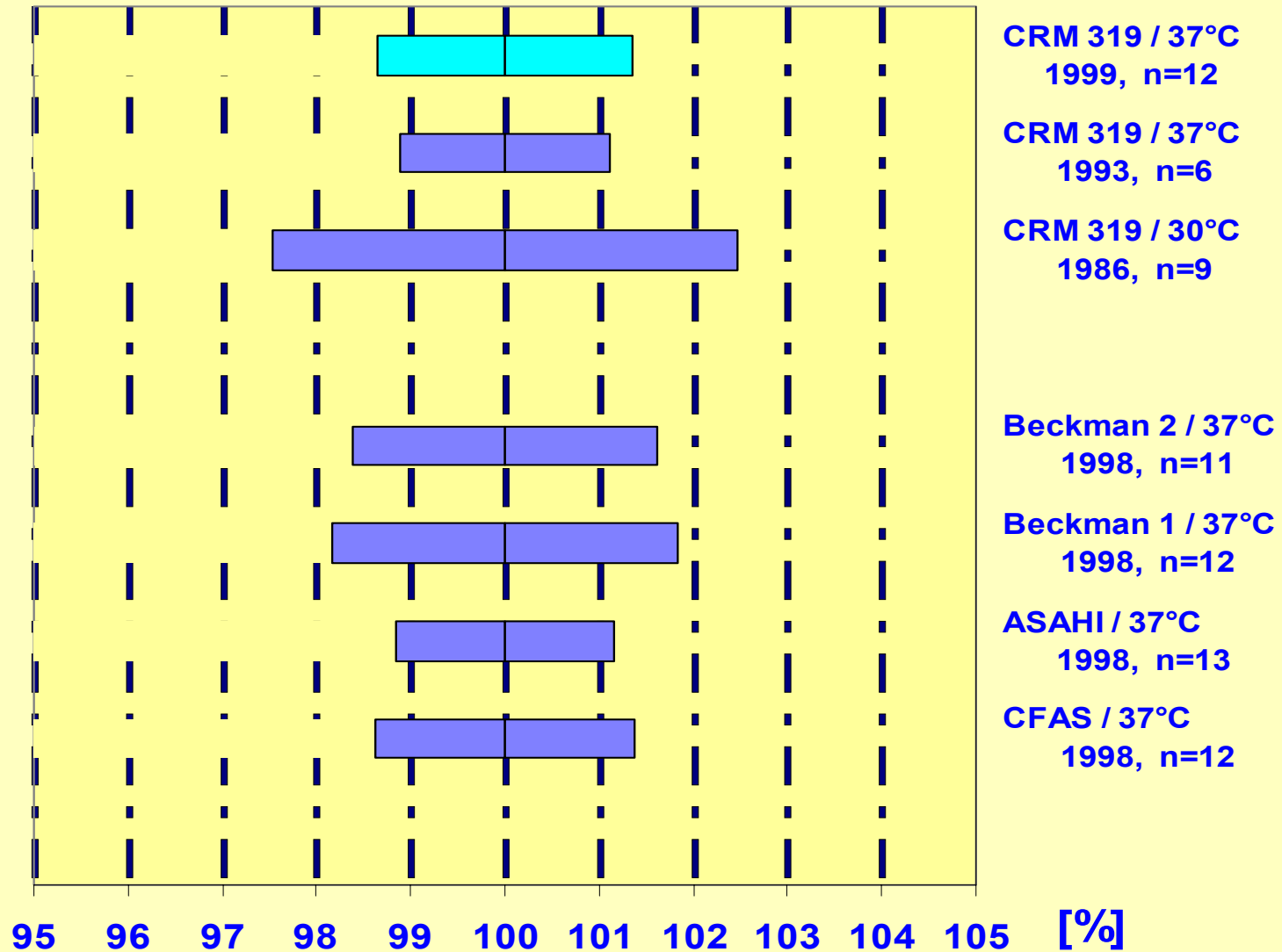
[%]

Creatine kinase (CK)



Relative Recovery and 95%-Confidence Interval (mean=100%)

Gamma glutamyl transferase (GGT)



Relative Recovery and 95%-Confidence Interval (mean=100%)

Network of Enzyme Reference Laboratories

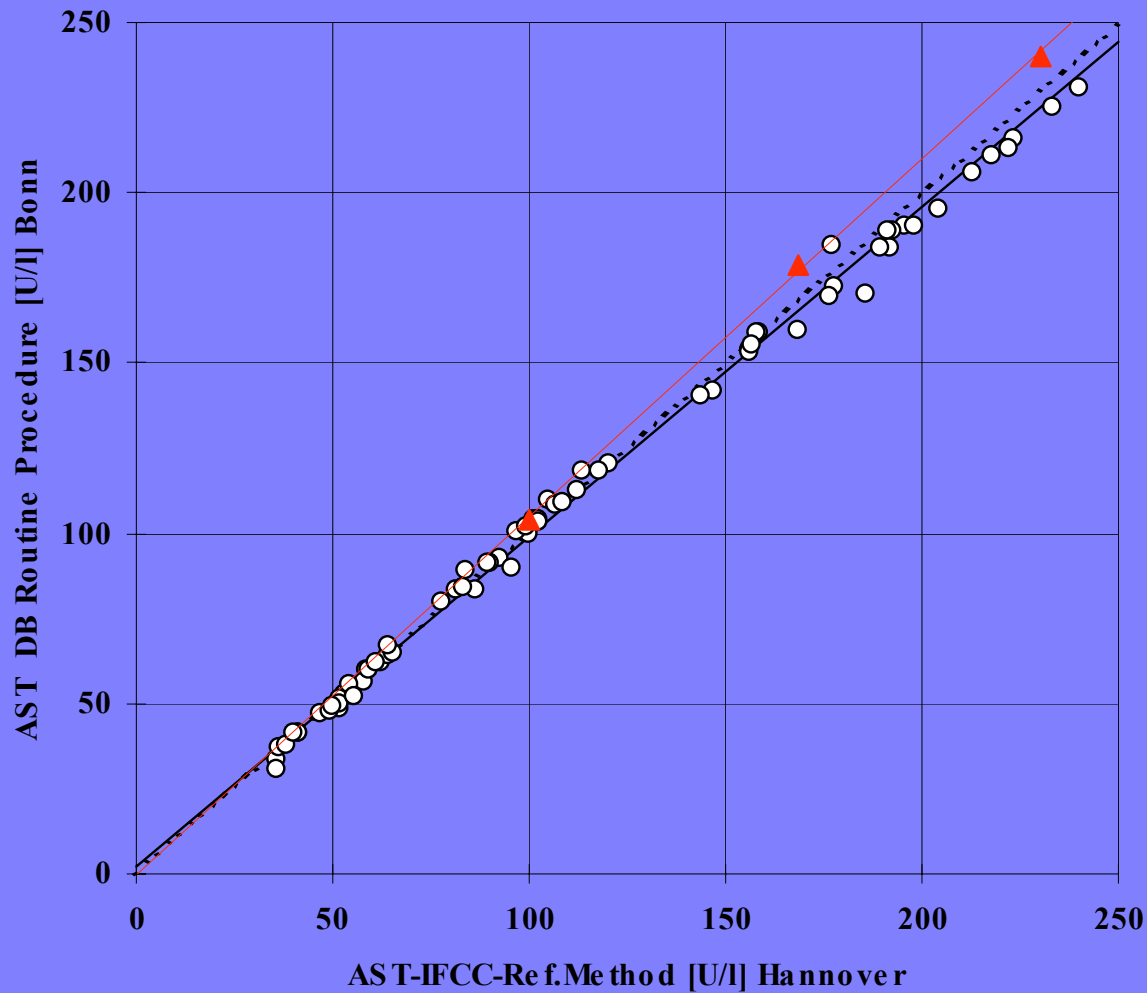
| | | | |
|--------------------------------------|---|------------------------------|------------------------|
| Dr. F. Ceriotti | Istituto Scientifico San Raffaele | Milano | Italy |
| Dr. G. Ehlers | Ortho - Clinical Diagnostics | Rochester | U.S.A. |
| Prof. G.Ferard/ Dr. Lessinger | Centre Traumatologie et Orthopedie | Illkirch Grafenstaden | France |
| Dr. F. H. Franck | Ziekenhuis Leyenburg | Den Haag | The Netherlands |
| Prof. J. Gella | Biosystems S.A. | Barcelone | Spain |
| Prof. W. Hölzel | Roche - Boehringer Mannheim GmbH | Tutzing | Germany |
| Dr. P. Joergensen | Dept. of Clinical Chemistry | Odense | Denmark |
| Prof. T. Kanno | Hamamatsu University Hospital | Hamamatsu | Japan |
| Dr. A. Kessner | Beckman -Coulter, Inc. | Brea, CA | U.S.A. |
| Dr. M. Panteghini | 1 Laboratory of Clinical Chemistry | Brescia | Italy |
| Dr. F. Schiele | Centre du Medecine Preventive | Nancy | France |
| PD Dr. G. Schumann | Med. Hochschule Hannover | Hannover | Germany |
| Dr. A. Vialle | Hopital Debrousse | Lyon | France |
| Dr. G. Weidemann | Klinikum der Stadt Nürnberg | Nürnberg | Germany |
| Dr. K.Yoshinari | ASAHI Chemical Industry Co.,Ltd. | Tokyo | Japan |

Approval of Reference Laboratories

by:

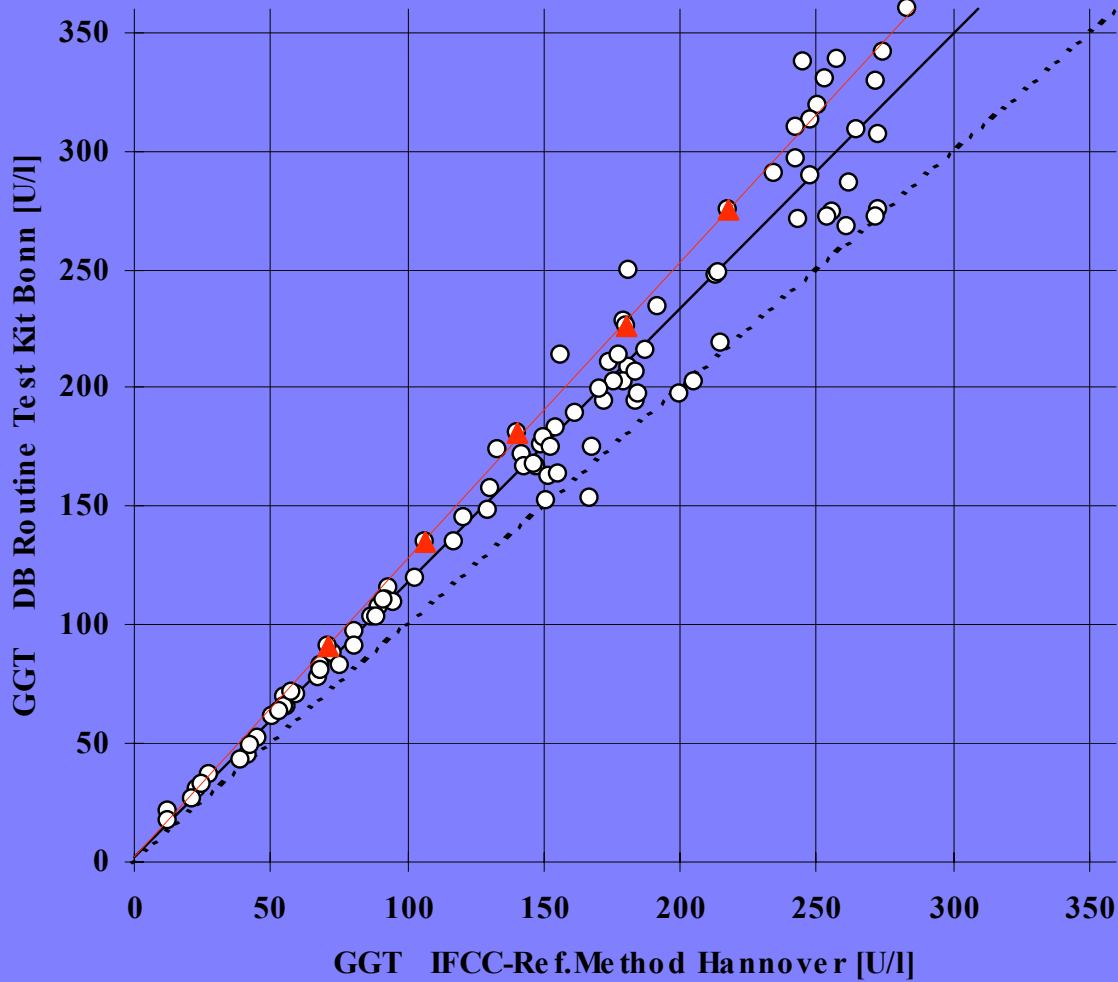
- **establishing a quality management system according to ISO 17025 or prEN/ISO 15195,**
- **regular inspections of the reference laboratory,**
- **regular measurement comparisons using split sample measurements.**

Evaluation of an AST Routine Test Kit



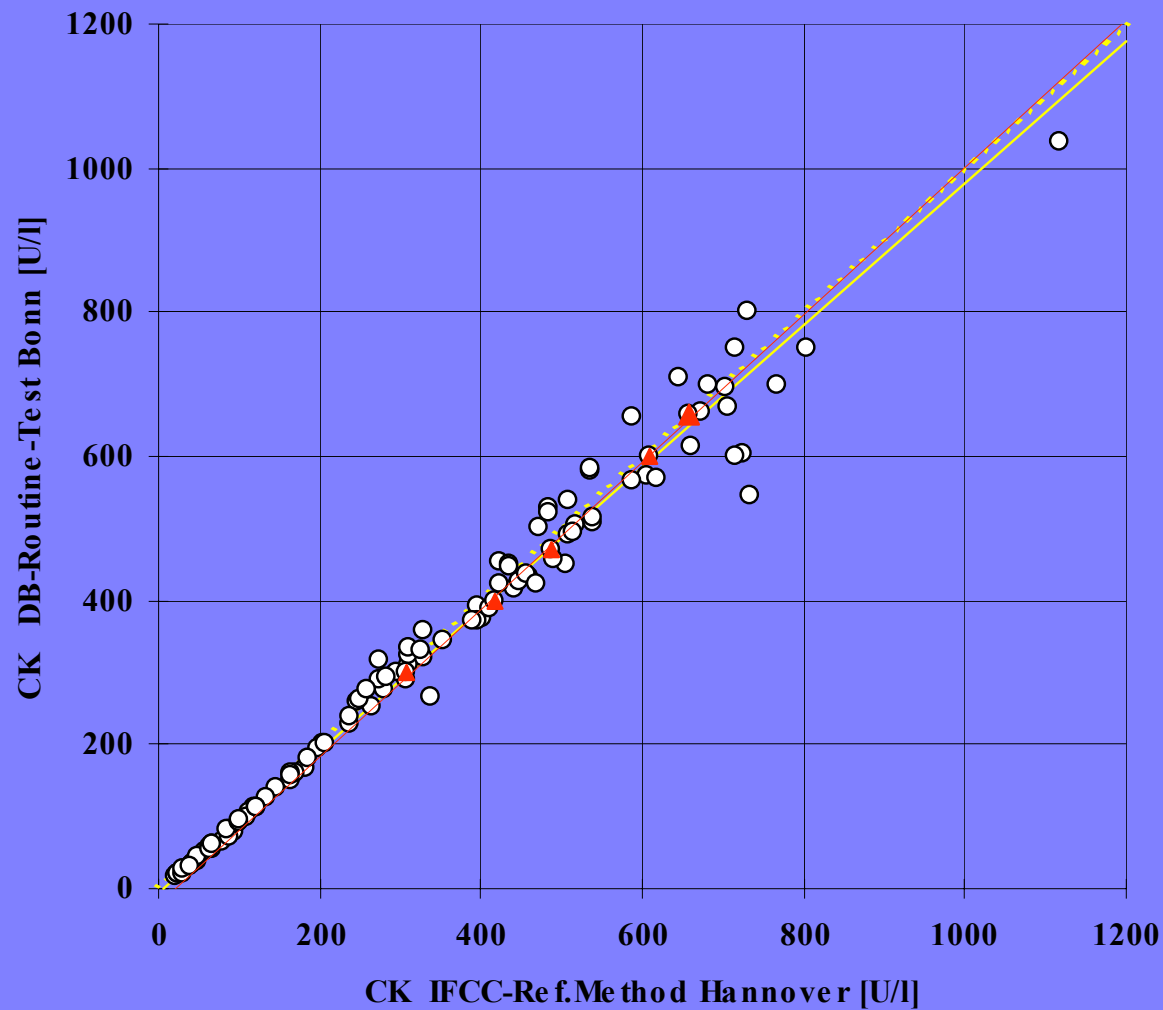
Method Comparison
with the IFCC
Reference Procedure

Evaluation of a GGT Routine Test Kit



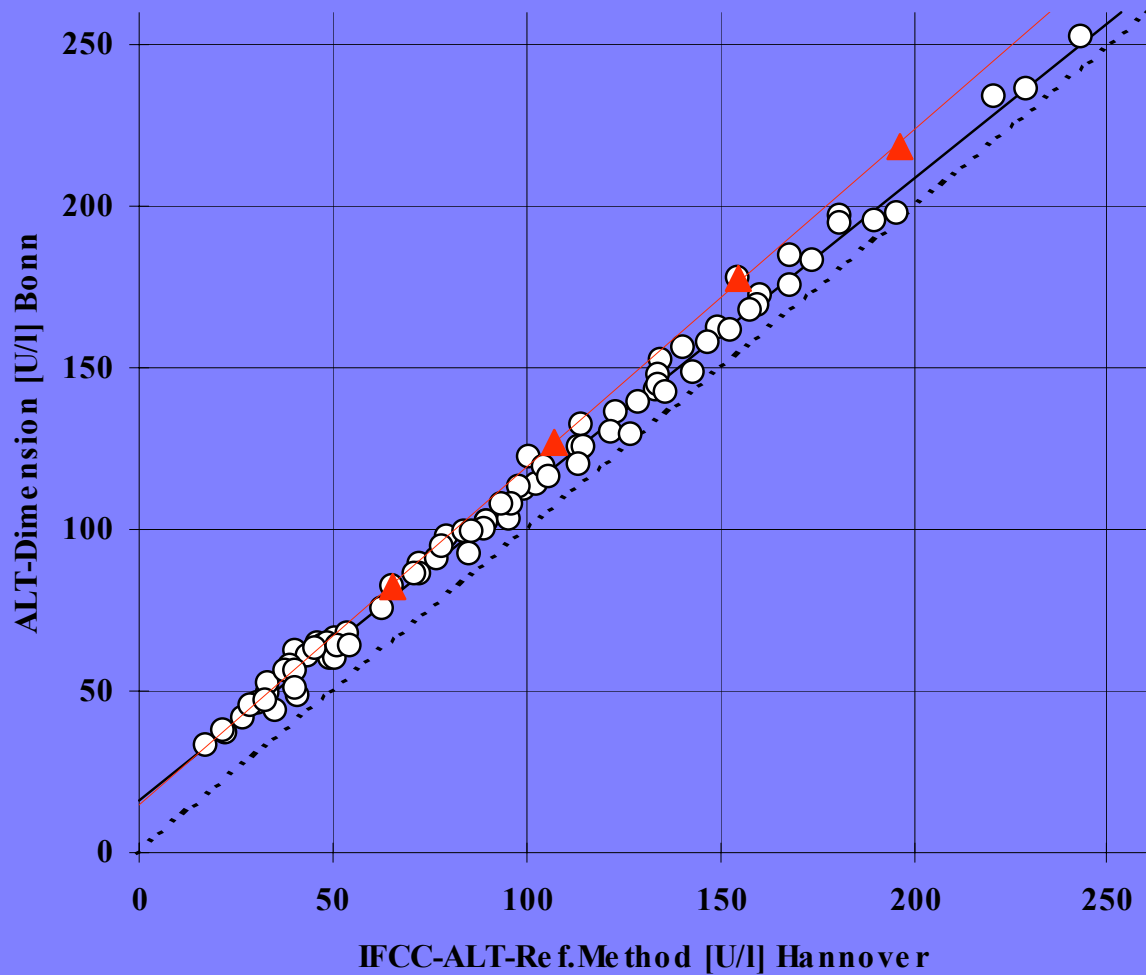
Method Comparison
with the IFCC
Reference Procedure

Evaluation of a CK Routine Test Kit



Method Comparison
with the IFCC
Reference Procedure

Evaluation of an ALT Routine Test Kit



Method Comparison
with the IFCC
Reference Procedure