



UNIVERSITÀ DEGLI STUDI
DI MILANO

STANDARDIZATION OF HETEROGENEOUS
ANALYTE MEASUREMENTS: THE EXAMPLE
OF HEMOGLOBIN A1c

Milano, 6 November 2007



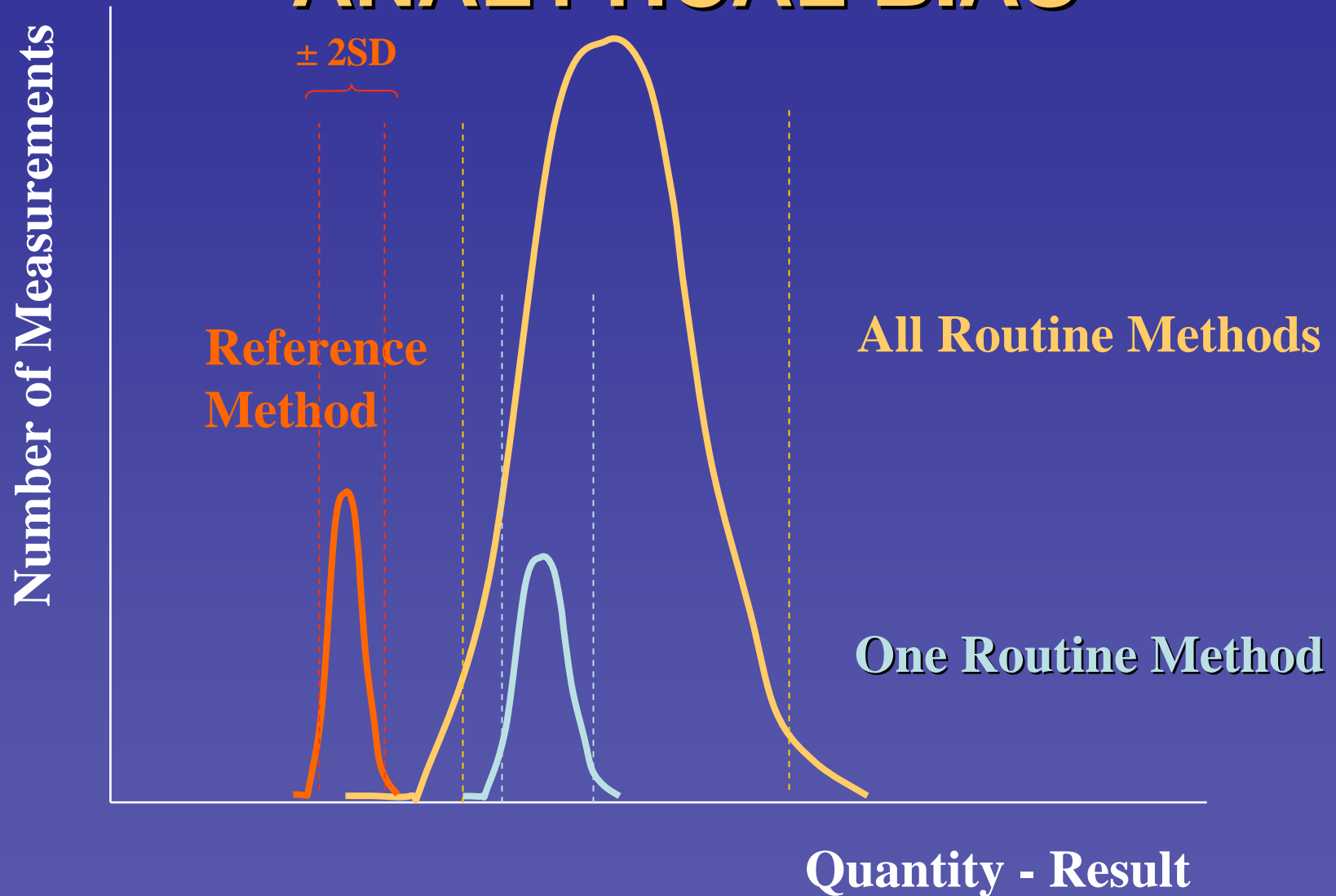
WHY WE NEED TRACEABILITY IN LABOARTORY MEDICINE

Mathias M. Müller

Inadequate or incorrect analytical performance has consequences for practical medicine and the health care system:

- **incorrect interpretation of results by the physician**
 - **wrong diagnosis and treatment**
 - **additional diagnostic procedures**
 - **impairment of the patient's situation and behavior**
- **increase in health care expenses**
 - **wrong political decisions**

ANALYTICAL BIAS



Comparison: Enzymes

ANALYTE	1970	1982	1992	2001	2003
Participants	36	269	603	1358	1812
AP	33.9	11.5	5.6	5.9	5.1
ALAT	67.8	17.2	5.0	5.9	5.7
ASAT	36.3	20.8	5.3	6.0	5.8
LDH	32.0	10.1	5.9	3.7	4.0

CV %



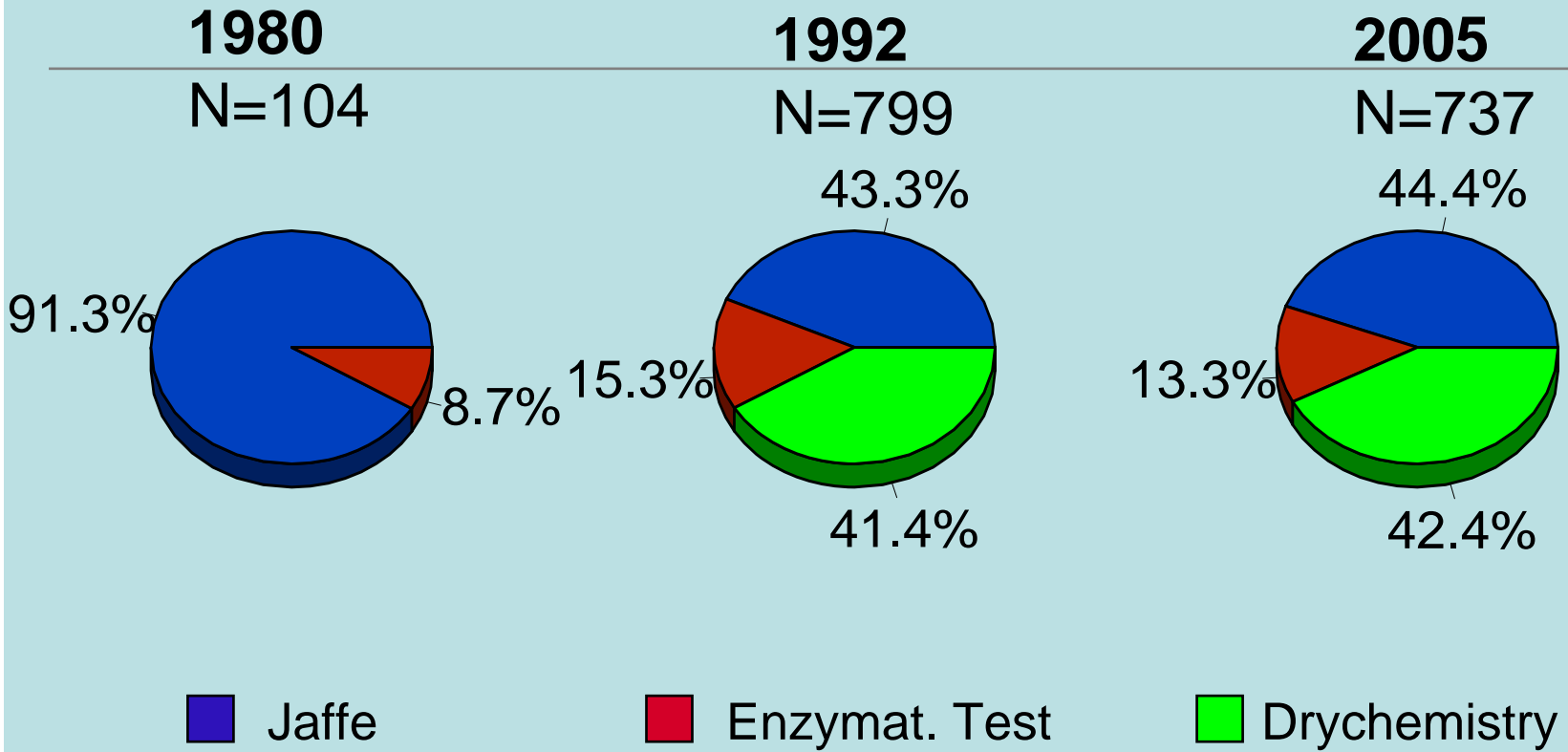
ÖQUASTA

Comparison: Substrates

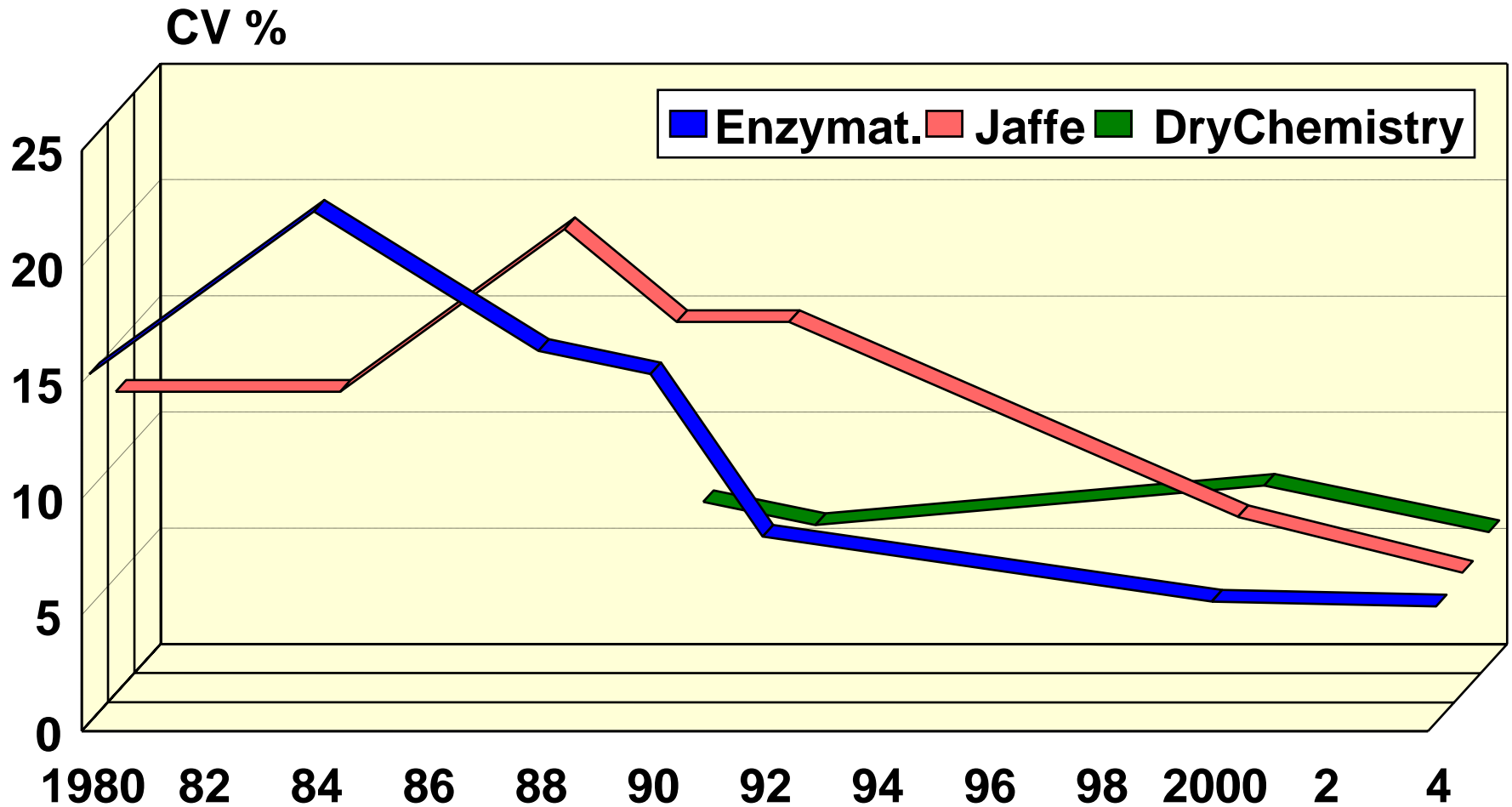
ANALYTE	1970	1982	1992	2004
Participants	36	269	603	2156
Na	7.3	2.5	1.9	1.9
K	8.6	8.4	3.0	2.1
Glucose	43.7	9.1	6.2	5.2
Creatinine	13.7	16.4	10.0	4.8

CV %

Creatinine: Methods



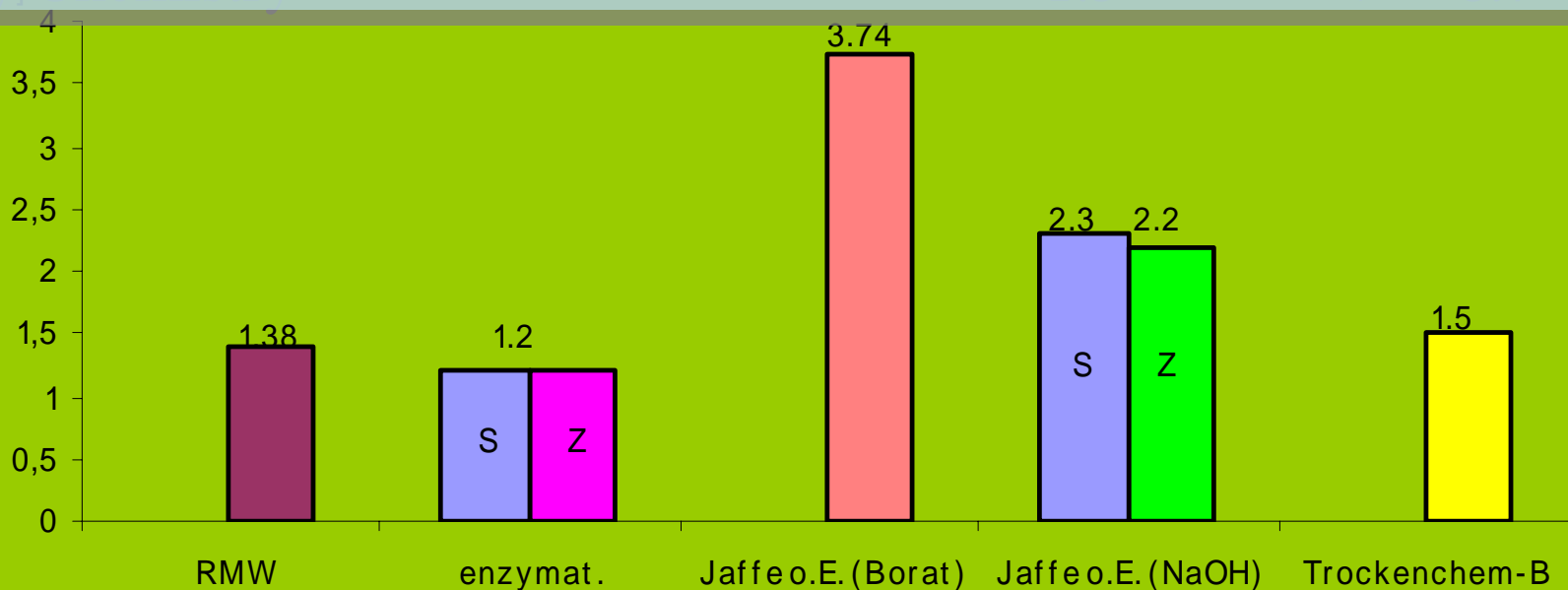
Creatinine: Method-Comparison



Creatinine: Method Comparison

Reference Target Value: 1.38 mg/dl

Method	TV	% Dev.	ConsV.	% Dev.
enzymatic	1.20	- 13.0	1.20	- 13.0
Jaffe o. E.(NaOH)	2.30	+ 66.7	2.20	+ 59.4
Jaffe o. E.(Borat)	-	-	3.74	+ 171.0
Dry Chemistry	-	-	1.5	+ 8.7



GFR Calculation

Creatinine Measurement

not IDMS calibrated

IDMS calibrated

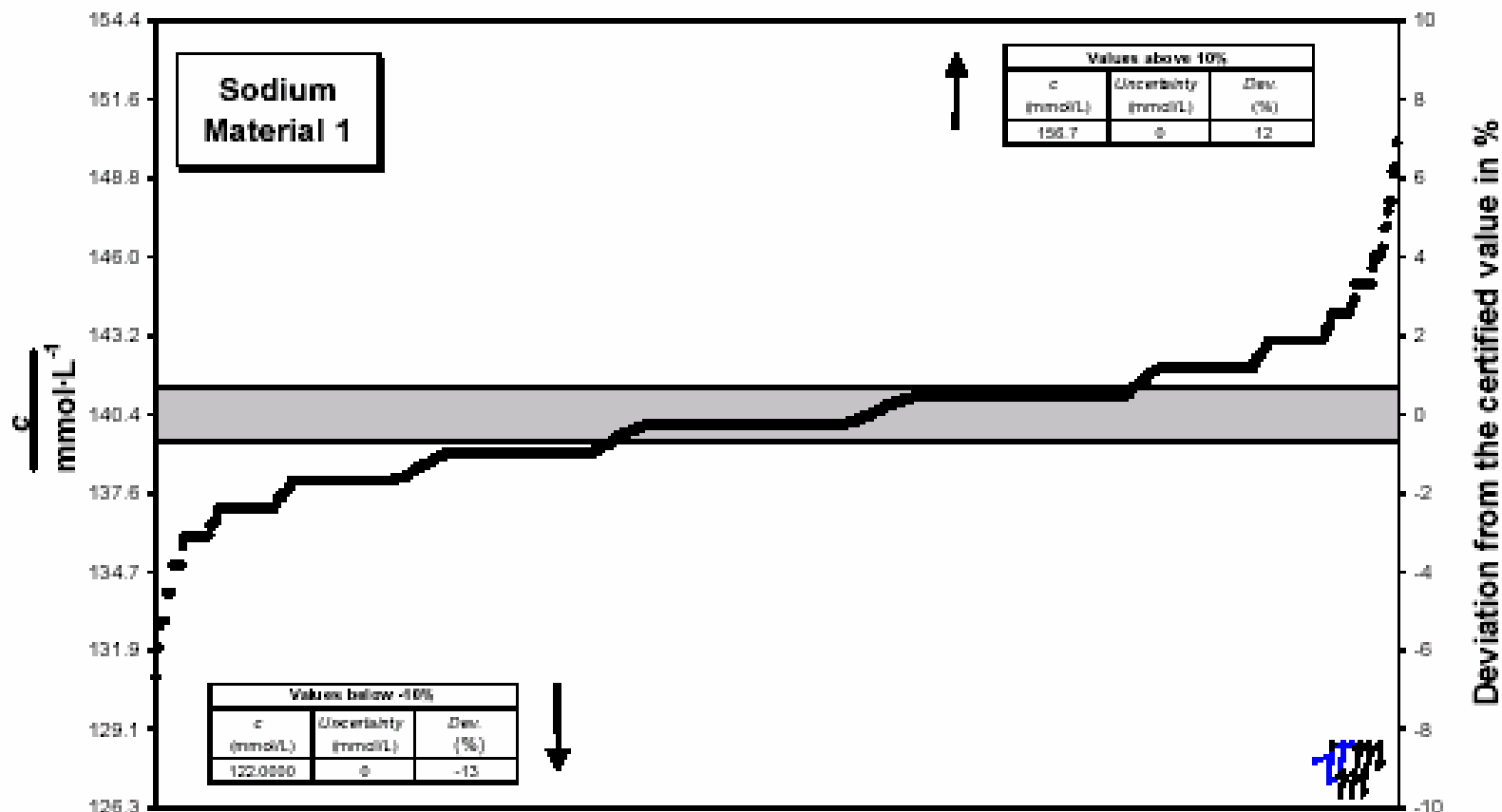
Reference Values:	GFR
80 -120 mL/min/1.73 m ²	mL/min/1.73 m ²
IDMS	55
Enzymatic Test	> 60
Jaffé (Borat)	18
Jaffé (NaOH)	34
Dry Chemistry	53

http://nkdep.nih.gov/professionals/gfr_calculators/index.htm

International Comparison

IMEP- 17: Trace and minor constituents in human serum

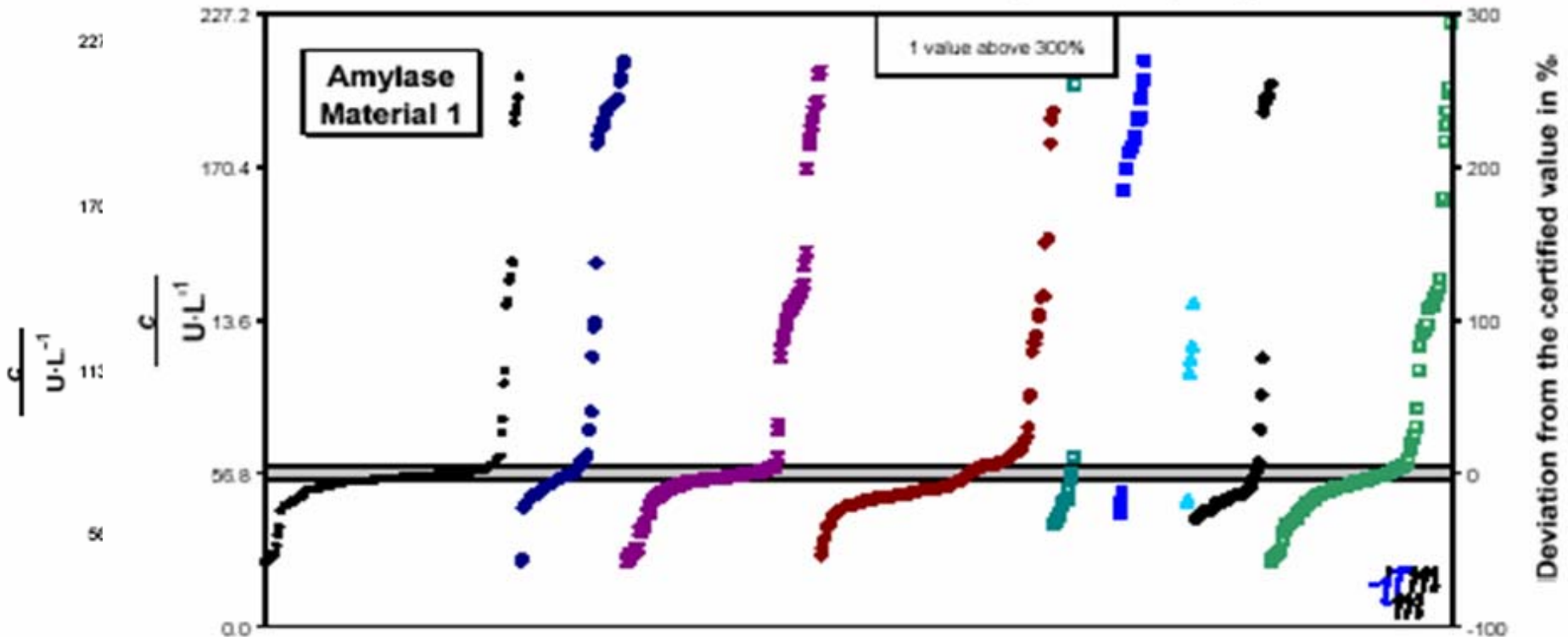
Certified value : $140.36 \pm 0.95 \text{ mmol}\cdot\text{L}^{-1}$ [$U=k\cdot u_c$ ($k=2$)]



Results from all participants (992 laboratories)

International Comparison Amylase

IMEP- 17: Trace and minor constituents in human serum
 Certified value : $56.8 \pm 2.6 \text{ U}\cdot\text{L}^{-1}$ [$U=k\cdot u_c$ ($k=2$)]

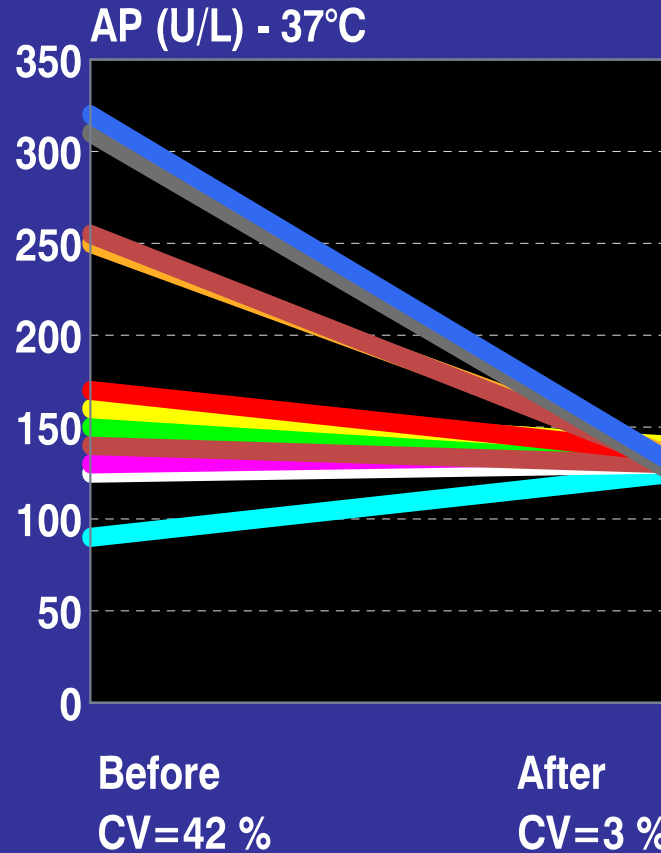
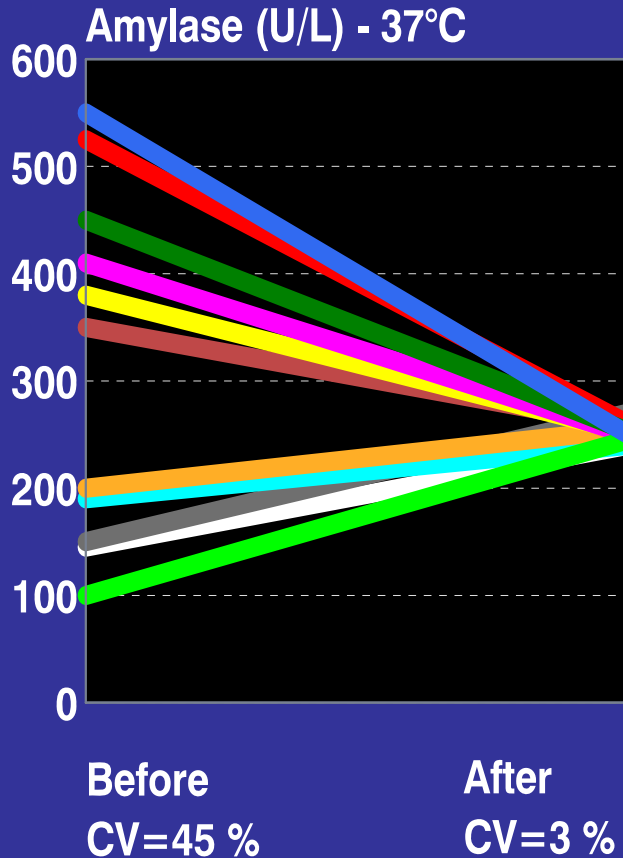


All reported results (863) arranged in method groups:
 IFCC comparable methods; Different methods, Scandinavian level;
 Different methods, Roche level; Different methods, Original level; Vitros 250-950, calculated to IFCC; Vitros 250-950, Scandinavian level; Vitros 250-950, calculated to Roche level;
 Vitros 250-950, original level and Other/No info

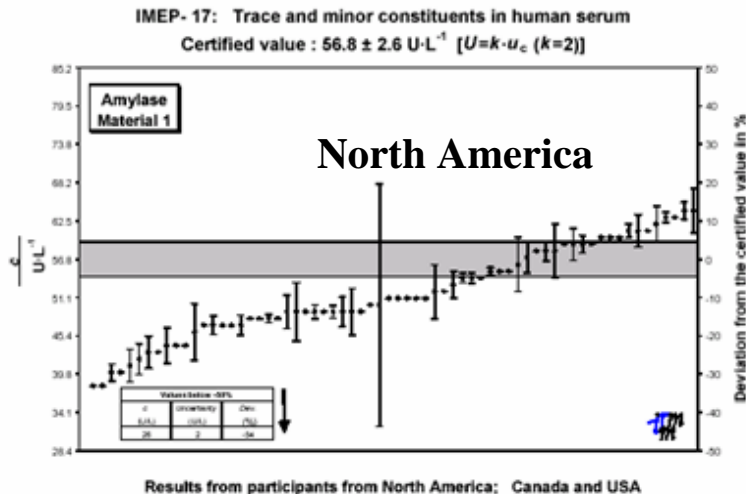
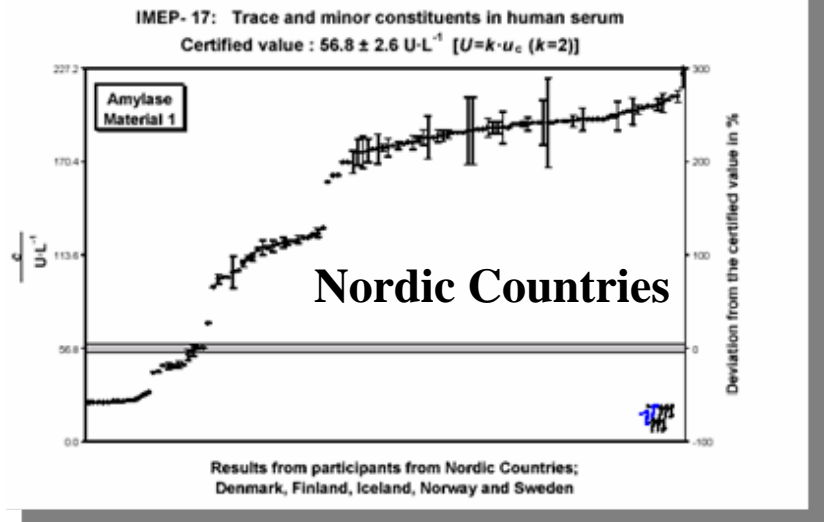
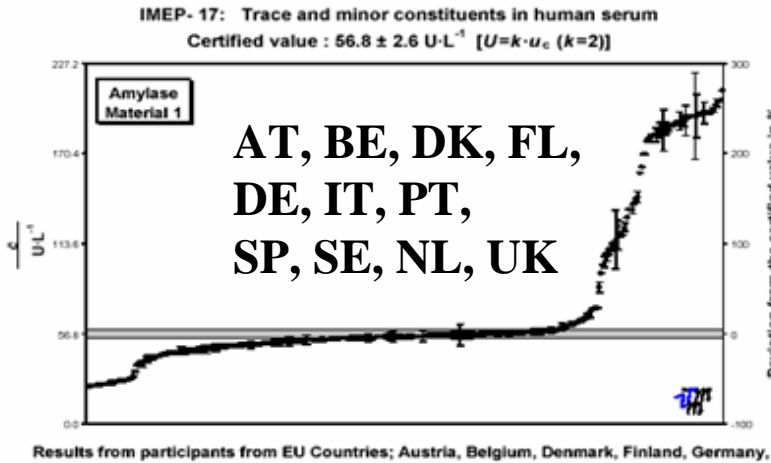
ifcc SD

WG-CALIBRATORS IN CLINICAL ENZYMOLOGY (WG-CCE)

Effect of Uniform Calibration on Various Methods



Amylase Comparison



- Difference in field methods
- Patient results not comparable



**Need for Harmonisation
 and Standardisation**

EQUAS Results

Clinical Guidelines for Decisions



NEED FOR INTERNATIONAL STANDARDISATION

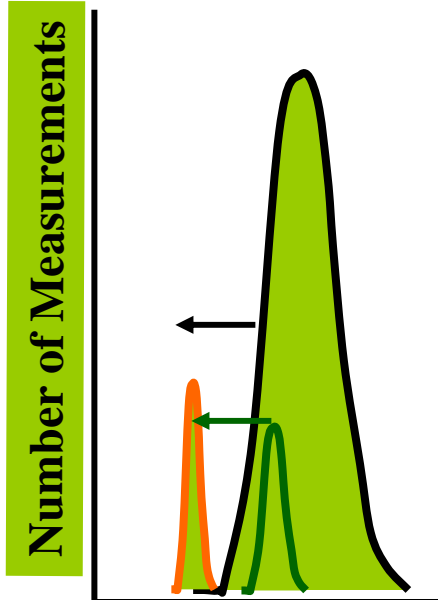


**REFERENCE
SYSTEM**

- **Characterisation of Analyte**
- **Clinical Needs**
- **Reference Procedure**
- **Reference Material**
- **Reference Laboratories**
- **Reference Ranges**

- **ISO/EN 15195**
Requirements for **reference measurement laboratories** in laboratory medicine
- **EN 12286**
Measurements of quantities in samples of biological origins – Presentation of **reference measurement procedures**
- **EN 12287**
Description of **reference materials**

TRACEABILITY



Property of the result related to national or international standards through an unbroken chain of comparisons all having stated uncertainties.

A: traceable to SI

B: non-traceable to SI

- Int'l Reference measurement procedure and int'l calibrator
- Int'l Reference measurement procedure but no int'l calibrator
- Int'l calibrator but no int'l reference measurement procedure
- Manufacturer's measurement procedure but neither int'l reference measurement procedure nor int'l calibrator

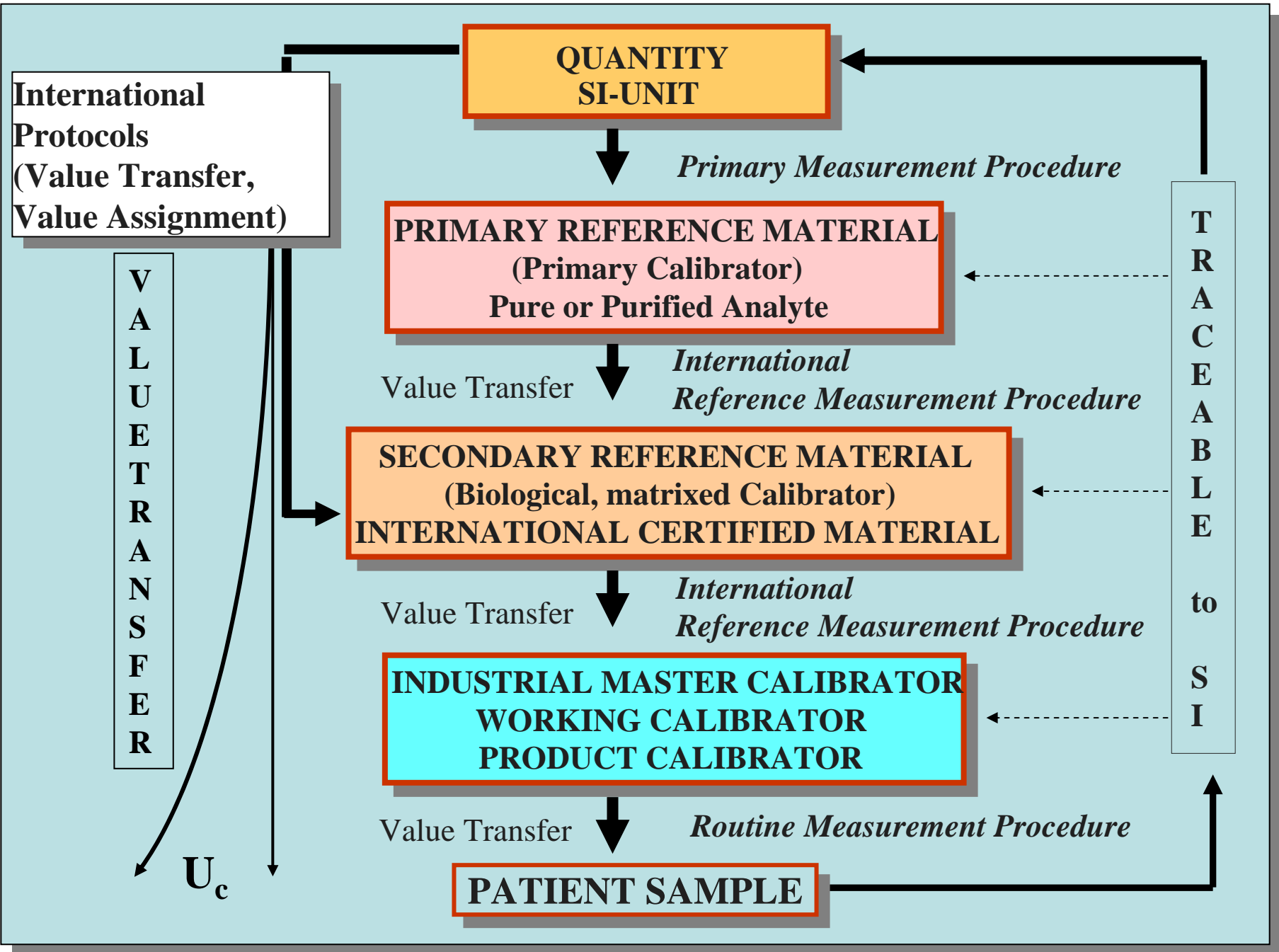
IVD-Directive 98/79

The **traceability** of values assigned to calibrators and or control materials must be assured through **reference measurement procedures and reference materials** of a higher order

ISO Standards

In vitro diagnostic medical devices - Measurements of quantities in biological samples

- **ISO 17511** – **Metrological traceability** assigned to calibrators and control materials.
- **ISO 18153** – **Metrological traceability** of values for catalytic concentration of enzymes assigned to calibrators and control materials.



JCTLM

Joint Committee of Traceability in Laboratory Medicine
Chair: J. C. Forest



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

A joint venture of professionals, metrology institutes, regulators and ivd-industry

JCTLM

Working Groups

1. Reference Materials and Reference Methods

Chair: H. Schimmel (IRMM), W. May (NIST)

Compilation of existing data (Lists)

2. Reference Laboratories - Networks

Chair: L. Siekmann (IFCC), L. Thienpont (IFCC)

Guidelines for reference laboratories

Identification of networks

http://www.bipm.org/enus/2_Committees/JCTLM.shtml

Glucose in Blood, Serum, Urine, CSF
SI-Unit: mmol/l

*Definition
of the measurand*

Section 1 –External to manufacturer, credentialing of the Certified Reference Material

SRM917b

NIST certification of SRM917b (purity)

Primary calibrator

SRM917b – weighed amount

Weighing procedure

Primary reference measurement procedure

Secondary calibrator

Human Patient Specimens, e.g. Blood, Serum, Urine, CSF

Higher Order Reference Procedure – e.g. Isotope Dilution - Mass Spectrometry or Procedure of Similar Trueness and Precision

Secondary reference measurement procedure

Section 2 –Internal to manufacturer, value assignment

Manufacturer's working calibrator

Manufacturer's Master Calibrator, Master Lot of Product Calibrator

Reference Procedure traceable to higher order reference procedure - e.g. Hexokinase/glucose-6-phosphate Dehydrogenase Procedure

Manufacturer's selected measurement procedure

Product Calibrator

New Lot Commercial Product Calibrator

Procedure applying same chemistry and equipment as routine procedure, but more precisely controlled conditions and more replicates to reduce uncertainty

Manufacturer's standing measurement procedure

Section 3 –External to manufacturer, End user's results are Traceable to Certified Reference Material and the Reference System

Commercially available system including product reagent and calibrator lots

End user's routine measurement procedure

Routine Sample – Human Patient Specimens, e.g. Blood, Serum, Urine or CSF

RESULT
Glucose in mmol/l

Result of the search for reference measurement methods/procedures : GLUCOSE



Sort by : Analyte Measurement principle/technique Matrix/Material

Select	Analyte	Measurement principle/technique	Matrix/Material
	glucose	Enzymatic	blood serum
	glucose	Isotope dilution mass spectrometry	blood plasma
	glucose	Isotope dilution mass spectrometry	blood serum
	glucose	Isotope dilution mass spectrometry	calibration solution
	glucose	Isotope dilution mass spectrometry	other

Isotope dilution mass spectrometry method for glucose in blood plasma

DGKL reference method for glucose

Applicable matrixe(s)	lyophilized, fresh or frozen
Full description of technique(s)	ID/GC/MS
Quantity	Amount-of-substance-concentration
Applicable range	0.5 mmol/l to 20 mmol/l
Expected uncertainty (level of confidence 95%)	1 % to [dependent from the matrix and the special properties of the analyte]
Reference(s)	<i>Biomedical Mass Spectrometry</i> , 1982, 9 , 395-405
Comment(s)	The expanded uncertainty is a relative one
JCTLM DB identification number	C3RMMP19

Results of the search for higher-order reference materials : GLUCOSE



Select	Analyte	Analyte category	Matrix/Material	Organization
	glucose	metabolites and substrates	frozen human serum	CENAM
	glucose	metabolites and substrates	glucose crystalline material	CENAM
	glucose	metabolites and substrates	glucose crystalline material	NIST

glucose in human serum

National Institute of Standards and Technology (NIST), United States

Phone: +1 301 975 6776

Email: sminfo@nist.gov

Fax: +1 301 948 3730

Web: <http://www.nist.gov/srm>

Name of the reference material	SRM 965a, glucose in frozen human serum
Quantity	Amount-of-substance concentration
Analyte certified/assigned value	1.918 mmol/l to 16.24 mmol/l
Expanded uncertainty (level of confidence 95%)	0.02 mmol/l to 0.19 mmol/l
Other relevant publication(s)	Method used for certification: <i>Biomed. Mass Spectrom.</i> , 1982, 9 , 395-405
Traceability	SI
CRM listing	List I



ÖQUASTA

Glucose: Method Comparison

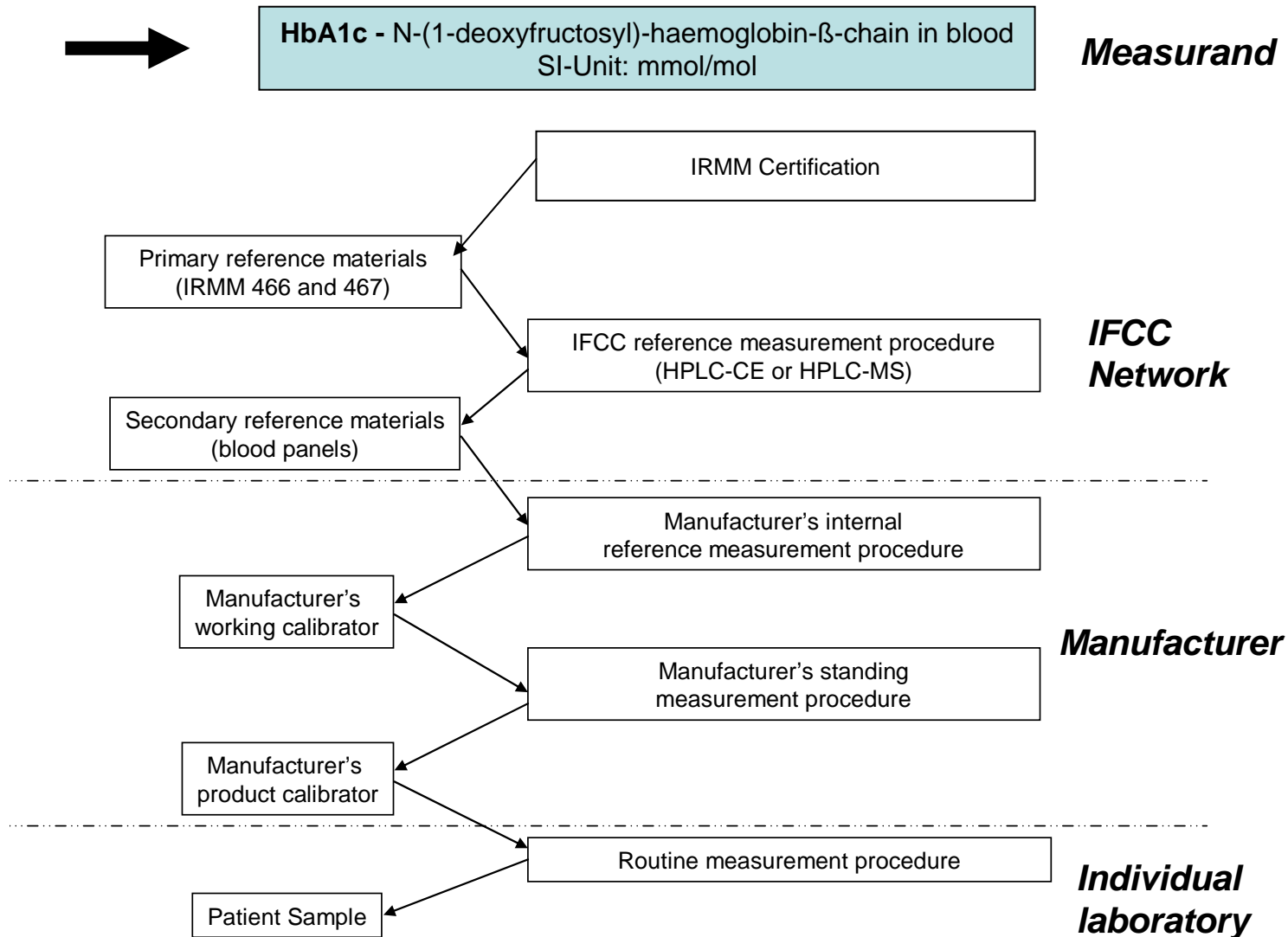
Reference Method TV: 226 mg/dl (192 – 260)

ENZYMAT.	N	Target	CV%	%Bias
109. RV	464	226	6.4	0
113. RV	444	223	6.0	- 1.3

REFLECTOM.	N	Target	CV%	%Bias
109. RV	259	227	8.8	0.4
113. RV	237	233	7.7	3.1

DRY CHEMISTRY	N	Target	CV%	%Bias
109. RV	486	238	6.0	5.3
113. RV	404	232	7.0	2.7

Reference System for HbA1c





Your search criteria: Reference measurement methods/procedures; Analyte: HbA1c; Analyte category: -; Matrix category:

Reference method for diabetes control and complications trial			
Applicable	Applicable matrix(s)	whole blood	
Full	Full description of technique(s)	Cation-exchange HPLC using Bio-Rex 70 resin	_C and roach
assess	Reference(s)	Goldstein, et al., Measurement of glycosylated hemoglobin: High performance liquid chromatographic and thiobarbituric acid colorimetric methods. In: Methods in diabetes research, Volume II: Clinical Methods, Clarke WL, Lerner J and Pohl SL, eds. NY: Wiley and Sons, Inc: 475-504, 1986	HbA1c. ber of usly eplace cisely) ile the ative to er's
JCTLM I	JCTLM DB identification number	C1RMP_P17	

Result of the search for reference materials: HbA1c
Your search criteria: Higher-order reference materials; Analyte: HbA1c

Sort by : Analyte Matrix/Material Organization

Se le ct	Ana lyt e	Ana lyt e c ateg ory	Ma trix/Ma teri al	Orga ni za ti on
	glyc ated hae mo glob in	pro te in s	hu ma n hae mo lysa te	IRMM
	HbA 1c	pro te in s	hem oglob in in buff er	HECT EF

glyc ated ha em oglob in in huma n h aemo lysa te

Instit ute for Re fer en ce Ma teri als and Mea su re ments (IRMM) , Eur ope an Uni on
Phon e: +32 (0)14 571 705

Ema il: jrc-irmm-rm-sales@ec.europa.eu

Fa x: +32 (0)14 590 406

Web: <http://www.irmm.jrc.be/>

Name of the refer ence ma teri al	BCR -40 5, glyc ated hae mo glob in
Quanti ty	Peak area fraction (HPLC)
Ana lyt e ce rti fied /a ssi g ned va lue	6.29 %
Expan de d unce rta in ty	0.18 %
Tracea bi li ty	SI
CRM listi ng	List I

HbA 1c in h em oglob in in buff er

HECTEF Stand ar d Ref er en ce Cen ter Founda ti on (HE CT EF) , Ja pa n
Phon e: +81 44 850 3140

Ema il: umem@hectef.com

Fa x: +81 44 850 3141

Web: <http://www.hectef-src.or.jp/>

Name of the refer ence ma teri al	JDS Lot 2, HbA 1c
Quanti ty	Mass fractio n
Ana lyt e ce rti fied /a ssi g ned va lue	4.04 % to 12.63 %
Expan de d unce rta in ty (level of confi de nce 95%)	0.08 % to 0.13 %
Re fer en ce(s) on comm utabi li ty	Japan Diabetes Soc iety Survey , IFC CH bA1c WG inte rco mpariso n s tudy
Tracea bi li ty	SI
CRM listi ng	List I

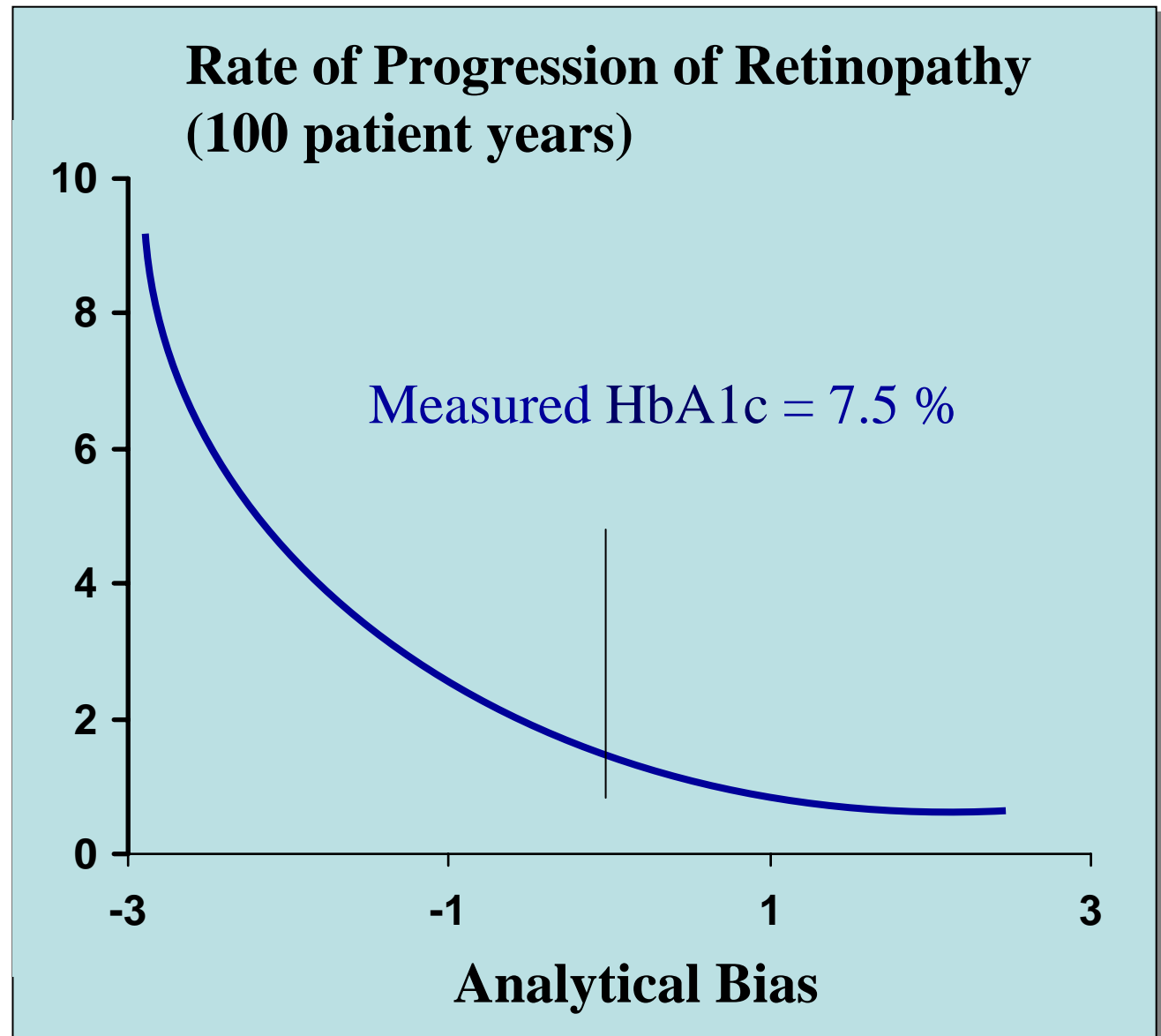
IFCC Traceable Reference Intervals Clinical Decision Levels

	Konventionl, DCCT Rel %	IFCC mmol/mol
Reference Interval	4 - 6	20 - 42
Target for Treatment	< 7	< 53
Change of Therapy in Diabetics	> 8	> 64

Analytical Bias - Therapeutic Consequence

HbA1c

**Insulin-dependent
patients**



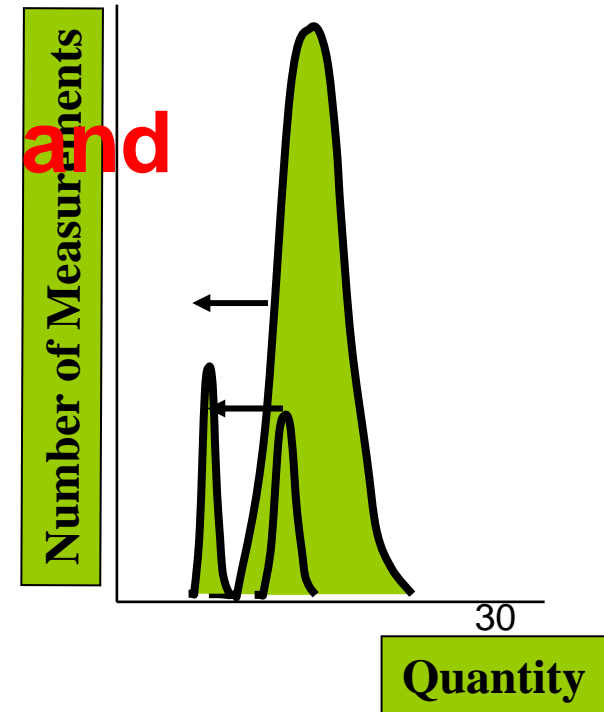
P. Hyltoft

Petersen et al

1997

IMPACT of Traceability on Laboratory Medicine

- Harmonisation and better comparability of field methods
- Change of numeric results
- Change of reference ranges
- **Impact on clinical decisions and classification of patients**



Focus on Standardisation and Traceability

- ◆ **Excellence in Analytical Performance based on modern concepts of metrology and science**
- ◆ **Needs for Patients**
- ◆ **Impact on Clinical Decisions**

**...will add QUALITY and VALUE to
CLINICAL CHEMISTRY
and
LABORATORY MEDICINE**





Forum of European
Societies of Clinical
Chemistry and Laboratory
Medicine



Osterreichische Gesellschaft für
Laboratoriumsmedizin und Klinische Chemie



EUROMEDLAB INNSBRUCK 2009



18th IFCC - FESCC European Congress of Clinical Chemistry and Laboratory Medicine

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Innsbruck, Austria
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