CIRME

The Centre for Metrological Traceability in Laboratory Medicine (CIRME): scope and activities

Mauro Panteghini

CIRME Director

UNIVERSITÀ DEGLI STUDI DI MILANO

Centro Interdipartimentale per la Riferibilità Metrologica in Medicina di Laboratorio (CIRME)

Direttore: Prof. Mauro Panteghini

sito web: http://users.unimi.it/cirme

Outline

- Introductory remarks
- Scope of CIRME
- CIRME activities
- Examples of ongoing projects

Comparability in Laboratory Medicine

- Interchangeability of results over time and space would significantly contribute to improvements in healthcare, since results of clinical studies undertaken in different locations or times could be universally applied

Standardize clinical decision limits (i.e., cutpoints for intervention)

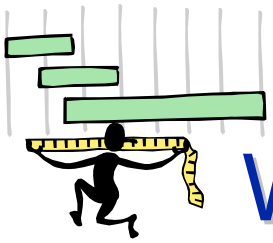
Effective application of evidence-based medicine

CIRME

Università degli Studi di Milano

Why comparability?

- Use of clinical guidelines is becoming more and more prominent in the clinical practice
- Analytes in these guidelines (glucose, HbA_{1c}, PSA, creatinine (eGFR), etc.) have specific cutoffs that are independent of the assay used
- To globally utilize these cutoffs, the assay results for the analyte in question must be comparable: TO BE COMPARABLE THEY MUST BE "TRACEABLE"



What is metrological traceability?

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

Objective → To enable the results obtained by the calibrated routine procedure to be expressed in terms of the values obtained at the highest available level of the calibration hierarchy

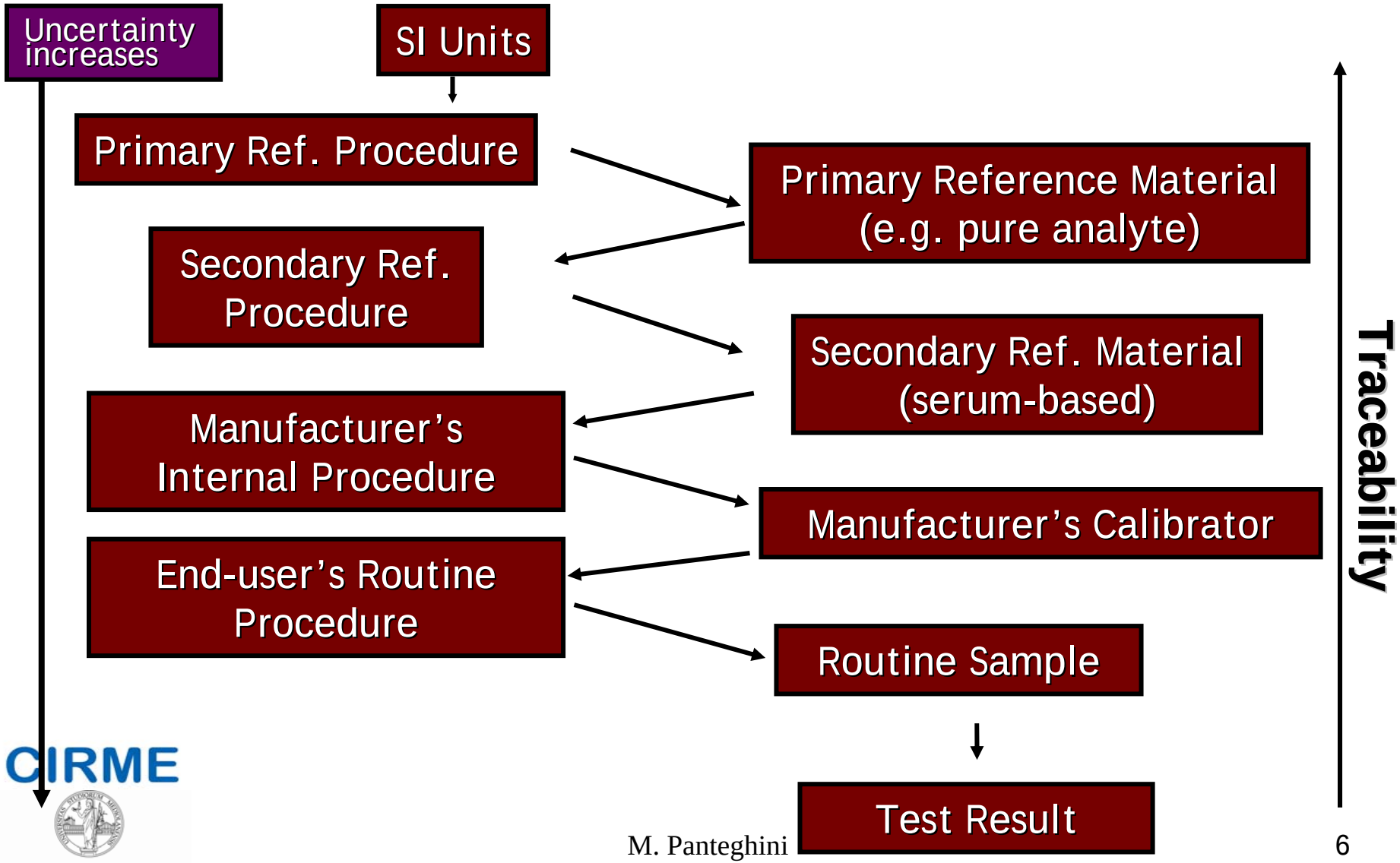
NOTES

1. The concept is often expressed by the adjective "traceable"
2. The unbroken chain of comparisons is called a "traceability chain"

CIRME

UNIVERSITÀ DEGLI STUDI DI MILANO

Reference Measurement System



CIRME

UNIVERSITÀ DEGLI STUDI DI MILANO

Legal Background for the Use of Metrologically Correct Measurement Systems in Laboratory Medicine

Requirement of the EU 98/79/EC-IVD Directive:

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

[Annex I - Essential Requirements (Part A. General Requirements)]

Official Journal of European Communities (1998)

CIRME

Università degli Studi di Milano

How to fulfill these essential requirements?

Through the availability of:

→ Reference materials

→ Reference methods

→ Reference laboratory services

Joint Committee on Traceability in Laboratory Medicine

Other key stakeholders:
- Producers of Reference Materials
- Regulatory Bodies
- IVD Industry
- EQAS Organizations

Objectives and Purpose

To support comparability and equivalence of measurement results in Laboratory Medicine for the purpose of improving healthcare, through worldwide accepted traceability effort following the principles of metrology

To support IVD manufacturers in registration and licensing the CE label conforming with the EU directive

CIRME

UNIVERSITÀ DEGLI STUDI DI MILANO

What has JCTLM delivered?

The World's only quality-assured database of:

a) Higher Order Reference Materials
b) Higher Order Reference Measurement Procedures
c) Laboratory Reference Measurement Services

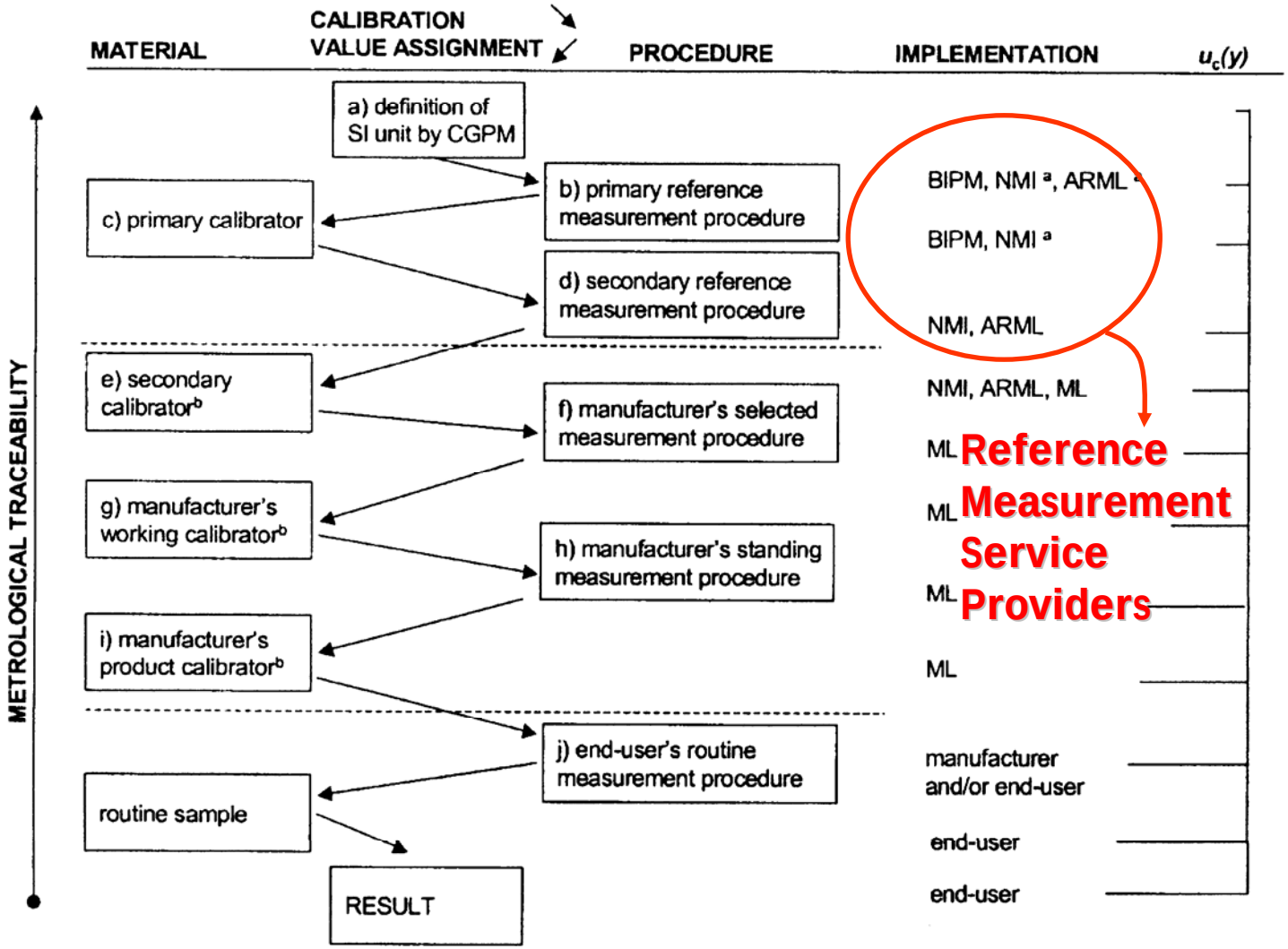
http://www.bipm.org/en/committees/jc/jctlm/jctlm-db/

For use by (primarily):

a) IVD industry (to ensure that results produced by IVDs are traceable to)
b) Regulators (to verify that results produced by IVDs are traceable to)

CIRME
Università degli Studi di Milano

Traceability implementation



ISO 17511:2003. In vitro diagnostic medical devices - Measurement of quantities in biological samples – Metrological traceability of values assigned to calibrators and control materials.

CIRI

UNIVERSITÀ DEGLI STUDI DI MILANO

Reference Measurement Service Providers

Laboratories have been assessed according to the following criteria:

a) metrological level of the employed reference measurement procedure (compliance with the JCTLM list).

b) accreditation to ISO 17025/15195 standards (for each individual measurand as calibration laboratory) or link to a national metrology institute.

c) demonstration of their competence by regular comparative measurements in specific EQAS.

CIRME

UNIVERSITÀ DEGLI STUDI DI MILANO

A Clearer Implementation of the IVD Directive

EU - IVD - Directive

JCTLM

Ref. Materials, Methods, Labs

IVD Industry
Conformity and Traceability
Uniform Calibration
Comparability of Patient Results

CIRME

Università degli Studi di Milano

CIRME

The Centre for Metrological Traceability in Laboratory Medicine (CIRME)

created on June 2006 with the scope to join in a sole entity scientists and activities of various Departments of the University of Milan interested in the development of reference methods and calibration materials of high metrological order in the field of biomedical diagnostics.

UNIVERSITÀ DEGLI STUDI DI MILANO

Centro Interdipartimentale per la Riferibilità Metrologica in Medicina di Laboratorio (CIRME)

Direttore: Prof. Mauro Panteghini

sito web: http://users.unimi.it/cirme

CIRME reference measurement services in the JCTLM list

List of reference measurement services

This file was created on 29 October 2007 from the JCTLM-DB website (http://www.bipm.org/jctlm/)
Your search criteria: Reference measurement services; Analyte: ALT; Analyte category: Enzymes; Matrix category: -

CIRME, Italy

Phone : +39 02 3904 2806
Fax : +39 02 5031 9835
Contact person : Prof. Mauro Panteghini
Email : mauro.panteghini@unimi.it

Analyte	alanine aminotransferase (ALT)
Material or matrix	blood serum, blood plasma
Applicable material or matrix	human serum or plasma (heparin); lyophilized, fresh, or frozen
Quantity	Catalytic activity concentration
Service measurement range	3.8 U/L to 250 U/L
Expanded uncertainty (level of confidence 95%)	(not available) to 2.0% The uncertainty of the lower limit of the measurement range is not available as this enzyme value is clinically unrelevant
Interlaboratory comparison results	RELA - IFCC External Quality assessment scheme for Reference Laboratories in Laboratory Medicine at http://www.dgkl-rfb.de:81/index.shtml Siekmann et al., *Clin. Chem. Lab. Med.*, 2002, **40**, 739-745
Measurement principle	Kinetic spectrophotometry
JCTLM reference measurement method/procedure	IFCC reference measurement procedure (37 °C) for ALT

CIRME
Università degli Studi di Milano

JCTLM

CIRME reference measurement services in the JCTLM list

List of reference measurement services

This file was created on 29 October 2007 from the JCTLM-DB website (http://www.bipm.org/jctlm/)
Your search criteria: Reference measurement services; Analyte: HbA1c; Analyte category: Proteins; Matrix category: -

CIRME, Italy

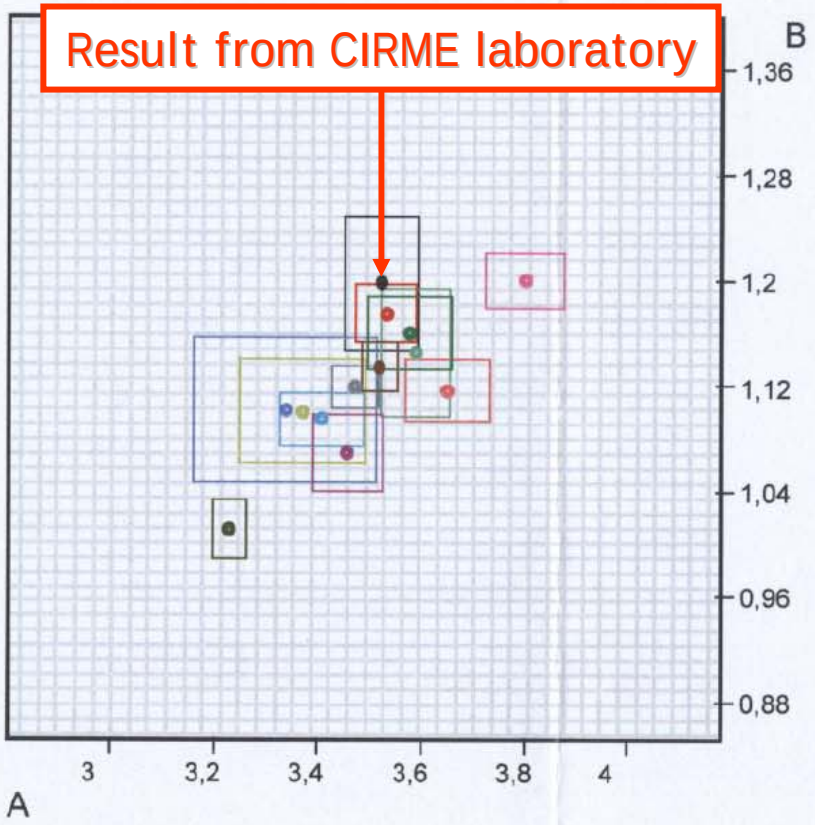
Phone : +39 02 5031 8323
Fax : +39 02 5031 8391
Contact person : Prof. Andrea Mosca
Email : andrea.mosca@unimi.it

Analyte	glycated hemoglobin (HbA1c)
Material or matrix	whole blood
Applicable material or matrix	human whole blood or human erythrocytes; lyophilized, fresh, or frozen
Quantity	Amount-of-substance fraction
Service measurement range	5 mmol/mol to 150 mmol/mol The lower limit of detection has been empirically determined in the laboratory
Expanded uncertainty (level of confidence 95%)	(Not applicable) to 2.0% The uncertainty of the lower limit of the measurement range is not available
Interlaboratory comparison results	RELA - IFCC External Quality assessment scheme for Reference Laboratories in Laboratory Medicine at http://www.dgkl-rfb.de:81/index.shtml IFCC Network of Reference Laboratories for HbA1c at http://www.ifcchba1c.net/
Measurement principle	Quantification of specific peptides isolated and quantified by Capillary Electrophoresis and Mass Spectrometry
JCTLM reference measurement method/procedure	IFCC method for HbA1c (Two equivalent detection methods)

CIRME

Università degli Studi di Milano

Participation to the IFCC ring trials for Reference Laboratories (RELA)



Evaluation for
LabCode 06

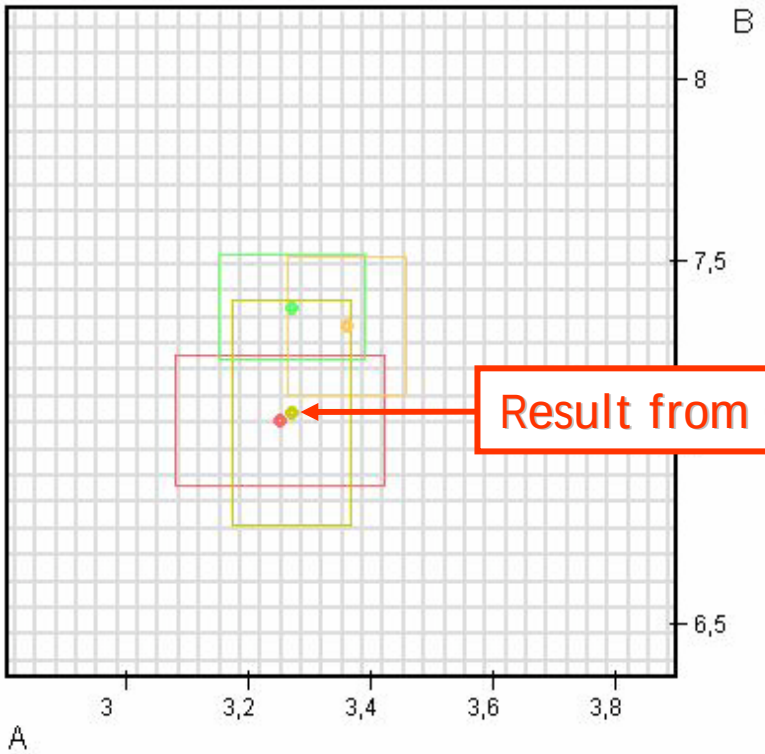
Lab	A	e.u.	B	e.u.	method
	3,648	0,080	1,115	0,023	kinetic spectrophotometry (IFCC)
06	3,522	0,070	1,197	0,050	kinetic spectrophotometry (IFCC)
	3,37	0,12	1,1	0,039	kinetic spectrophotometry (IFCC)
	3,587	0,065	1,143	0,048	kinetic spectrophotometry (IFCC)
	3,406	0,080	1,093	0,020	kinetic spectrophotometry (IFCC)
	3,336	0,177	1,101	0,055	kinetic spectrophotometry (IFCC)
	3,799	0,074	1,200	0,021	kinetic spectrophotometry (IFCC)
	3,471	0,045	1,119	0,016	kinetic spectrophotometry (IFCC)
	3,457	0,068	1,069	0,029	kinetic spectrophotometry (IFCC)
	3,531	0,058	1,174	0,022	kinetic spectrophotometry (IFCC)
	3,518	0,035	1,134	0,018	kinetic spectrophotometry (IFCC)
	3,228	0,033	1,010	0,023	kinetic spectrophotometry (IFCC)
	3,577	0,082	1,159	0,027	kinetic spectrophotometry (IFCC)

Results of IFCC ring trials are available at:
http://www.dgkl-rfb.de:81

Participation to the IFCC ring trials for Reference Laboratories (RELA)

RELA 2006

HbA1c [%]



Lab	A	e.u.	B	e.u.	method
10	3,25	0,171	7,06	0,179	IFCC
27	3,36	0,096	7,32	0,192	IFCC
43	3,27	0,096	7,08	0,312	IFCC
52	3,27	0,12	7,37	0,144	IFCC

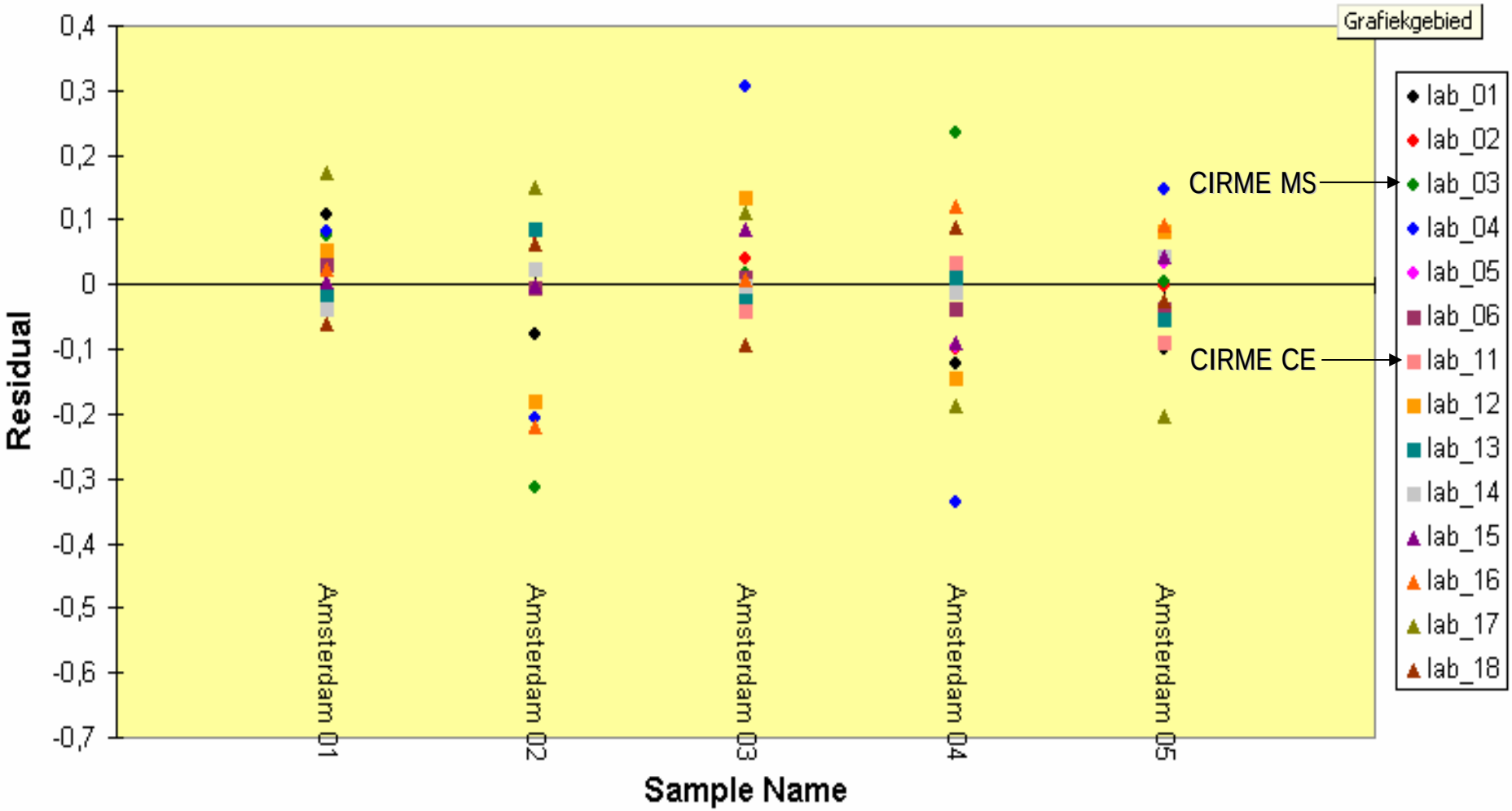
Result from CIRME laboratory

Results of IFCC ring trials are available at:
http://www.dgkl-rfb.de:81

CIRME

Università degli Studi di Milano

In addition, to warrant continuity, stability and further reliability, the CIRME reference laboratories participate in the IFCC network studies



SERVICES

Certification of Reference Materials

↓

In cooperation with producers of RMs

Target-setting for EQAS

↓

In cooperation with EQAS organizers

Validation of Diagnostic Kits/Instruments

↓

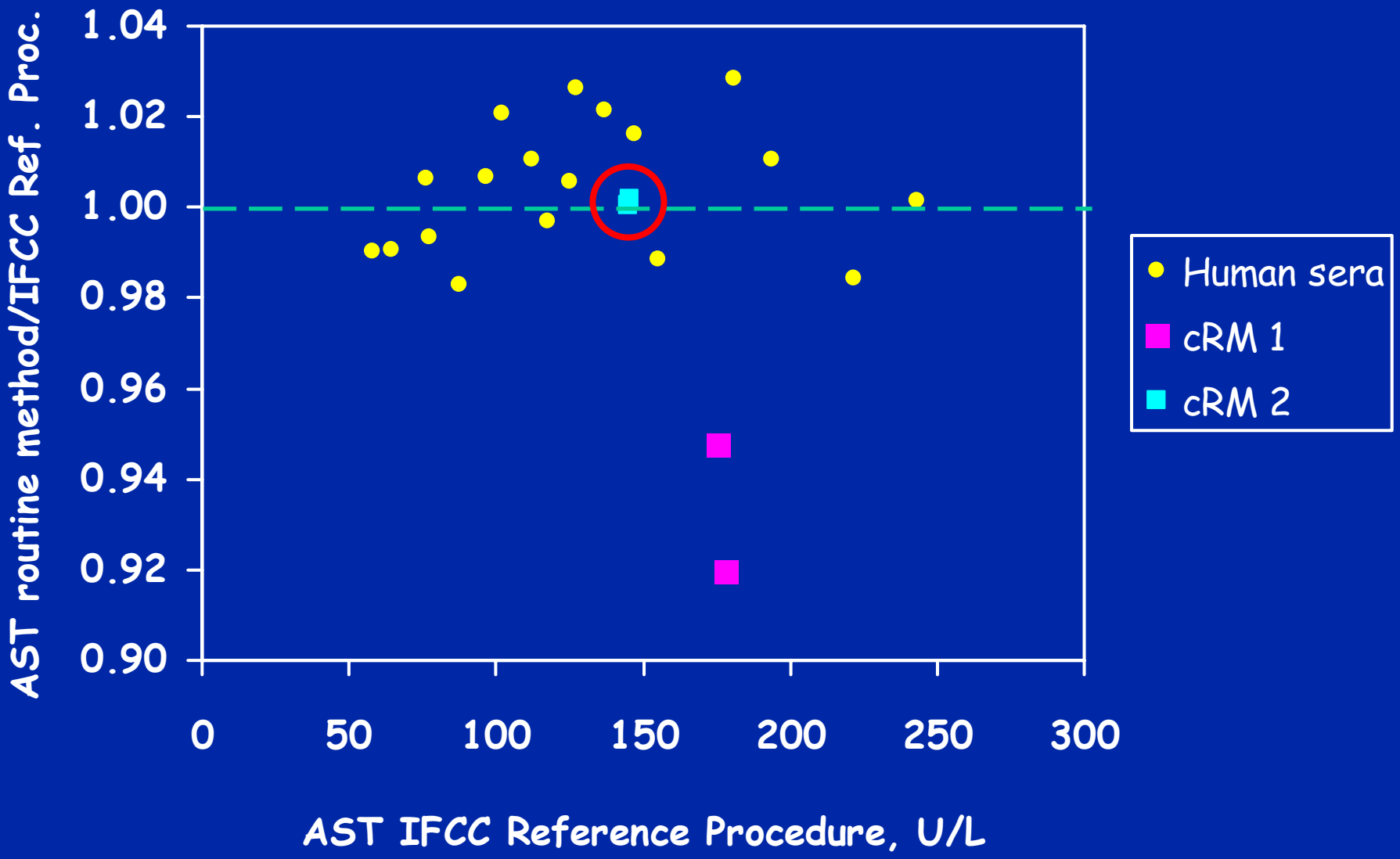
In cooperation with manufacturers

CIRME

UNIVERSITÀ DEGLI STUDI DI MILANO

CIRME Reference Laboratories

AST reference material



AST feasibility study

I. Summary

Average results obtained by 12 labs	**Pool of serum**	**RELA A**	**RELA B**
Average (U/L)	110.08	233.10	191.29
StDev of average (U/L)	3.25	8.53	6.30
RSD of average (%)	2.95	3.66	3.29
Standard u_{char} (U/L)	0.94	2.46	1.82
Relative u_{char} (%)	0.85	1.06	0.95

Where StDev= standard deviation; RSD = relative standard deviation, u_{char} = uncertainty contribution from characterisation (standard uchar corresponds to the standard error of the average; relative u_{char} corresponds to the relative standard error of the average).

The feasibility results combined with the results of the homogeneity study allow us to expect an expanded uncertainty of around 4% for the assigned value of the candidate reference material, which is similar to the expanded uncertainty of the other IRMM reference materials for enzymes.

Reference material ERM-DA470 Human Serum Proteins

Cooperation with IRMM and the IFCC Committee on Plasma Proteins for the preparation of the new reference material for plasma proteins [ERM-DA470]:

- serum collection, *completed*;
- selection of 18 laboratories participating in the value transfer, *completed*;
- lab feasibility study, *completed*;
- value transfer campaign for 14 plasma proteins (Autumn 2007);
- availability of the certified material (June 2008).

CIRME

UNIVERSITÀ DEGLI STUDI DI MILANO

IFCC Project for Standardization of Myoglobin Immunoassays

PURPOSE → to determine the suitability of 5 candidate secondary RMs in relation to linearity, recovery rate and commutability

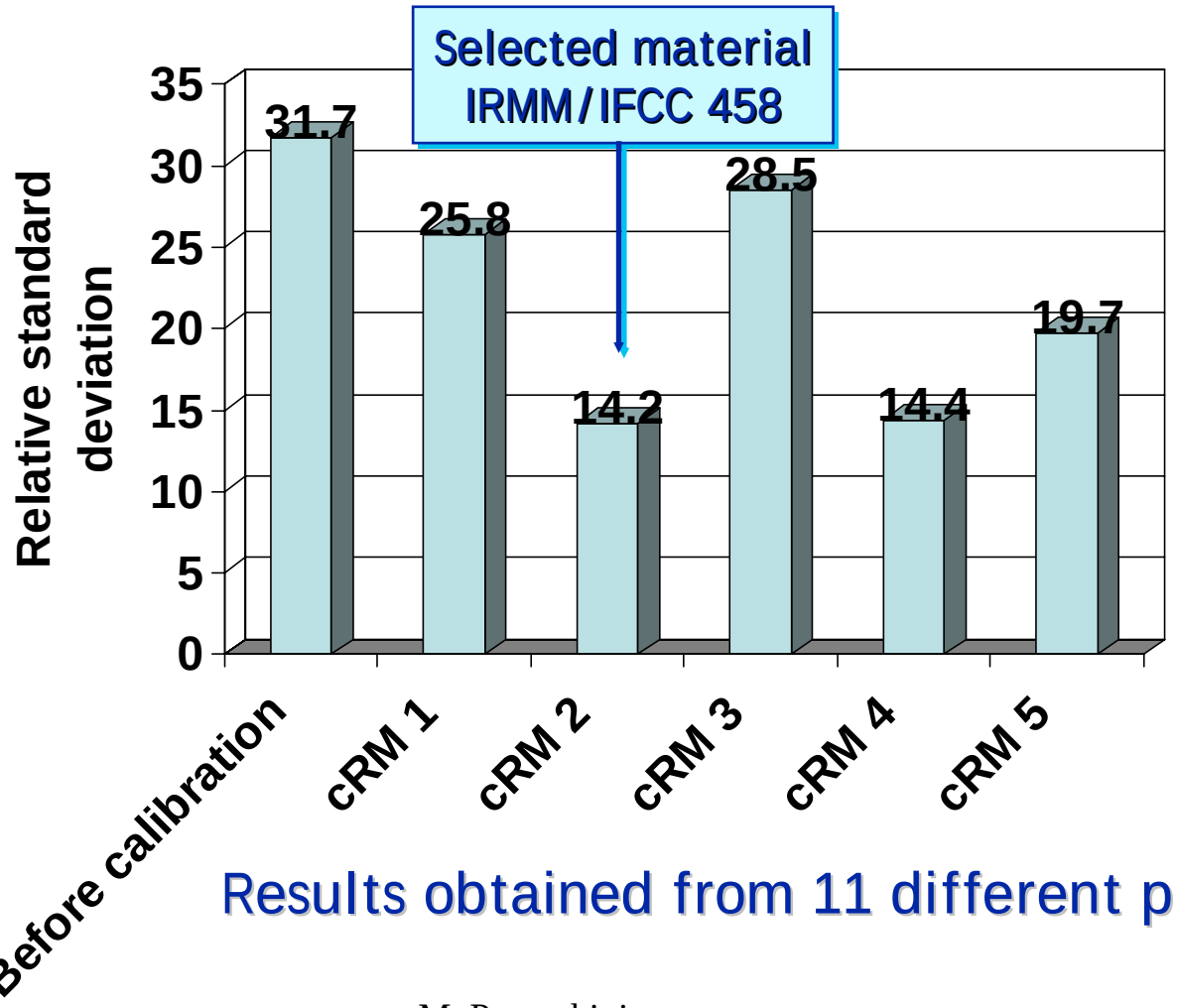
Sources of candidate reference materials for myoglobin

Material	Form	Composition	Nominal value (µg/l)	Manufacturer
cRM#1	lyophilized	myoglobin in bovine serum albumin	700	BioMerieux
cRM#2	lyophilized	human serum spiked with myoglobin	600	HyTest
cRM#3	lyophilized	human serum spiked with myoglogin	650	BioProcessing
cRM#4	frozen liquid	human serum spiked with myoglobin	677	Scipac
cRM#5	frozen liquid	myoglobin in bovine serum albumin	750	Spectral

CIRME

Università degli Studi di Milano

IFCC Project for Standardization of Myoglobin Immunoassays



Results obtained from 11 different platforms

Panteghini M et al., Clin Chim Acta 2004

CIRME

Università degli Studi di Milano

Current status of the project

- After production, the material was stored at IRMM and part of the batch was stored at different temperatures under isochronous conditions for a stability study (short and long-term). These samples are now ready for analysis as well as some samples foreseen for homogeneity testing. These studies are performed by CIRME.
- After this phase, the value assignment of the material will be performed using an LC-MS-MS method developed in a collaborative project with IRMM.

CIRME

UNIVERSITÀ DEGLI STUDI DI MILANO

Reference Materials for Hemoglobins A_0 and A_2

Available c/o CIRME (Dept. Science and Biomedical Technology)

Primary:

- Pure HbA_0 and HbA_2 (three batches produced so far)

Secondary:

- Lyophilized hemolysates;
- Production process started on May 2007;
- Preliminarily tested for stability and commutability for 3 HPLC methods;
- In cooperation with IRMM and IFCC

CIRME

Università degli Studi di Milano

Reference Measurement Procedure for HbA_2

Principle

HbA_2 ratio to whole hemoglobin is determined as ratio of a delta chain specific peptide to an alpha chain specific peptide.
Peptides are obtained by treating total red blood cell lysate with trypsin.
Peptide mixture is analyzed by HPLC-ESI/MS.

Calibration is performed against primary ref. materials (mixtures of HbA_2 and HbA_0 materials).

The SOP has been defined and implemented in 2 reference laboratories: one is from CIRME.

CIRME

UNIVERSITÀ DEGLI STUDI DI MILANO

Reference Measurement Procedure for Alkaline Phosphatase (ALP)

Optimum reaction conditions for the determination of the catalytic concentration of ALP at the measurement temperature 37 °C

The optimized reaction conditions of the IFCC reference procedure at a measurement temperature of 30 °C[1] were reinvestigated for a measurement temperature 37 °C.

[1] Tietz, N.W., Rinker, A.D., Shaw, L.M. (1983) IFCC Methods for the Measurement of Catalytic Concentrations of Enzymes. Part 5. IFCC Method for Alkaline Phosphatase. IFCC Document Stage 2, Draft 1. J. Clin. Chem. Clin Biochem. *21*, 731-748

IFCC/IRMM study

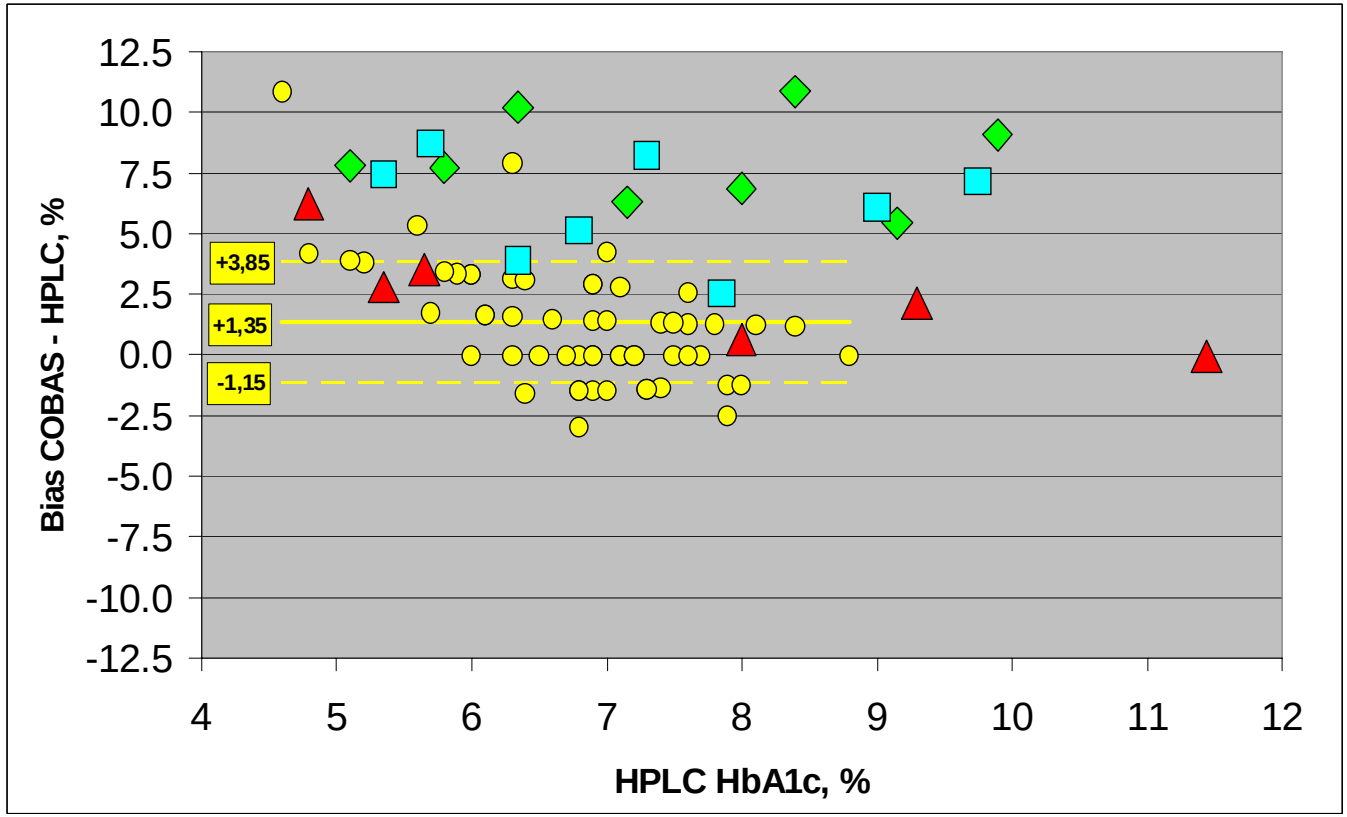
Testing transferability of the candidate reference procedure for measurement of the catalytic concentration of ALP

- The state of the evaluation of the IFCC reference procedure for ALP at 37 °C requires now a multicentre study.
- For that purpose, 7 laboratories with experiences in spectrometric kinetic reference analysis of catalytic concentration of enzymes have been recruited to take part in the study.

CIRME

UNIVERSITÀ DEGLI STUDI DI MILANO

Evaluation of commutability of EQAS materials



CIRME

Università degli Studi di Milano

IFCC Committee on Reference Intervals and Decision Limits (C-RIDL)

Multicentre Reference Interval Study for AST, ALT & γGT

- Preparation of a protocol for collaborative experiments on the establishment of reference values using assays traceable to reference systems
- Production of "standardized" reference intervals for AST, ALT, and γGT

CIRME

UNIVERSITÀ DEGLI STUDI DI MILANO

IFCC Committee on Reference Intervals and Decision Limits (C-RIDL)

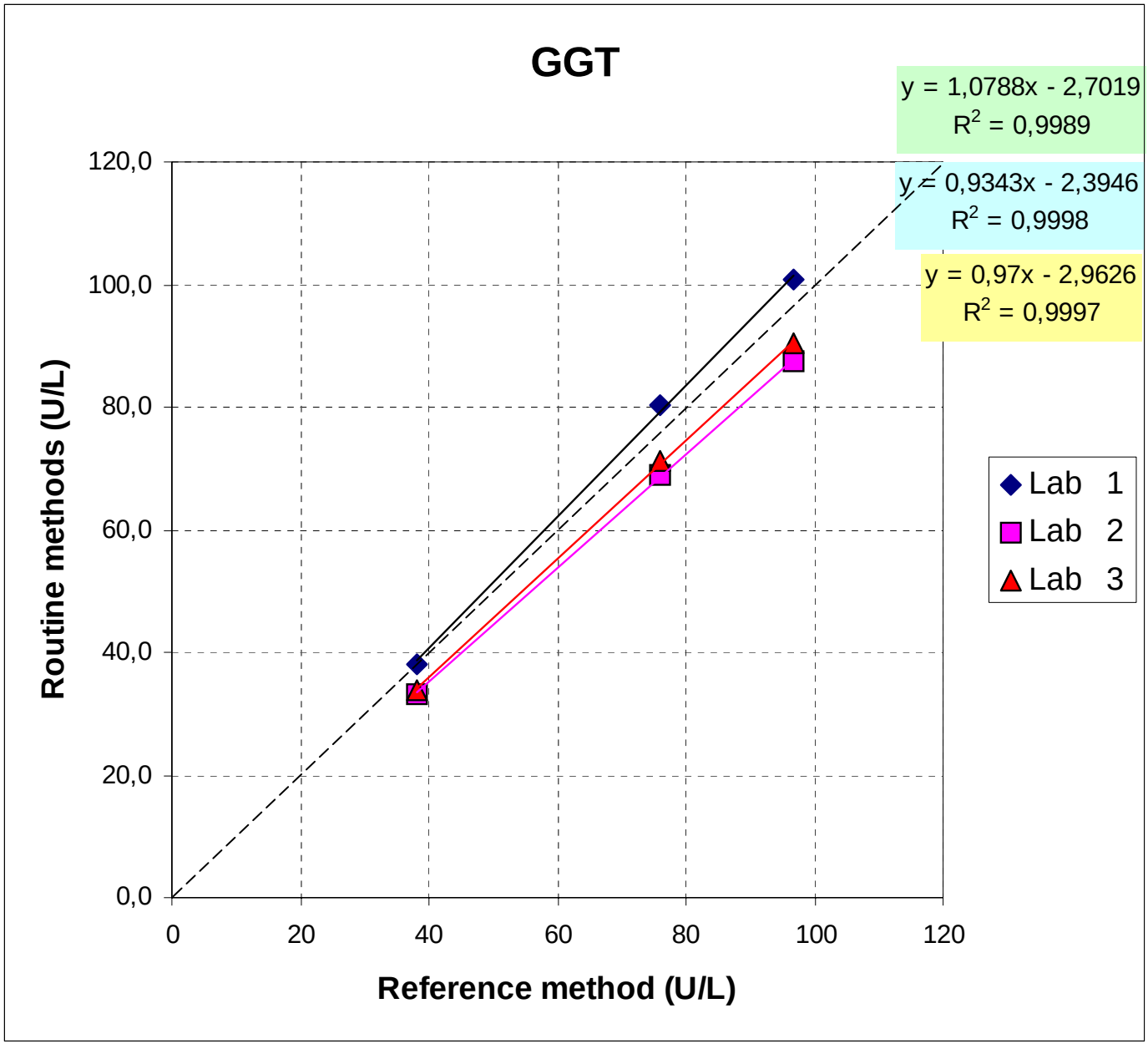
Multicentre Reference Interval Study for AST, ALT & γGT

1. Preparation of the material for trueness/traceability control
   - 3 serum pools at borderline concentrations, minimally processed (filtering, freezing at -80 °C);
   - value assignment by the reference method by three reference laboratories according to a defined protocol (three batches, two or three replicate measurements per batch).

CIRME

UNIVERSITÀ DEGLI STUDI DI MILANO

Verification of trueness of the participating laboratories



CIRME

Università degli Studi di Milano

Traceability as a mean to obtain worldwide useful reference intervals

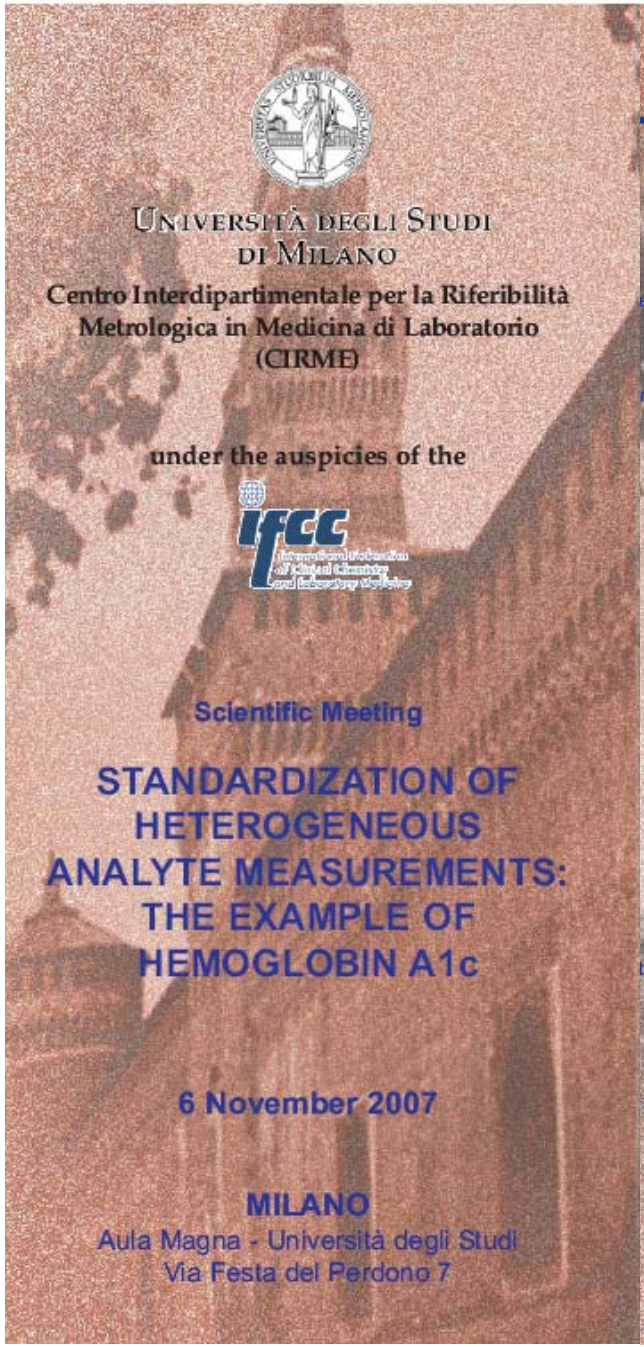
Premise

Use of commercial assays that provide traceable results permits to employ "common" reference intervals (at least within homogeneous ethnic groups)

AST (U/L)	→	F 15–34 M 18–42
ALT (U/L)	→	F 7–41 M 9–55
GGT (U/L)	→	F 13–43 M 14–59

CIRME

UNIVERSITÀ DEGLI STUDI DI MILANO



Programme

10.00 **Meeting inauguration**
Academic Authorities

MORNING SESSION
Chairpersons: M.M. Mueller, F. Ceriotti

10.30 **The Centre for Metrological Traceability in Laboratory Medicine (CIRME): scope and activities**
M. Panteghini (Milano, IT)

11.00 **Defining HbA1c: the indispensable decision to approach measurement standardization**
J.O. Jeppsson (Malmoe, SE)

11.30 **The HbA1c network: structure, performance and rules**
C. Weykamp (Winterswijk, NL)

12.00 **Implementation of the IFCC reference system for HbA1c in clinical practice: how to educate clinicians**
G. John (Norwich, UK)

12.30 **Discussion**

13.00 Break

AFTERNOON SESSION
Chairpersons: J. Hicks, A. Pontiroli

14.00 **Appropriate ways of adopting the HbA1c standardization in clinical practice: the diabetologist's view**
E. Ferrannini (Pisa, IT)

14.30 **Why we need traceability in Laboratory Medicine**
M.M. Mueller (Vienna, AT)

15.00 **Standardization of hemoglobin A2: does HbA1c history repeat itself?**
A. Mosca (Milano, IT)

15.30 **Discussion**

16.00 **Meeting conclusions**
J. Hicks (Washington DC,US)

CIRME

Università degli Studi
di Milano