

REALIZING TRACEABILITY IN LABORATORY MEDICINE : A COORDINATED APPROACH

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IFCC



INTRODUCTION

- **Summary of IFCC activities related to standardization**
- **Examples of collaborative strategies :**
 - IFCC WG for Standardisation of HCG**
 - IFCC WG for Standardisation of HbA1c**



STANDARDIZATION ACTIVITIES

Guidelines and Recommendations

- More than 40 guidelines and recommendations.
- List available on the website of the IFCC.



STANDARDIZATION

Reference measurement procedures : some recent examples

**IFCC Reference Methods for Measurement of pH,
gases and electrolytes in blood. Scand. J Clin Lab
Invest suppl. 1993; 214 : 83-94**

**IFCC Method for Alpha-amylase. Clin Chem Lab Med
1998; 36 : 185-203**

**Approved IFCC Reference Method for the
Measurement of HbA1c in human blood. Clin Chem
Lab Med 2002; 40 : 78-89**

**IFCC Primary Reference Procedures for the
Measurement of Catalytic Activity Concentrations of
Enzymes at 37°C. Parts 1-6 : CK, LD, ALT, AST, GGT,
Clin Chem Lab Med 2002, 40 : (in press)**

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REFERENCE MATERIALS

Apolipoprotein A1 (SP1-WHO), Apolipoprotein B (SP3-WHO), Plasma Proteins-CRM 470 (IRMM), PSA-free (96/668WHO), PSA complexed (96/700-WHO), Cortisol (IRMM/IFCC 451) GGT (IRMM/IFCC 452), LD-1 (IRMM/IFCC 453), ALT (IRMM/IFCC 454), CK-MB (IRMM/IFCC 455), alpha-Amylase (IRMM/IFCC 456), HCG-related reference reagents for immuno procedures with assigned values in molar terms (WHO) (details available on Website of IFCC)

Materials in preparation : HbA1c, Myoglobin, Troponin I, Homocysteine



REFERENCE LABORATORY NETWORKS

Committee on Reference System of
Enzymes

Working Group on Standardization
of HbA1c

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WG-HCG

**Lack of universally accepted nomenclature
for hCG and hCG-related molecules**

**Existing hCG standards had been assigned
arbitrary units**

**Difficulty in comparing the extent of
recognition of different hCG-related
molecules in different immunoassays**

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WG -HCG

**IFCC 4th Bergmeyer Conference (1992) :
Improvement of comparability and
compatibility of laboratory assay results
in life sciences. Scand J Clin Lab Invest:
53(S216).**

**Different approaches for chemically well-
defined analytes, and for heterogeneous
molecules (e.g. glycoproteins).**



WG - HCG

Background

EQAS results demonstrate considerable variation in

- **Recognition of various hCG-related molecules in different immunoassays**
- **Assay standardisation**

The variation causes undesirable clinical consequences.

The hCG Working Group was set up at the 4th Bergmeyer Conference (1992) with the aim of:

- **Improving standardisation**
- **Preparing new standards**
- **Establishing uniform nomenclature**
- **Improving quality control materials**

WG-HCG

MEMBERSHIP :

Ulf-Hakan Stenman, Chair (Finland)

Catharine Sturgeon, Secretary (UK)

Jean-Michel Bidart (France)

Peter Berger (Austria)

Steven Birken (USA)

Robert Norman (Australia)



WG - HCG

Preparation of standards :

Intact, or “holo-”, hCG [hCG]

Nicked hCG [hCG_n]

Alpha-subunit of hCG [hCG α]

Beta-subunit of hCG [hCG β]

Nicked hCG β [hCG β _n]

hCG β -core fragment [hCG β cf]

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WG-HCG

PROJECT ORGANISATION :

- Project finance
- Choice of starting material
- Purification procedure
- Characterization and value assignment
- Processing, ampouling, distribution
- Submission, certification
- Promotion, education

IFCC WG-HCG

PROJECT FINANCE :

- Historically, IS preparations donated by Laboratories with special expertise
- Important issue: how best to finance preparation and validation of International Reference Materials



IFCC WG-HCG

PROJECT FINANCE :

- **Innovation: financial partnership**
 - IFCC Scientific Division
 - IFCC Corporate Members(support of thirteen companies, which in return received early access to the purified preparations, prior to their anticipated adoption as International Reference Mat.)

IFCC WG-HCG

CHOICE OF STARTING MATERIAL :

- hCG from pregnancy urine (300 million units of partially purified urinary hCG from Diosynth, Oss, The Netherlands)

IFCC WG-HCG

PURIFICATION PROCEDURE :

- Purification of the preparations done at Columbia University (Dr Birken)

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IFCC WG-HCG

CHARACTERIZATION :

- Collaborating Laboratories (following various techniques: amino acid terminal sequencing, SDS gel electrophoresis, immunoblotting procedures, amino acid composition....)



WG - HCG

What was accomplished ?

Completion of purification of six important hCG-related molecules by Dr Steven Birken [Columbia University, NY].

Purity of the six preparations confirmed by sequence analyses, SDS gel electrophoresis, HPLC, and mass spectrometry.

Amino acid content determined.

Bioactivity of non-nicked hCG demonstrated.

Preparations ampouled and coded at NIBSC [Potters Bar, UK].

Values assigned in international collaborative study organised by Dr Adrian Bristow [NIBSC].

Submission to WHO in November 2001.

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WG - HCG

Submission and Certification :

- Six preparations submitted to WHO-ECBS, November 2001
- Accepted as reference reagents for immuno procedures
- With assigned values in molar terms

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WG - HCG

Promotion and Education :

- Manuscripts related to the project focussing respectively on the preparation, evaluation, and application of the reference materials
- Other educational activities to encourage better understanding of the clinically relevant forms of HCG related molecules and interpretation of HCG results



WG - HCG

Accomplishment of a significant step forward in the standardization of the measurement of HCG in biological fluids

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HbA1c

Clinical importance

- 1. Independent parameter of glycemic control**
- 2. Risk parameter for the development of late complications**
- 3. Independent risk parameter for CVD in non diabetics**

IFCC WG-HbA1c

HbA1c values of routine assays differ considerably between methods ;

To overcome this problem, IFCC has established a WG on glycated hemoglobin standardisation in 1995.



IFCC WG - HbA1c

Kor Miedema, chair, NL
Wieland Hoelzel, secretary, D
Jan-Olof Jeppsson, S
Andrea Mosca, I
Garry John, UK
David Goldstein, USA
Randie Little, USA
Gary Myers, USA
Tadao Hoshino, J
Ian Goodaal, A



IFCC WG-HbA1c

PROJECT ORGANIZATION :

- Project finance
- Definition of the analyte : beta-N-1-desoxy fructosyl hemoglobin;
- Development of both primary and secondary reference materials;
- Establishment of a validated reference method
- Promotion, education
- International consensus on utilisation of new medical decision targets



IFCC WG-HbA1c

PROJECT FINANCE :

EU Project Contract No. SMT CT 98-2248 :

« Development of a reference method for the determination of HbA1c in human blood and establishing a European Network of Reference Laboratories for this method » started November 1998 and ended May 2001.

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IFCC WG-HbA1c

PROJECT FINANCE :

Contribution of IVD Industry :

Major support (Boehringer/Roche) : primary reference material, reference method, supporting meetings. Complementary support from various manufacturers for the meetings of the WG and the Network of RLs (NRL). Collaboration with all important HbA1c manufacturers in the implementation of the reference system.



IFCC WG-HbA1c

REFERENCE METHOD :

Specifically measures the glycated beta-N-terminal residue of the b chain of Hb ;

The assay principle is peptide mapping after proteolytic cleavage of the molecule and measurement of the ratio of the glycated and non-glycated b-N-terminal hexapeptide by HPLC/MS or HPLC/CE

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IFCC WG-HbA1c

SECONDARY REFERENCE MATERIAL

- Panels of blood samples (mixtures covering relevant range) with values assigned by Network Ref Labs (matrix-based ref. mat. can be used as calibrators for routine methods)
- Lyophilised hemolysate candidates with assigned value by Network (some limitations, suited as calibrators for most routine methods but not all)

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IFCC Network of HbA1c Reference Laboratories

Network Coordinator Dr. Cas Weykamp, Winterswijk

CE - De Weezenlanden, Zwolle, NL

CE - University Malmoe, S

MS - University Gent, B

CE - CDC, Atlanta, USA

MS - CDC, Atlanta, USA

MS - Roche Diagnostics, Penzberg, D

MS - University Milano, I

CE - University Milano, I

CE - Medical center Keio, J

CE - SR Center, Kawasaki, J

CE - Streekziekenhuis, Winterswijk, NL



Performance HbA1c reference laboratories

Analytical criteria :

With-in Laboratory Precision :
average CV $<3.0\%$ in a set of 4-10 samples
no individual CV $>5.0\%$

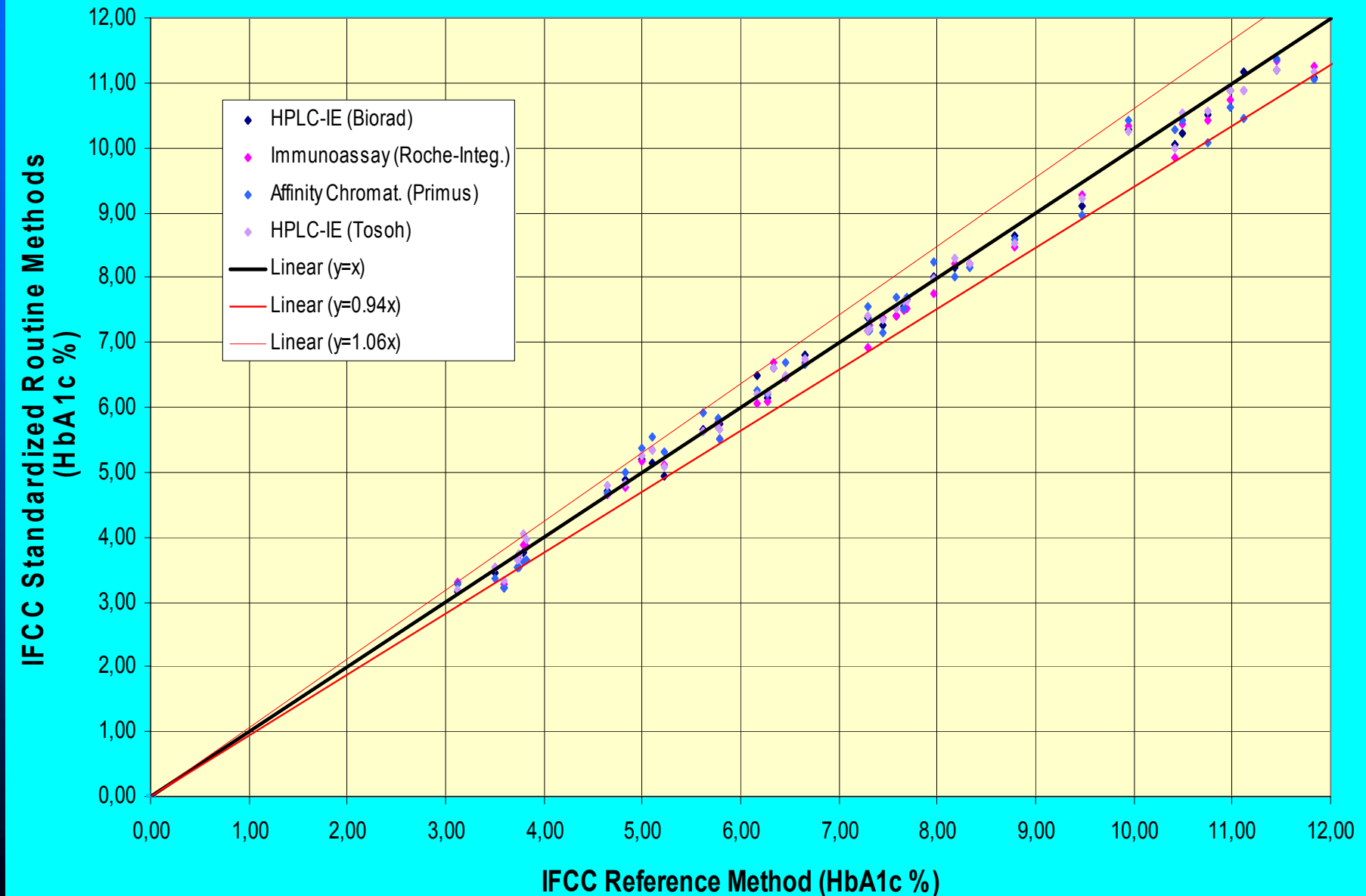
Allowable Systematic Deviation :
95 % of all values within $\pm 2.0\%$ of overall mean

Outliers :
in a set of 4-10 samples, outlier is allowed within
3.0% of overall mean

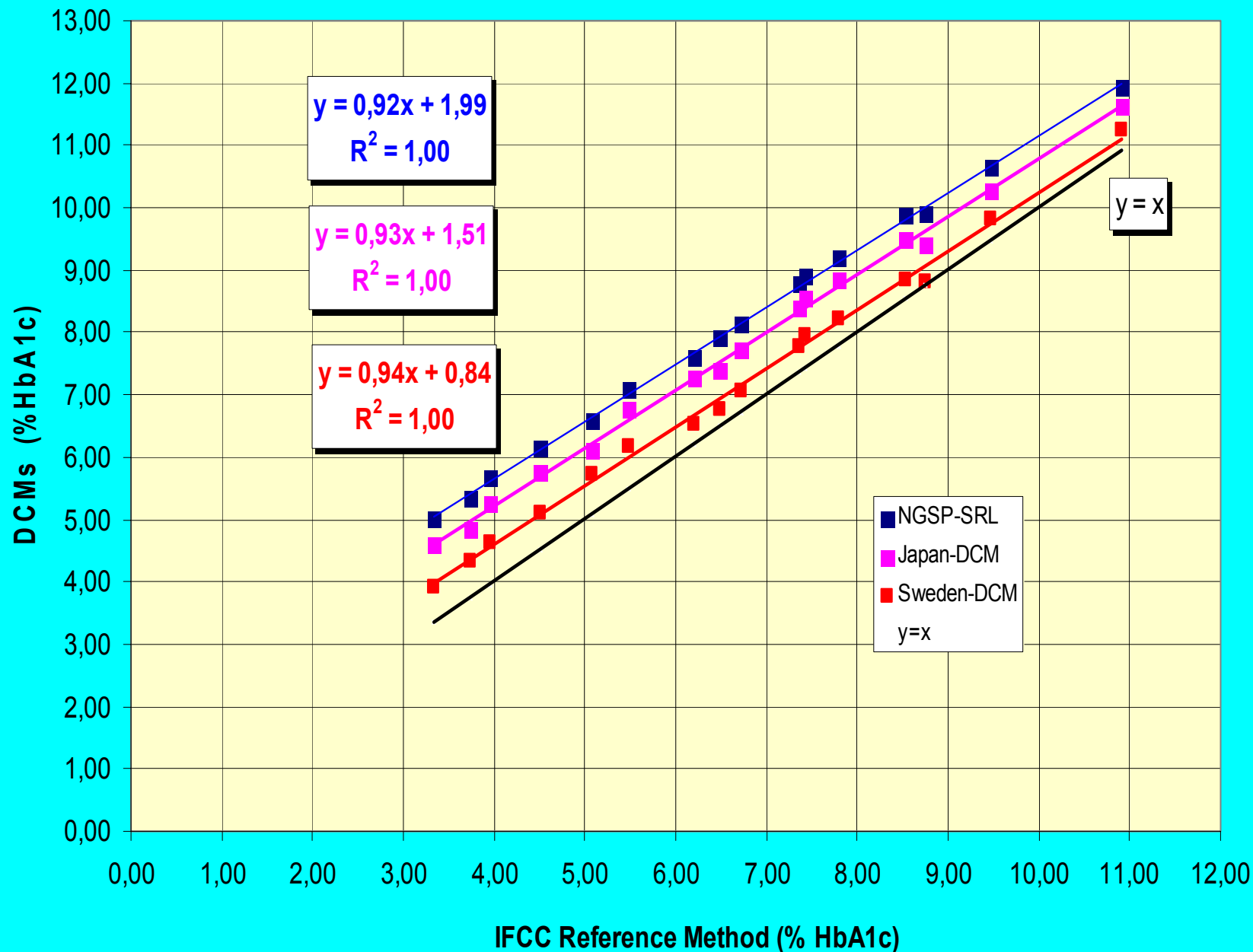
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Method Comparison IFCC Reference Methods versus Standardized Routine Methods

(Immunoassay, HPLC-IE, Affinity Chromatography)



Comparison IFCC Reference Method versus Designated Comparison Methods USA, Japan, Sweden (studies Flo I and II)



WG – HbA1c

PROMOTION AND EDUCATION :

- Translation to clinical utilization
- IFCC Diabetes Global Campaign
- Collaboration with European Association for the Study of Diabetes (EASD), International Diabetes Federation (IDF), American Diabetes Association (ADA), ...



CONCLUSION

IFCC has experienced over the years partnership through its Committees and Working Groups with numerous individual collaborators and organisations to promote the development and implementation of reference systems for biological analytes of clinical significance, covering the multiple aspects of such endeavors, from definition of clinical needs to translation in medical practice

