

10<sup>th</sup> International Scientific Meeting

**CIRME**

Centre for Metrological Traceability in Laboratory Medicine

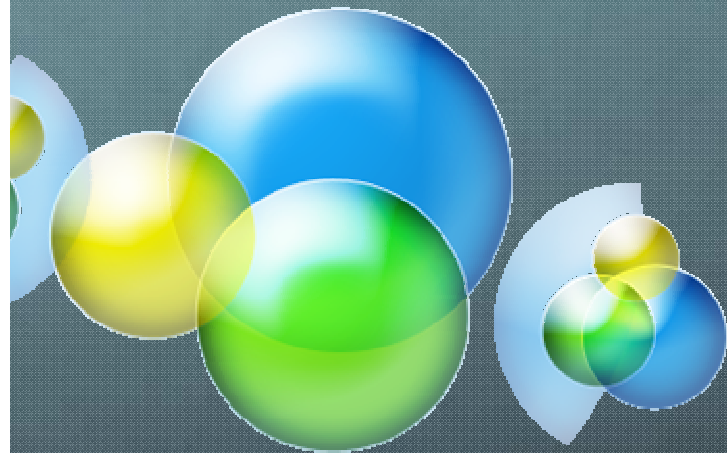
**LABORERS**

**MILANO, ITALY**

**November 17-18, 2016**

**AULA MAGNA - LITA SEGRATE**

Via Fratelli Cervi 93 - Segrate, Milano



# **Standardization of HbA<sub>1c</sub>: are all the pieces in place?**

**Andrea Mosca**

**CIRME, Dept. Physiopathology and transplantation  
University of Milano, Milano (IT)**

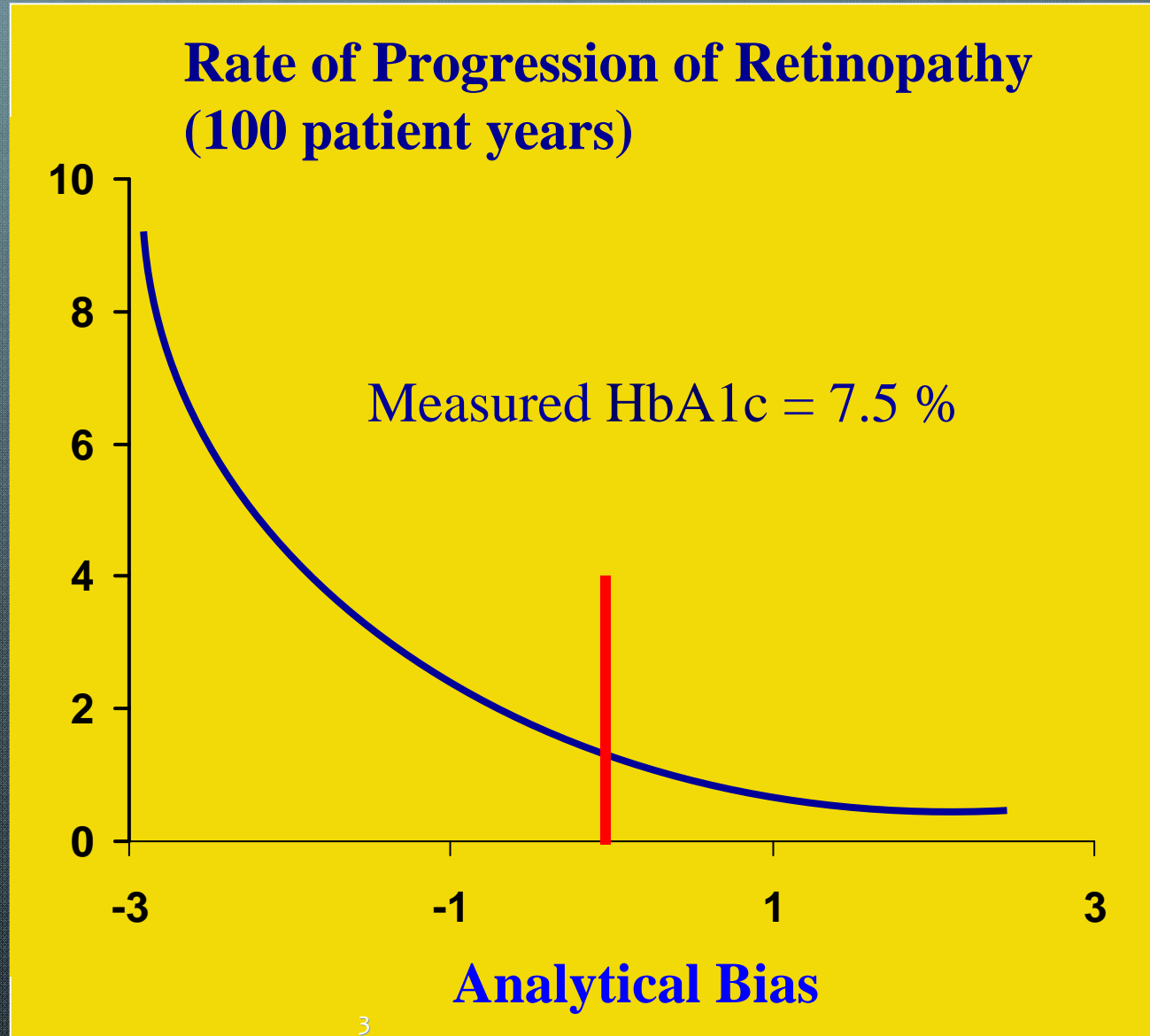
# prologue

# Analytical Bias - Therapeutic Consequence

HbA<sub>1c</sub>

Insulin-dependent patients

P. Hyltoft  
Petersen et al  
1997





UNIVERSITÀ DEGLI STUDI  
DI MILANO

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

under the auspices of the



Scientific Meeting

**STANDARDIZATION OF  
HETEROGENEOUS  
ANALYTE MEASUREMENTS:  
THE EXAMPLE OF  
HEMOGLOBIN A1c**

6 November 2007

**MILANO**

Aula Magna - Università degli Studi  
Via Festa del Perdono 7

## 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 (Malmö, 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)

**International  
Protocols  
(Value Transfer,  
Value Assignment)**

V  
A  
L  
U  
E  
T  
R  
A  
N  
S  
F  
E  
R

$U_c$

**QUANTITY  
SI-UNIT**

*Primary Measurement Procedure*

**PRIMARY REFERENCE MATERIAL  
(Primary Calibrator)  
Pure or Purified Analyte**

Value Transfer

*International  
Reference Measurement Procedure*

**SECONDARY REFERENCE MATERIAL  
(Biological, matrixed Calibrator)  
INTERNATIONAL CERTIFIED MATERIAL**

Value Transfer

*International  
Reference Measurement Procedure*

**INDUSTRIAL MASTER CALIBRATOR  
WORKING CALIBRATOR  
PRODUCT CALIBRATOR**

Value Transfer

*Routine Measurement Procedure*

**PATIENT SAMPLE**

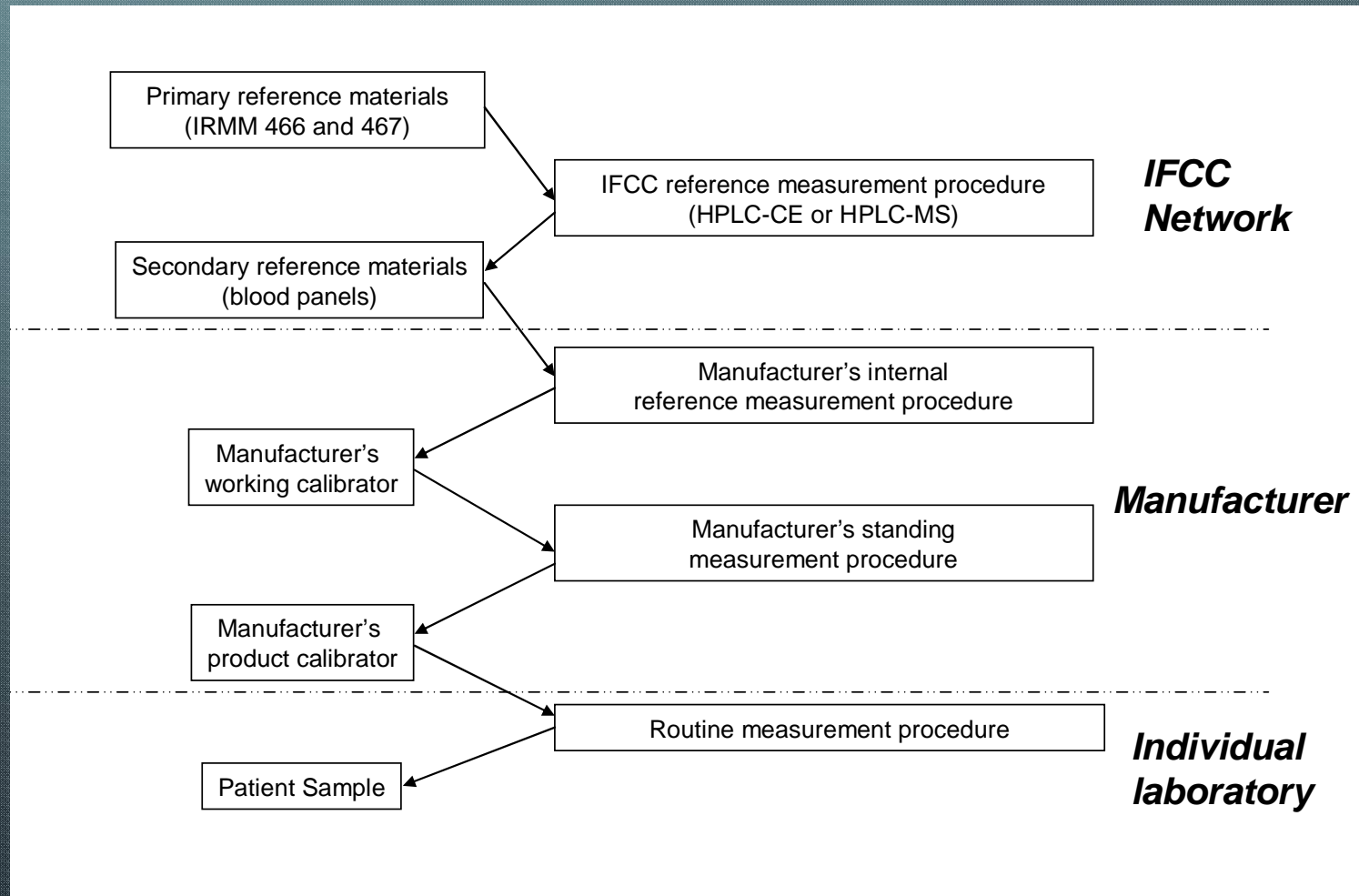
T  
R  
A  
C  
E  
A  
B  
L  
E  
  
to  
  
S  
I

# Reference System for HbA<sub>1c</sub>



**HbA<sub>1c</sub>** - N-(1-deoxyfructosyl)-haemoglobin-β-chain in blood  
SI-Unit: mmol/mol

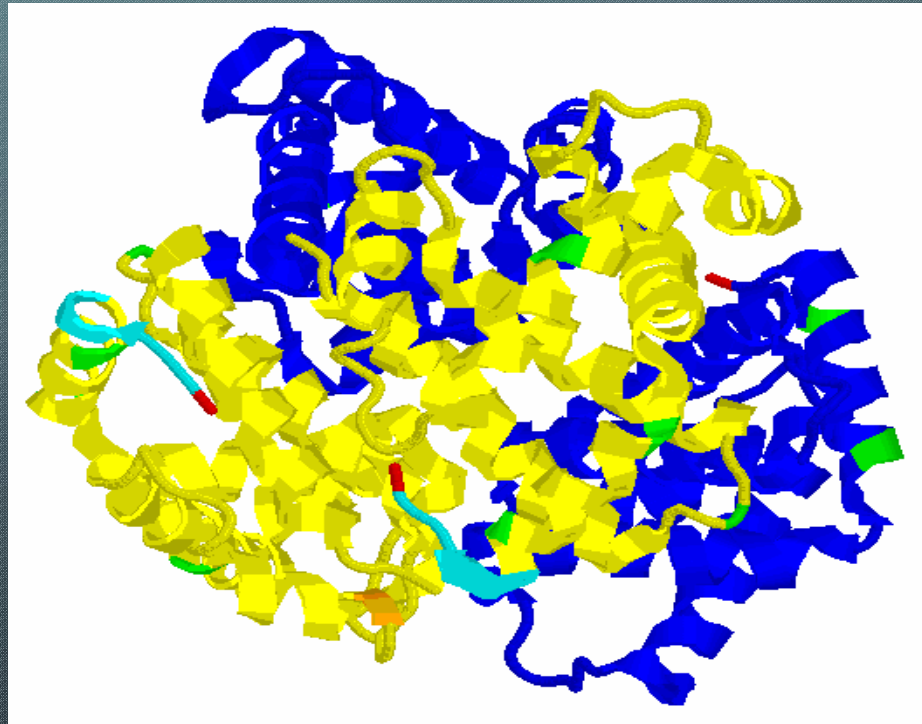
*Measurand*



# Glycation sites of Hemoglobin

$\beta$ -Chains: Yellow  
 $\alpha$ -Chains: Blue

N-terminal  
Valine: red  
Lysine green



Glutathione

HbA<sub>3</sub> or HbA<sub>1d</sub>



NH<sub>2</sub>

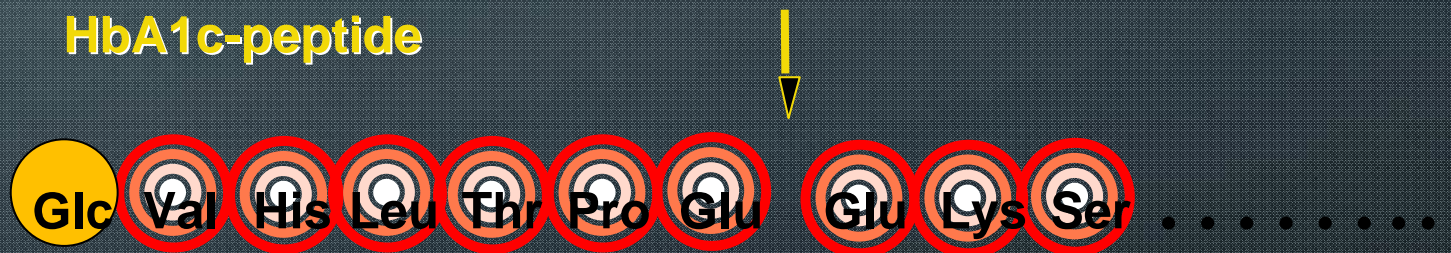
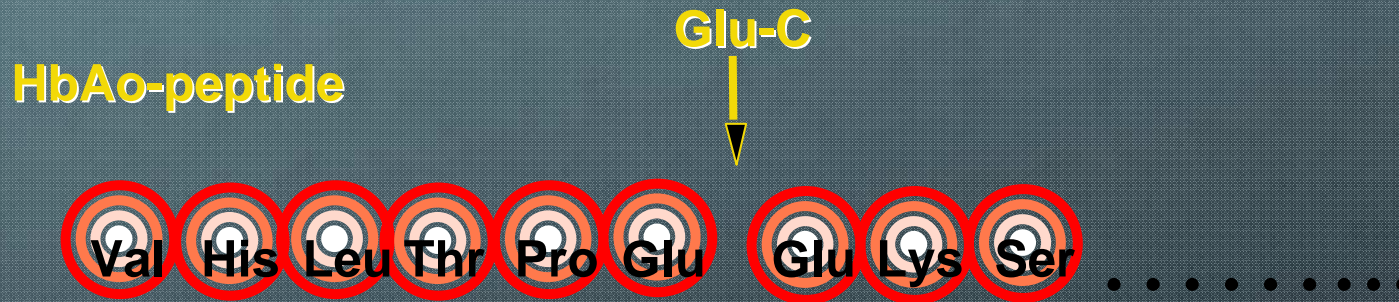
CH<sub>2</sub>SH

# top of the chain

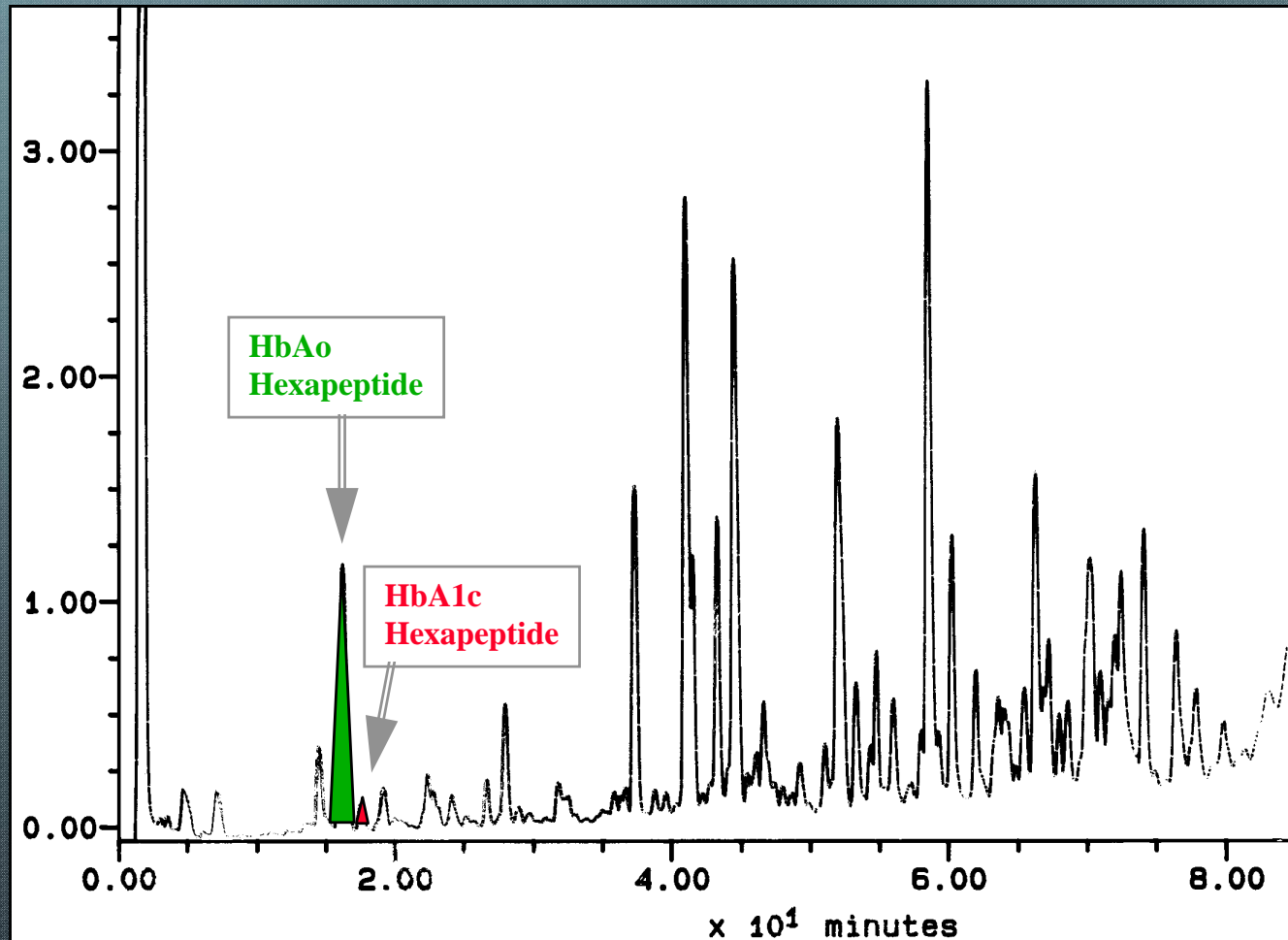


# The Analytical Challenge

Proteolytic cleavage of  $\beta$ -chain (146 amino acids)

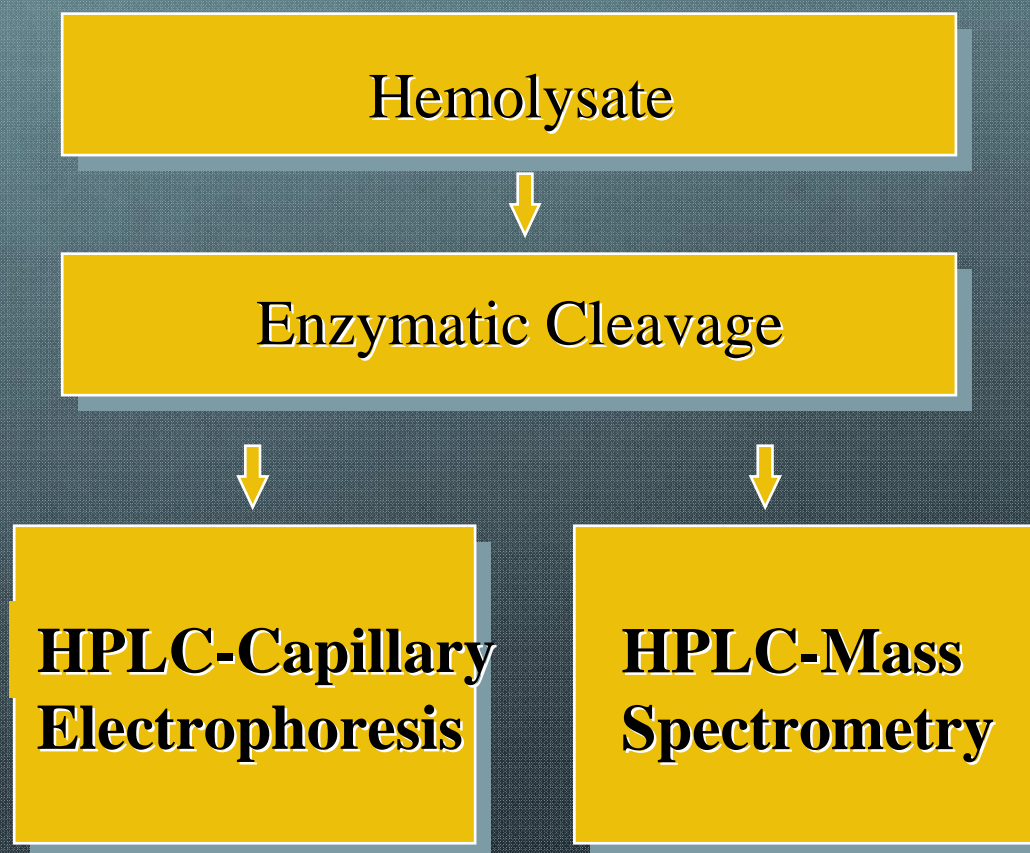


# Photometric detection of peptides



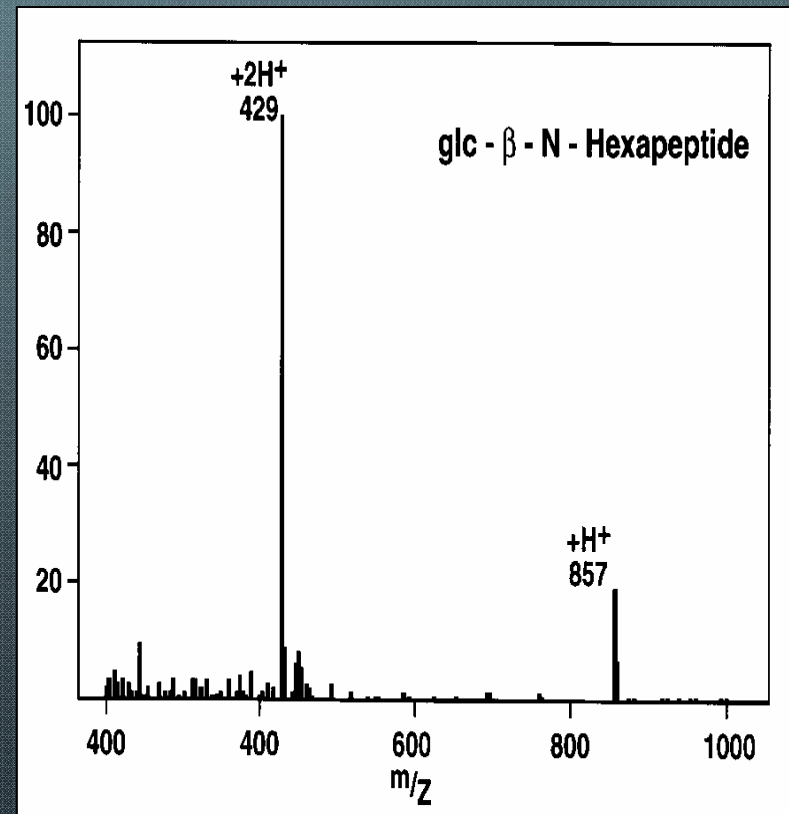
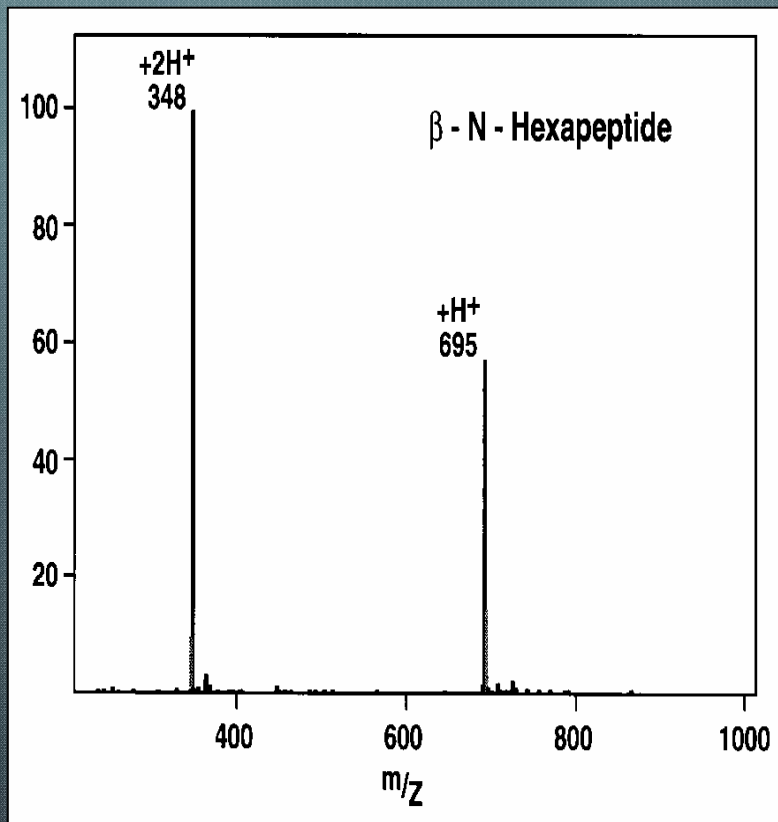
C18 Chromatography

# Flow Chart for Reference Methods



Approved by IFCC 2001

# ESI mass spectra of nonglycated and glycated bN-terminal hexapeptides

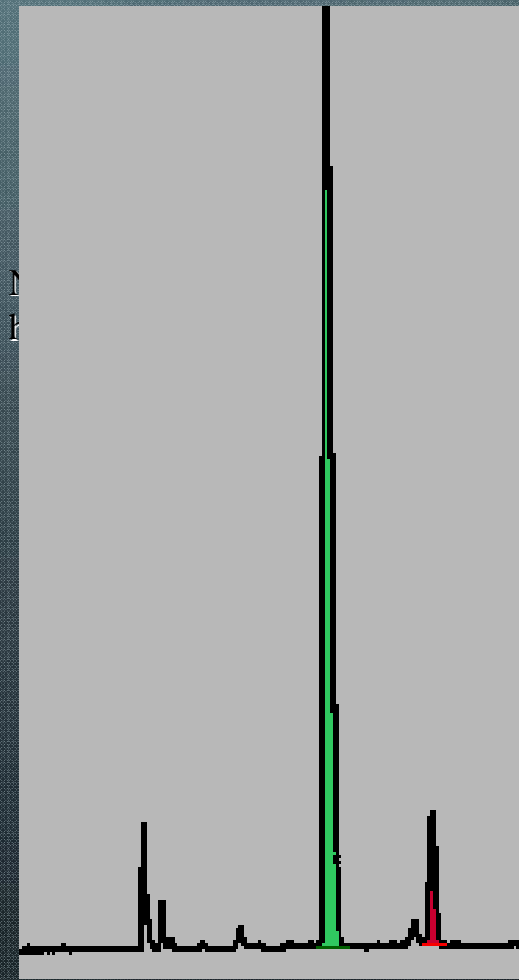
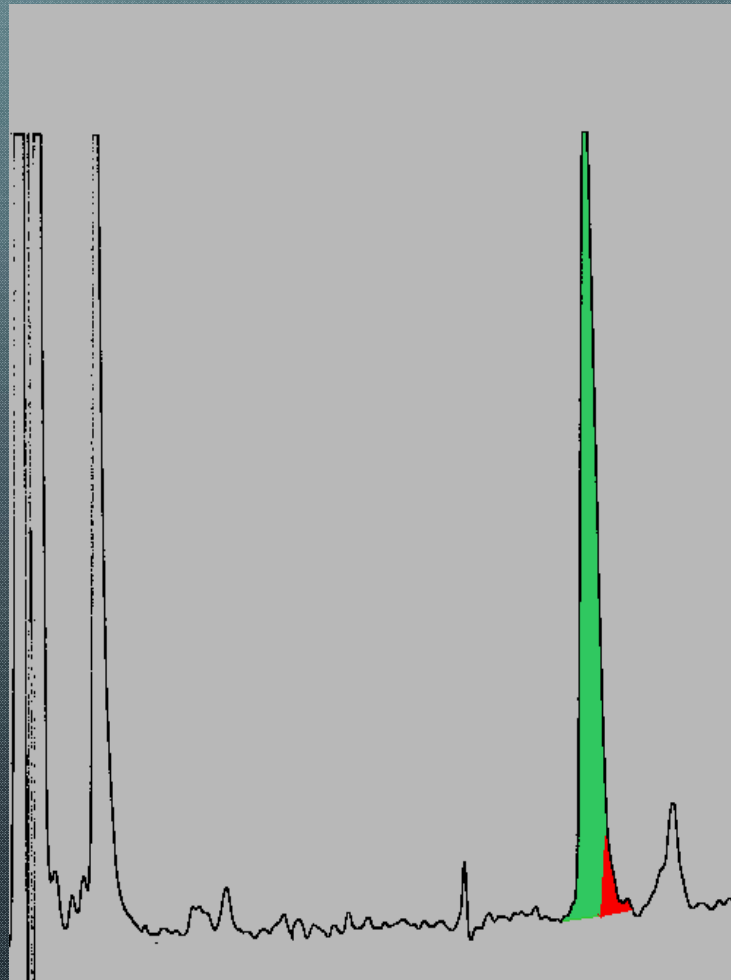


*Kobold et al Clin Chem 1977;43;1944*

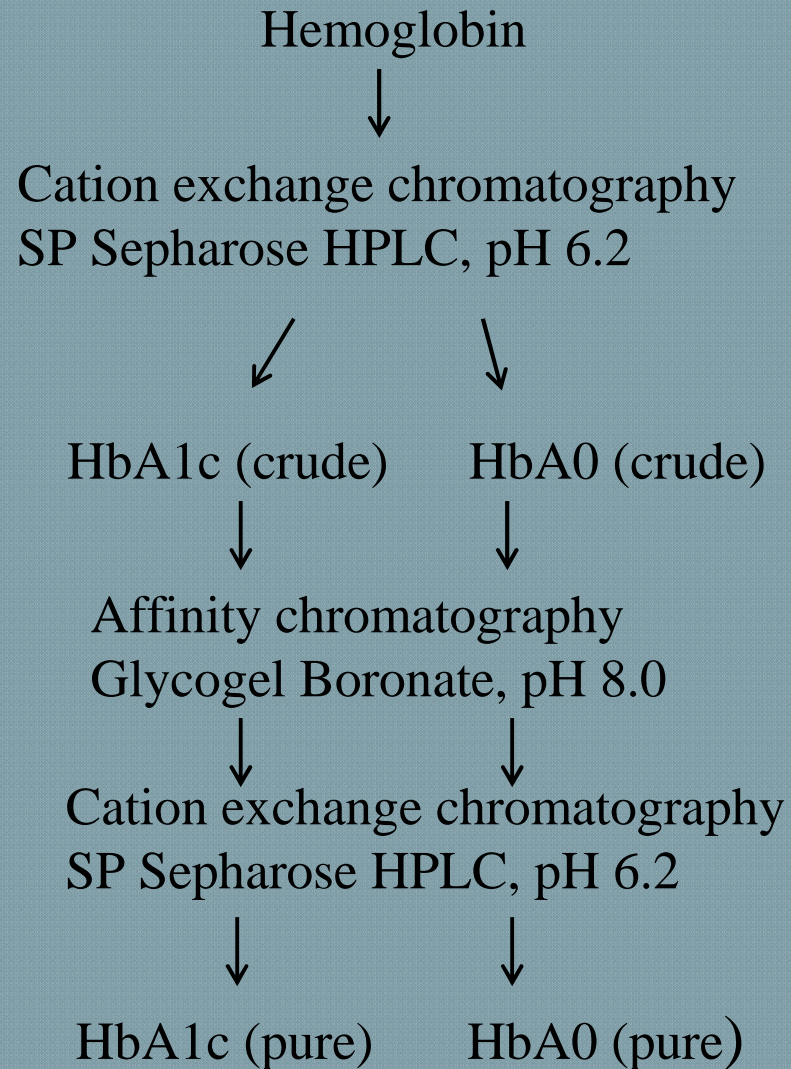
# Two-dimensional separation of N-terminal hexapeptides of hemoglobin

C 18 Chromatography

Capillary Electrophoresis



# Preparation of calibrators



HbA<sub>0</sub>

HbA<sub>1c</sub>



EUROPEAN COMMISSION  
JOINT RESEARCH CENTRE  
Institute for Reference Materials and Measurements



**CERTIFIED REFERENCE MATERIAL  
IRMM/IFCC- 467**

CERTIFICATE OF ANALYSIS

HAEMOGLOBIN ISOLATED FROM WHOLE BLOOD	
	Amount-of-substance fraction
	Certified value <sup>1)</sup> [mmol/mol]
HbA0/(HbA1c + HbA0) <sup>2)</sup>	> 976
<p>1) The certified value was calculated from the average of the results for the amount-of-substance fraction of HbA1c for two accepted datasets and converted into amount-of-substance fraction HbA0 (1000 - HbA1c mmol/mol). Measurements were carried out using the IFCC reference measurement procedure and were further confirmed by other methods. The certified value, expressed as mmol HbA0 per mol HbA1c plus HbA0, is traceable to the SI. With a 95 % probability, the true value of the material is above this level.</p> <p>2) HbA1c is defined as the beta-N-(1-deoxyfructos-1-yl) haemoglobin. HbA0 is defined as the non-glycated haemoglobin.</p>	

This certificate is valid for one year after purchase.

Sales date:

The minimum amount of sample to be used is 20 µL.

Geel, March 2007

Signed:   
Prof. Dr. Hendrik Emons  
Unit for Reference Materials  
EC-JRC-IRMM  
Retieseweg 111  
2440 Geel, Belgium



EUROPEAN COMMISSION  
JOINT RESEARCH CENTRE  
Institute for Reference Materials and Measurements



**CERTIFIED REFERENCE MATERIAL  
IRMM/IFCC- 466**

CERTIFICATE OF ANALYSIS

HAEMOGLOBIN ISOLATED FROM WHOLE BLOOD		
	Amount-of-substance fraction	
	Certified value <sup>1)</sup> [mmol/mol]	Uncertainty <sup>2)</sup> [mmol/mol]
HbA1c/(HbA0 + HbA1c) <sup>3)</sup>	934	22
<p>1) The certified value was calculated from the average of the results for the amount-of-substance fraction of HbA0 versus HbA0 plus HbA1c for three accepted datasets and converted into amount-of-substance fraction HbA1c (1000 mmol/mol - HbA0 mmol/mol). Measurements were carried out using the IFCC reference measurement procedure and were further confirmed by other methods. The certified value, expressed as mmol HbA1c per mol HbA1c plus HbA0, is traceable to the SI.</p> <p>2) The certified uncertainty is the expanded uncertainty estimated in accordance with the Guide to the Expression of Uncertainty in Measurement (GUM) with a coverage factor <math>k = 2</math>, corresponding to a level of confidence of about 95 %.</p> <p>3) HbA1c is defined as the beta-N-(1-deoxyfructos-1-yl) haemoglobin. HbA0 is defined as the non-glycated haemoglobin.</p>		

This certificate is valid for one year after purchase.

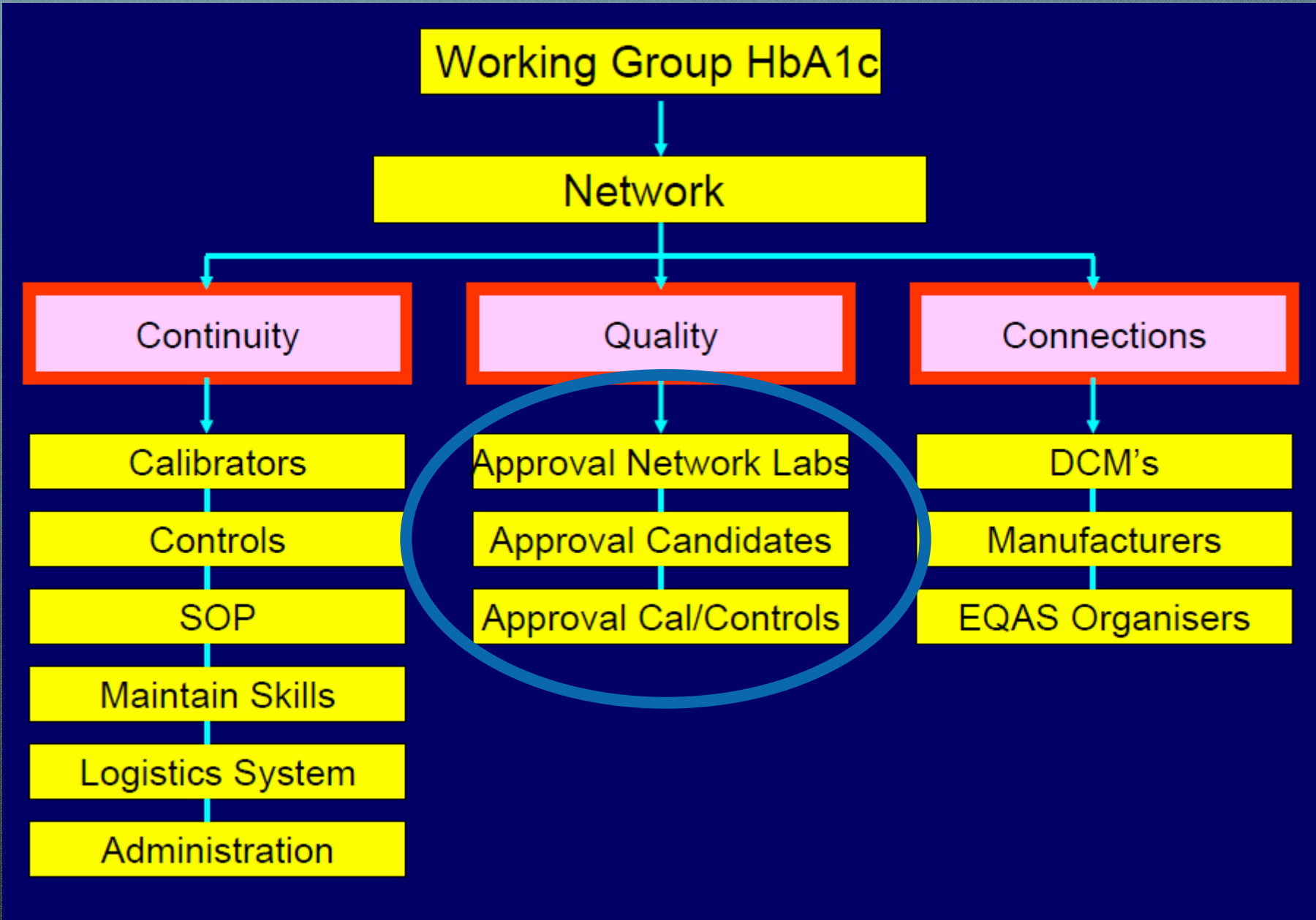
Sales date:

The minimum amount of sample to be used is 20 µL.

Geel, March 2007

Signed:   
Prof. Dr. Hendrik Emons  
Unit for Reference Materials  
EC-JRC-IRMM  
Retieseweg 111  
2440 Geel, Belgium

U<sub>c</sub> = 1.18 %





# Clinical Chemistry

Clin  
Chem  
2004; 50:  
166-74

## IFCC Reference System for Measurement of Hemoglobin A<sub>1c</sub> in Human Blood and the National Standardization Schemes in the United States, Japan, and Sweden: A Method-Comparison Study

WIELAND HOELZEL,<sup>1</sup> CAS WEYKAMP,<sup>2</sup> JAN-OLOF JEPPSSON,<sup>3</sup> KOR MIEDEMA,<sup>4\*</sup> JOHN R. BARR,<sup>5</sup>  
IAN GOODALL,<sup>6</sup> TADAO HOSHINO,<sup>7</sup> W. GARRY JOHN,<sup>8</sup> UWE KOBOLD,<sup>1</sup> RANDIE LITTLE,<sup>9</sup>  
ANDREA MOSCA,<sup>10</sup> PIERLUIGI MAURI,<sup>11</sup> RITA PARONI,<sup>12</sup> FRANSISCUS SUSANTO,<sup>13</sup>  
IZUMU TAKEI,<sup>14</sup> LINDA THIENPONT,<sup>15</sup> MASAO UMEMOTO,<sup>16</sup> and HSIAO-MEI WIEDMEYER,<sup>9</sup> on  
behalf of the IFCC WORKING GROUP ON HbA<sub>1c</sub> STANDARDIZATION

CERTIFIED REFERENCE MATERIAL  
IFCC HbA<sub>1c</sub> NETWORK PURE HbA<sub>0</sub>

CERTIFICATE OF ANALYSIS

HbA <sub>0</sub> <sup>(a)</sup> isolated from human whole blood		
Batch:	14224300	
Expiry Date:	February 2019	
<i>Parameter</i>	<i>Certified Value</i>	<i>Uncertainty</i>
HbA <sub>0</sub>	≥ 999.8 mmol/mol <sup>(b)</sup>	
Total Hb	146.0 mg/gram <sup>(c)</sup>	2.11 mg/gram <sup>(d)</sup>

(a) HbA<sub>0</sub> is defined as the non-glycated hemoglobin.  
 (b) The certified value of HbA<sub>0</sub> is expressed as mmol HbA<sub>0</sub> per mol (HbA<sub>1c</sub> + HbA<sub>0</sub>) and measured according to the SOP of the IFCC HbA<sub>1c</sub> Reference Measurement Procedure (1,2).  
 (c) Total Hb is expressed as mg/gram and measured according to the SOP of the IFCC HbA<sub>1c</sub> Reference Measurement Procedure (1,2).  
 (d) The uncertainty is the expanded uncertainty at the 95% level of confidence (k=2).

Winterswijk, 3 February 2012

Signed: 

Dr. C.W. Weykamp  
IFCC HbA<sub>1c</sub> Network Coordinator

CERTIFIED REFERENCE MATERIAL  
IFCC HbA<sub>1c</sub> NETWORK PURE HbA<sub>1c</sub>

CERTIFICATE OF ANALYSIS

HbA <sub>1c</sub> <sup>(a)</sup> isolated from human whole blood		
Batch:	30226400	
Expiry Date:	February 2019	
<i>Parameter</i>	<i>Certified Value</i>	<i>Uncertainty</i>
HbA <sub>1c</sub>	889.5 mmol/mol <sup>(b)</sup>	1.4 mmol/mol <sup>(d)</sup>
Total Hb	38.52 mg/gram <sup>(c)</sup>	0.12 mg/gram <sup>(d)</sup>

(a) HbA<sub>1c</sub> is defined as the beta-N-(1-deoxyfructos-1-yl) hemoglobin. HbA<sub>0</sub> is defined as the non-glycated haemoglobin.  
 (b) The certified value of HbA<sub>1c</sub> is expressed as mmol HbA<sub>1c</sub> per mol (HbA<sub>1c</sub> + HbA<sub>0</sub>) and measured according to the SOP of the IFCC HbA<sub>1c</sub> Reference Measurement Procedure (1, 2).  
 (c) Total Hb is expressed as mg/gram and measured according to the SOP of the IFCC HbA<sub>1c</sub> Reference Measurement Procedure (1,2).  
 (d) The uncertainty is the expanded uncertainty at the 95% level of confidence (k=2).

U<sub>c</sub> = 0.8 %

Winterswijk, 3 February 2012

Signed: 

Dr. C.W. Weykamp  
IFCC HbA<sub>1c</sub> Network Coordinator

**Intended Purpose**

The material has only one purpose: to be used together with IFCC HbA<sub>1c</sub> Network pure HbA<sub>0</sub> to manufacture the primary calibrators (Pcal) for the IFCC HbA<sub>1c</sub> Network of Reference Laboratories operating the IFCC HbA<sub>1c</sub> Reference Measurement Procedure.

**Product description**

The material is prepared from human whole blood obtained from non-diabetic volunteers. The material is deep frozen (<-70°C) and contains 50 mmol/L MES, 10 mmol/L KCN, 2 mmol/L EDTA and the pH is 6.2.

## IFCC HbA<sub>1c</sub> NETWORK PRIMARY CALIBRATORS PRODUCT CERTIFICATE

Calibrator set IFCC HbA <sub>1c</sub> Reference Measurement Procedure					
Batch:		Pcal 2012			
Expiry Date:		February 2019			
<b>Raw materials</b>					
IFCC HbA <sub>1c</sub> Network pure HbA <sub>1c</sub>		Batch: 30226400			
IFCC HbA <sub>1c</sub> Network pure HbA <sub>0</sub>		Batch: 14224300			
<b>Assigned Values</b>					
Vial #	HbA <sub>1c</sub> <sup>(a)</sup> mmol/mol Hb	Ratio <sup>(b)</sup> [HbA <sub>1c</sub> ]/[HbA <sub>0</sub> ]	U <sup>(c)</sup>	Total Hb <sup>(d)</sup> (mg)	Volume <sup>(d)</sup> (μL)
Pcal 2012 - A	0.0	0.00000	0.01	1	30.0
Pcal 2012 - B	29.3	0.03018	0.16	1	30.0
Pcal 2012 - C	58.7	0.06236	0.32	1	30.0
Pcal 2012 - D	87.5	0.09589	0.46	1	30.0
Pcal 2012 - E	117.4	0.13302	0.59	1	30.0
Pcal 2012 - F	146.9	0.17220	0.71	1	30.0
<p>(a) HbA<sub>1c</sub> is defined as the beta-N-(1-deoxyfructos-1-yl) hemoglobin and expressed as mmol HbA<sub>1c</sub> per mol (HbA<sub>1c</sub> + HbA<sub>0</sub>). The concentration is calculated from the mixed amounts of pure HbA<sub>1c</sub> (1) and pure HbA<sub>0</sub> (2) according to the SOP of the IFCC HbA<sub>1c</sub> Reference Measurement Procedure (3,4,5).</p> <p>(b) The Ratio [HbA<sub>1c</sub>]/[HbA<sub>0</sub>] is the ratio of the HbA<sub>1c</sub> concentration in mmol/mol and the total hemoglobin concentration in mmol/mol (1000 - HbA<sub>1c</sub> concentration in mmol/mol) according to the SOP of the IFCC HbA<sub>1c</sub> Reference Measurement Procedure (3,4,5).</p> <p>(c) The uncertainty (U) is the expanded uncertainty at the 95% level of confidence (k=2) and calculated according to ref. 6.</p> <p>(d) Before dispensing the calibrator solutions in the vials, the total hemoglobin concentration is adjusted to 33.3 mg/mL. Then 30.0 μL is dispensed which results in 1 mg hemoglobin/vial. This concept facilitates convenient and precise handling of the calibrators: for the enzymatic digestion the content does not have to be removed from the vials as simply 50 μL of the enzyme solution can be added to the vial.</p>					
<b>Approval Measurements</b>					
Prior to release the calibrator set has been evaluated and approved by the following network laboratories as part of the 2012 California IFCC HbA <sub>1c</sub> intercomparison study (7).					
CHU de Reims (HPLC-ESI/MS)		Roche Diagnostics GmbH (HPLC-ESI/MS)			
INSTAND e.v. (HPLC-ESI/MS and HPLC-CE)		School of Medicine Keio University (HPLC-ESI/MS)			
Isala (HPLC-CE)		Shanghai Center for Clinical Laboratory (HPLC-CE)			
Korea Centers for Disease Control and Prevention (HPLC-ESI/MS)		Siemens HealthCare Diagnostics (HPLC-ESI/MS)			
Queen Beatrix Hospital (HPLC-CE)		Suraksha Diagnostics Pvt. Ltd (HPLC-CE)			
Institute of Biopathological medicine (HPLC-ESI/MS and HPLC-CE)		Universita degli Studi di Milano (HPLC-ESI/MS and HPLC-CE)			
ReCCs (HPLC-ESI/MS)		University of Missouri School of Medicine (HPLC-CE)			

U<sub>c</sub> = 0.27 %

U<sub>c</sub> = 0.26 %



Network on HbA<sub>1c</sub> Standardization

# REPORT

## Intercomparison Studies Shanghai-1 and Shanghai-2

of the

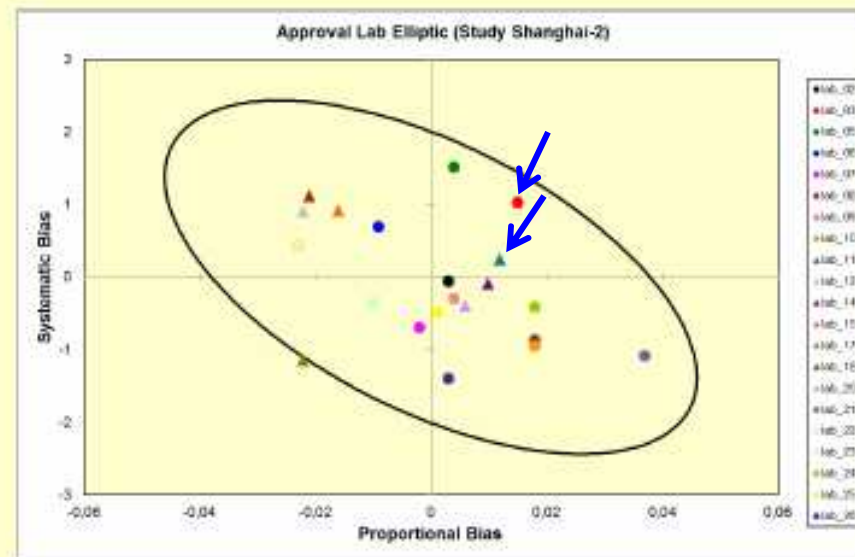
IFCC Network on Standardisation of HbA<sub>1c</sub>

Dr. Cas Weykamp, IFCC Network Coordinator  
Carla Siebelder, BSc, Associate IFCC Network Coordinator

1 November 2016

20

Lab	Systematic Bias (abscis)	Proportional Bias (slope)	Combined Statistical Test Critical Value 10.6
lab_02	-0,06	0,003	0,1
lab_03	1,03	0,015	6,9
lab_05	1,52	0,004	7,1
lab_06	0,70	-0,009	0,9
lab_07	-0,69	-0,002	1,5
lab_08	-0,87	0,018	1,9
lab_09	-0,30	0,004	0,2
lab_10	-0,95	0,018	2,1
lab_11	0,24	0,012	1,7
lab_12	0,91	-0,022	2,5
lab_14	-0,09	0,010	0,7
lab_15	0,92	-0,016	1,8
lab_17	-1,15	-0,022	11,2
lab_18	1,12	-0,021	2,9
lab_20	-0,40	0,006	0,3
lab_21	-1,08	0,037	6,7
lab_22	0,43	-0,023	2,8
lab_23	-0,35	-0,010	1,5
lab_24	-0,41	0,018	1,7
lab_25	-0,48	0,001	0,5
lab_26	-1,39	0,003	4,4



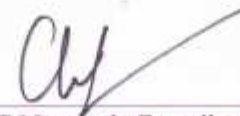
# Certificate

## *Approval of Network Laboratories operating the IFCC Reference Measurement Procedure for HbA<sub>1c</sub>*

This certifies that *Centro per la Riferibilità metrologica in Medicina di Laboratorio (CIRME), Università degli Studi di Milano, Milano, Italy* participated in and passed the criteria\* of the intercomparison studies of the Network of Reference Laboratories operating the IFCC Reference Measurement Procedure for HbA<sub>1c</sub> and has the status of approved Network Laboratory in 2017.

Date of issue: 1 November 2016

Certification expires: 31 December 2017



IFCC Network Coordinator

\* According to Könnert e.a. in "Statistical Rules for Laboratory Networks. Journal of Testing and Evaluation. March 2006, vol 34. Paper ID/JTE 14082"

Working Group HbA1c

Network

Continuity

Calibrators

Controls

SOP

Maintain Skills

Logistics System

Administration

Quality

Approval Network Labs

Approval Candidates

Approval Cal/Controls

Connections

DCM's

Manufacturers

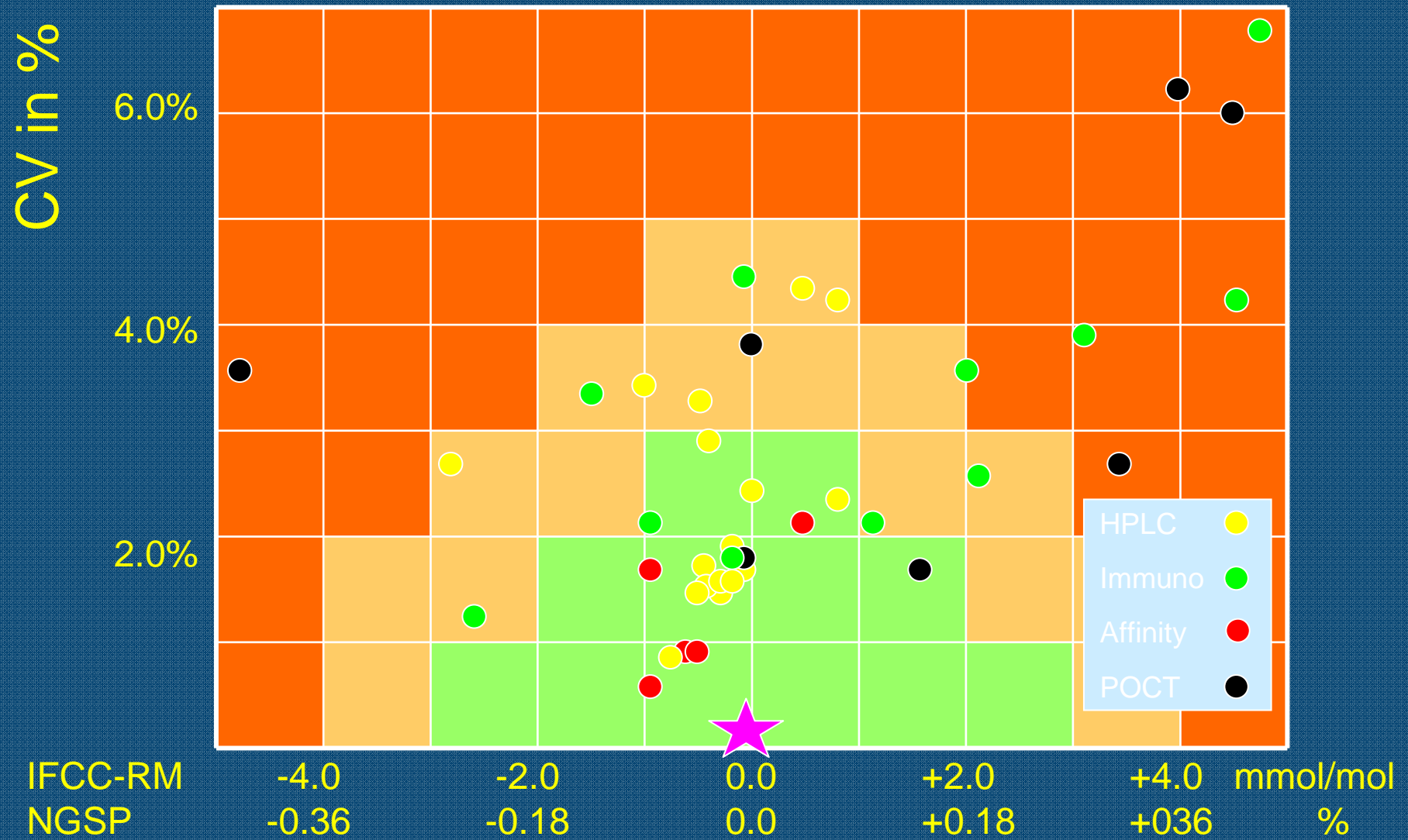
EQAS Organisers

#### 4.2.1.4 *Monitoring Programme*

To prove traceability (especially in respect to the IVD directive), manufacturers appreciate the availability of an independent monitoring programme. This programme is available from the IFCC network. In summary, participating manufacturers receive 24 frozen whole blood samples once a year. Following the list with deadlines, every two weeks a specimen is assayed. Results are submitted via the interactive website of the IFCC HbA<sub>1c</sub> Network, immediately after the deadline for submission is passed the report can be requested from the website. The report shows the submitted result in comparison to the target value. Additionally trend reports (either related to time or to HbA<sub>1c</sub> content) can be requested and once the annual cycle is completed the annual report deals with precision and linearity. Results are strictly confidential: only the manufacturer has access to his results but he is free to use the results. Manufacturers get a certificate of traceability on which their performance in terms of deviation from the IFCC target, precision and linearity are specified. Details and certificate/ instructions can be downloaded from the website.



# Monitoring Programme Manufacturers 2009



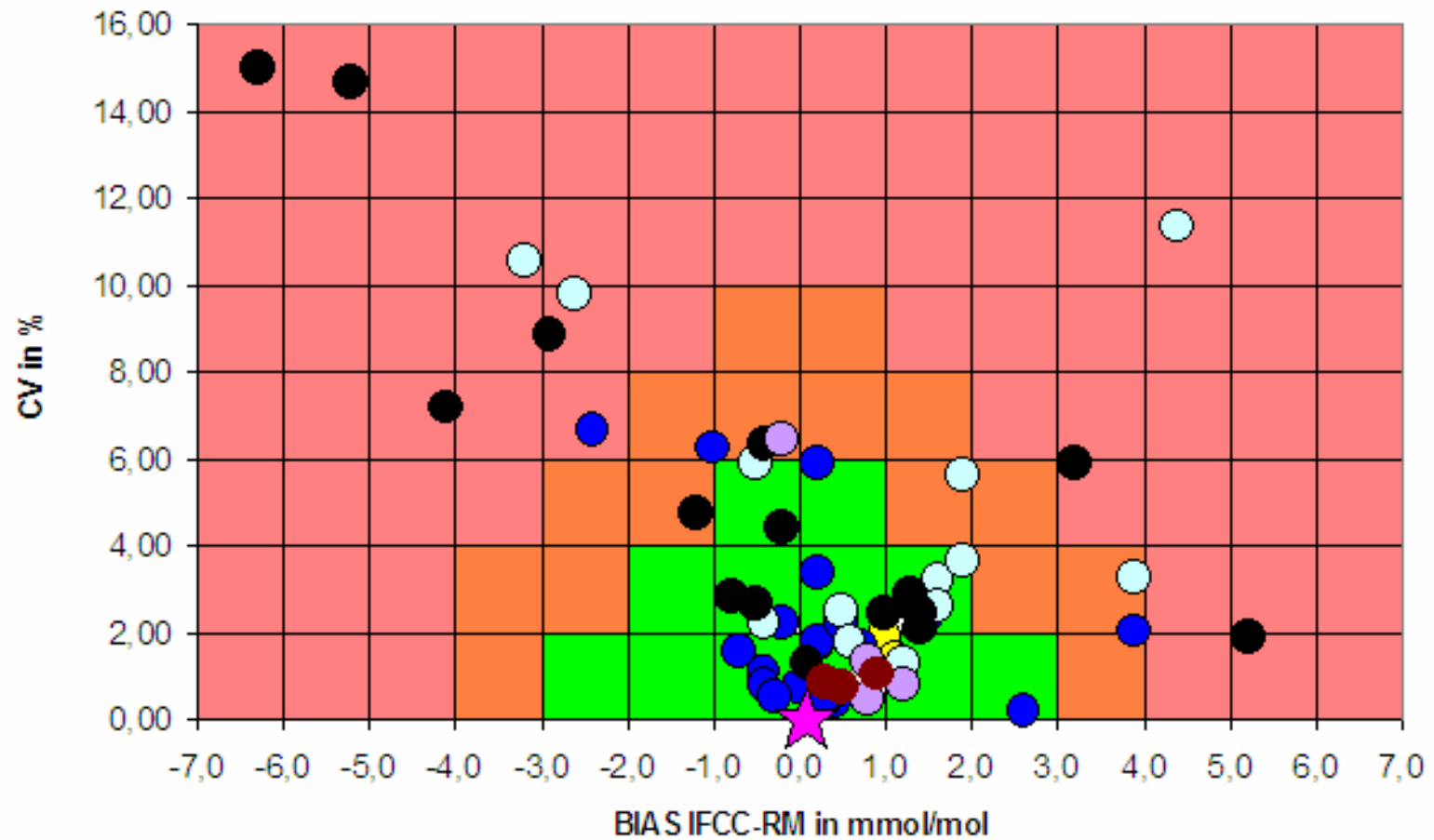
IFCC-RM  
NGSP

-4.0 -2.0 0.0 +2.0 +4.0 mmol/mol  
-0.36 -0.18 0.0 +0.18 +0.36 %

★ Best Performance

Bias

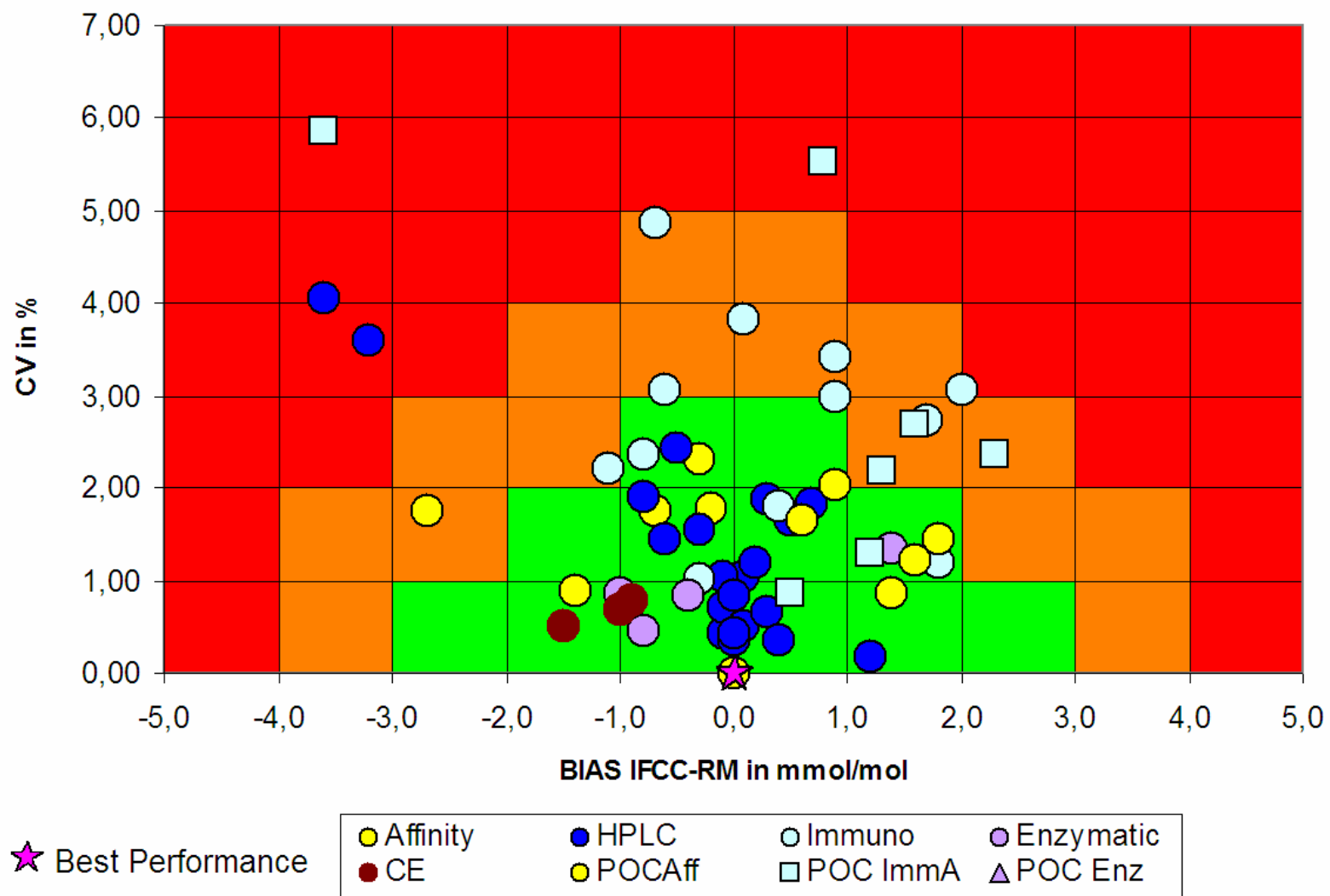
## Monitoring Programme Manufacturers 2013



★ Best Performance




● Affinity ● HPLC ○ Immuno ● POCT ● Enzymatic ● CE

## Monitoring Programme Manufacturers 2015



# middle of the chain

# What information should be provided

-  which kind of higher-order materials and methods to assign traceable values to the calibrators
-  which kind of internal calibration hierarchy
-  uc of commercial calibrators

**Table 1**

Metrological traceability and uncertainty information derived from calibrator package inserts of commercial systems measuring blood glucose marketed by four IVD companies.

Company	Platform	Principle of commercial method	Calibrator	Declared standard uncertainty <sup>a</sup>	Higher-order reference employed		Type of traceability chain used <sup>b</sup>	Combined standard uncertainty associated with the used chain <sup>c</sup>
					Method	Material		
Abbott	Architect	ND	Multiconstituent calibrator	2.70%	IDMS	NIST SRM 965	A	1.22–1.45% <sup>d</sup>
Beckman	AU	Hexokinase	System calibrator	ND	ND	NIST SRM 965	A	1.22–1.45% <sup>d</sup>
	Synchron	Hexokinase	Synchron multicalibrator	ND	ND	NIST SRM 917a	D	1.60–3.00% <sup>e</sup>
Roche	Cobas c	Hexokinase	C.f.a.s.	0.84%	IDMS	ND	B	1.70%
	Integra	Hexokinase	C.f.a.s.	0.62%	IDMS	ND	B	1.70%
	Modular	Hexokinase	C.f.a.s.	0.84%	IDMS	ND	B	1.70%
		GOD	C.f.a.s.	0.84%	IDMS	ND	B	1.70%
Siemens	Advia	Hexokinase	Chemistry calibrator	1.30%	Hexokinase	NIST SRM 917a	C	1.88–3.26% <sup>f</sup>
		GOD	Chemistry calibrator	0.80%	Hexokinase	NIST SRM 917a	C	1.88–3.26% <sup>f</sup>

## Verification of in vitro medical diagnostics (IVD) metrological traceability: Responsibilities and strategies

Federica Braga\*, Mauro Panteghini

Clin Chim Acta 2014;432:55-61

# Certificate

## *Traceability of Manufacturers to the IFCC Reference Measurement Procedure for HbA1c*

This certifies that [REDACTED] using [REDACTED], participates in the Monitoring Programme to demonstrate traceability. In the Monitoring Programme of 2015 the following performance was seen:

Deviation from IFCC-target	at 30 mmol HbA1c/mol Hb :	0.1
	at 60 mmol HbA1c/mol Hb :	0.0
	at 90 mmol HbA1c/mol Hb :	-0.1
Reproducibility, coefficient of variation		0.42%
Linearity, correlation coefficient		0.9995

Date of issue: 4 December 2015

Certification expires: 31 December 2016

  
Dr. C.W. Weykamp  
IFCC HbA1cNetwork Coordinator

## Product certificate HbA1c

Product name



Product number

4755

Product code

Level	Product code	Colour cap
Low	Level 1	Green
High	Level 2	Red

Batch number and  
Expiry date

Level	Batch number	Exp. Date stored at 30°C / -16°C
Low	05046/01	2021/03
High	06046/01	2021/03

Reconstitution volume

0.6 mL with a precision of  $\pm 1.0\%$

Haemoglobin

Level	Hb (mmol/L)
Low	7.1
High	7.0




Assigned Values\*

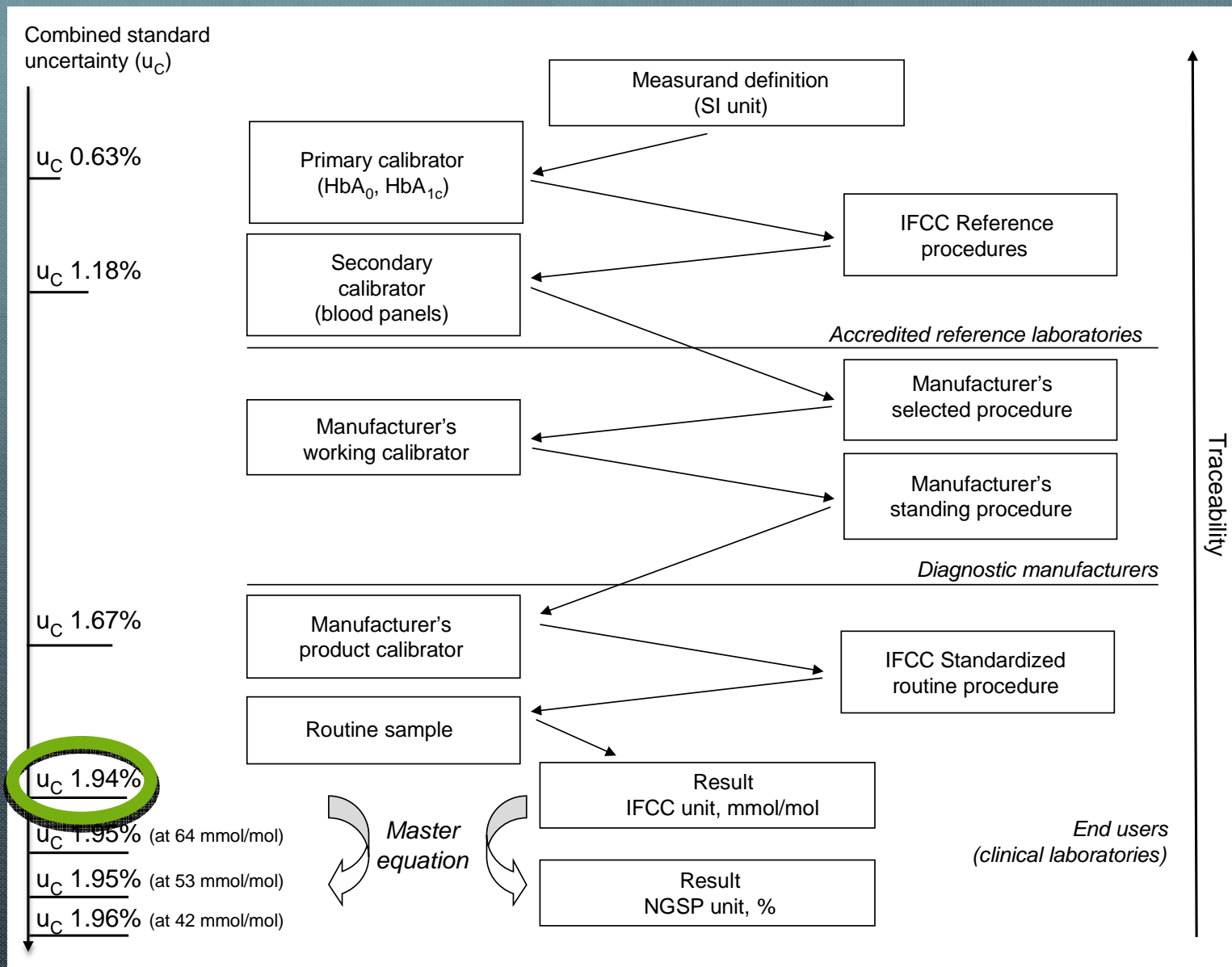
Level	Certified Value (Expanded Uncertainty, k=2)	
	IFCC mmol/mol	DCCT %
Low	36.8 (0.7)	5.52 (0.06)
High	86.8 (1.5)	10.09 (0.14)

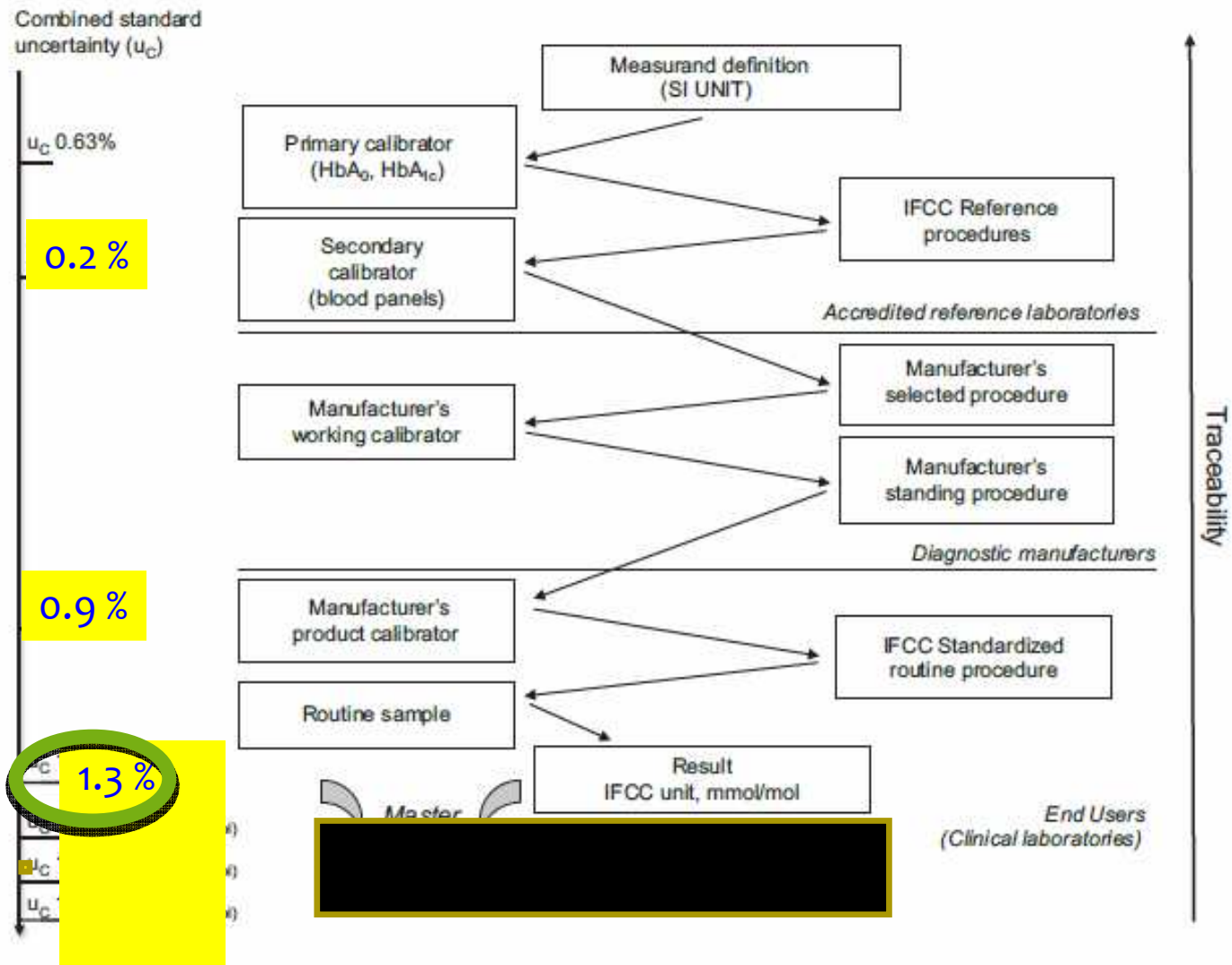


# bottom of the chain

# What should be done to prove the metrological traceability - uncertainties -

-  The use of a traceable method
-  The uncertainty of the commercial calibrators
-  The long-term imprecision of the routine method





**Figure 1** HbA<sub>1c</sub> reference measurement system and associated combined standard uncertainty. NGSP, National Glycohemoglobin Standardisation Program; SI, International System of Measurement.

## Other factors contributing to the uncertainty

 Bias

 Biological variation

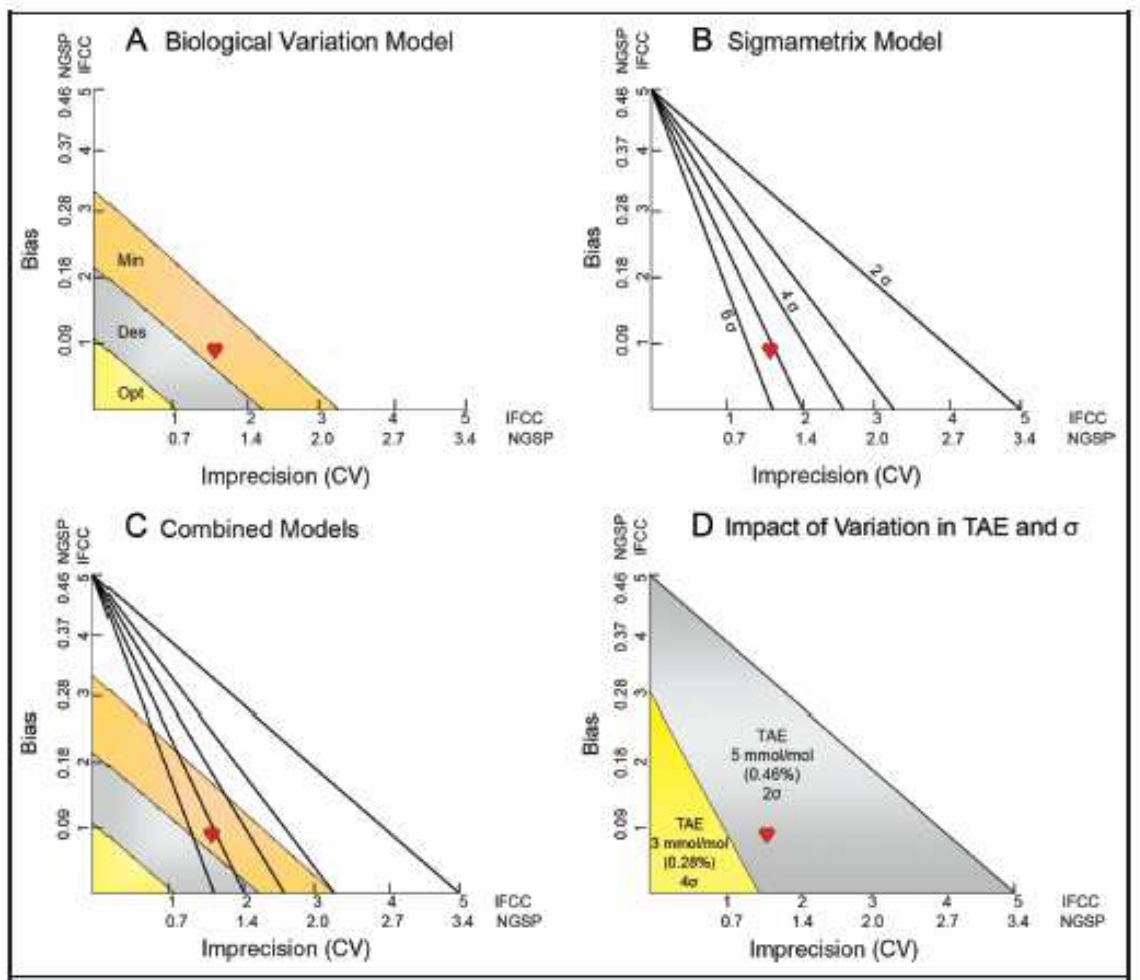
$$u(\text{composite}) = (u^2\text{IQC} + u^2\text{EQC} + u^2\text{intra-individual})^{1/2}$$

EDMA position paper – Estimation of uncertainty of measurement in medical laboratories. [www.edma-ivd.be](http://www.edma-ivd.be); 2006/09

# What should be done to guarantee the long-term performance

 analytical goals

 EQAS



**Fig. 1. Quality target models.** Imprecision (CV) on the x axis and bias on the y axis. Quality targets are shown in colors for the BV model (A) [optimum (opt), desirable (des), minimum (min)] and with lines for the SM model (B) ( $2\sigma$ - $6\sigma$ ). (C), Both models. The red heart represents the performance of a laboratory with a bias of 1 mmol/mol (0.9%), an imprecision of 1.5% (1.0%), and a TE of 2.5 mmol/mol (0.23%) and meets the minimum performance criterion in the BV model and the  $5\sigma$  criterion in the SM model. (D), Impact of varying the set TAE and acceptable  $\sigma$  value on performance.

## Revaluation of biological variation of glycated hemoglobin (HbA<sub>1c</sub>) using an accurately designed protocol and an assay traceable to the IFCC reference system

Federica Braga <sup>a,b,\*</sup>, Alberto Dolci <sup>b</sup>, Martina Montagnana <sup>c</sup>, Franca Pagani <sup>d</sup>, Renata Paleari <sup>a</sup>, Gian Cesare Guidi <sup>c</sup>, Andrea Mosca <sup>a</sup>, Mauro Panteghini <sup>a,b</sup>

**Table 3**

Analytical goals for HbA<sub>1c</sub> measurement derived from data on biological variation.

Quality level	Imprecision, %	Bias, %	Total error, %
Optimal	≤0.6	≤±0.9	≤±2.0
Desirable	≤1.3	≤±1.9	≤±3.9
Minimal	≤1.9	≤±2.8	≤±5.9

*Clin Chim Acta* 2011;412:1412





**HbA1c-Ciclo 2006**

Analita: **HbA1c** %

	N.	Out	Media	C.V.	S.D.	Med.na
Tutti	142	2	8.22	8.4	0.7	8.10
Tuo Metodi HbA1c	19	1	8.06	2.7	0.2	8.05

Campione	3 (Scad. 07/04/2006)
Valore assegnato DCCT	8.0
Tuo risultato	7.9

	Diff. S	Diff. %
Tutti	-0.47	-3.89
Tuo Metodi HbA1c	-0.80	-1.99

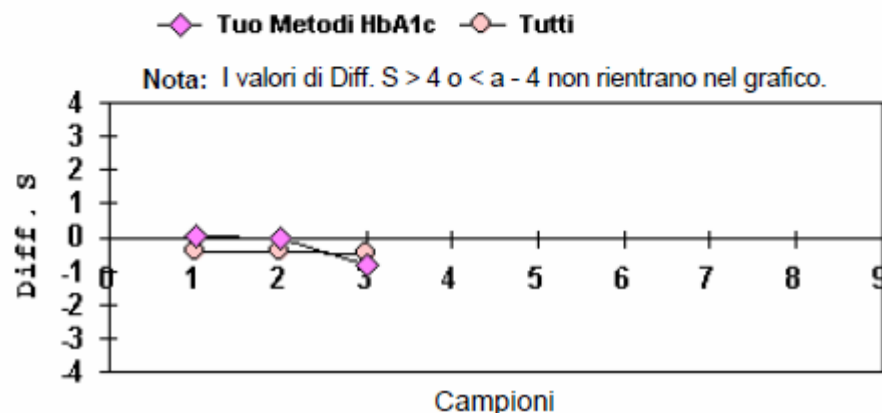
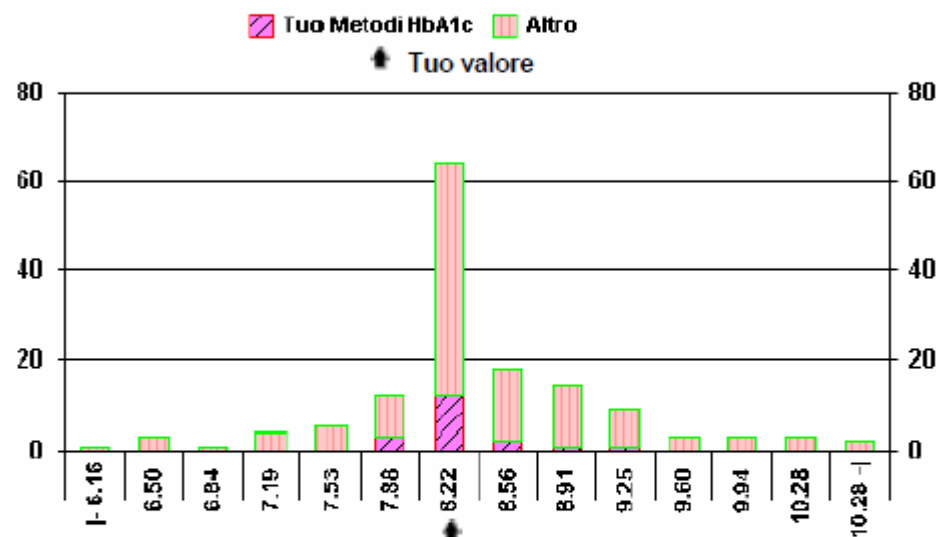
Campioni							
1	2	3	4	5	6	7	8
O	O	O					

O = Interno X = Esterno rispetto ai limiti dichiarati

N. risultati quantitativi	142
N. risultati semiquantitativi/qualitativi	

Riepilogo x Metodi HbA1c risultati quantitativi (> 7 Centri)				
Metodi HbA1c	N.	Out	M.	C.V.
Bio-Rad Variant II Dual kit	19	1	8.06	2.7
Nyocard Reader II	16	1	8.37	11.5
Tosoh G7	16	0	8.02	5.4
Menarini HA 8180	13	0	8.15	2.4
Far scam. ionico	8	0	7.65	16.5
Menarini HA 8140	8	0	7.93	10.5
Roche tina-quant su strum. Hitachi	8	0	8.28	5.3

Tuo Metodi HbA1c  
Bio-Rad Variant II Dual kit





Analita: **HbA1c (IFCC)**

mmol/mol

	N.	Out	Media	C.V.	S.D.	Med.na
Tutti	319	5	65.15	5.7	3.7	65.00
Tuo Metodo HbA1c	19	0	67.79	3.5	2.4	67.00

Campione	3 (Scad. 27/04/2016)	
Tuo risultato	71.0	

	Diff. S	Diff. %
Tutti	1.58	8.98
Tuo Metodo HbA1c	1.34	4.74

Valutazione errore totale							
1	2	3	4	5	6	7	8
○	○	○					

○ = Interno X = Esterno rispetto ai L.A. L.A. camp. corrente: 6

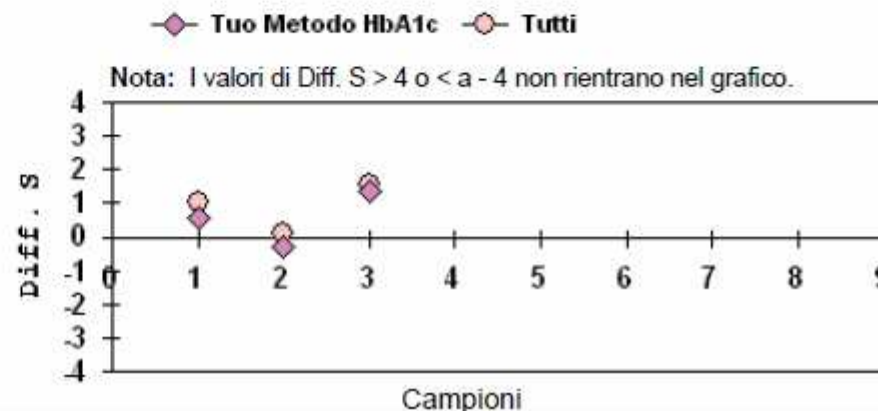
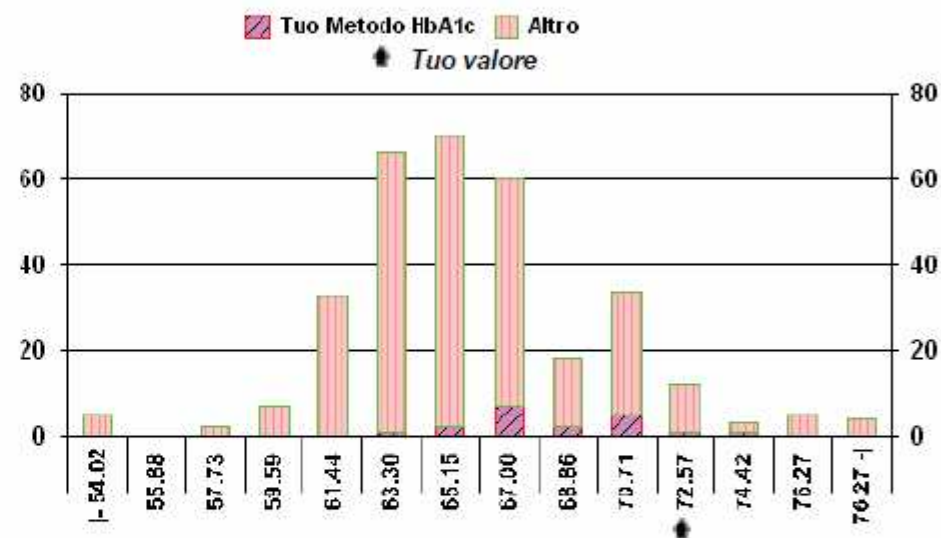
N. risultati numerici	319
N. risultati semiquantitativi/qualitativi	

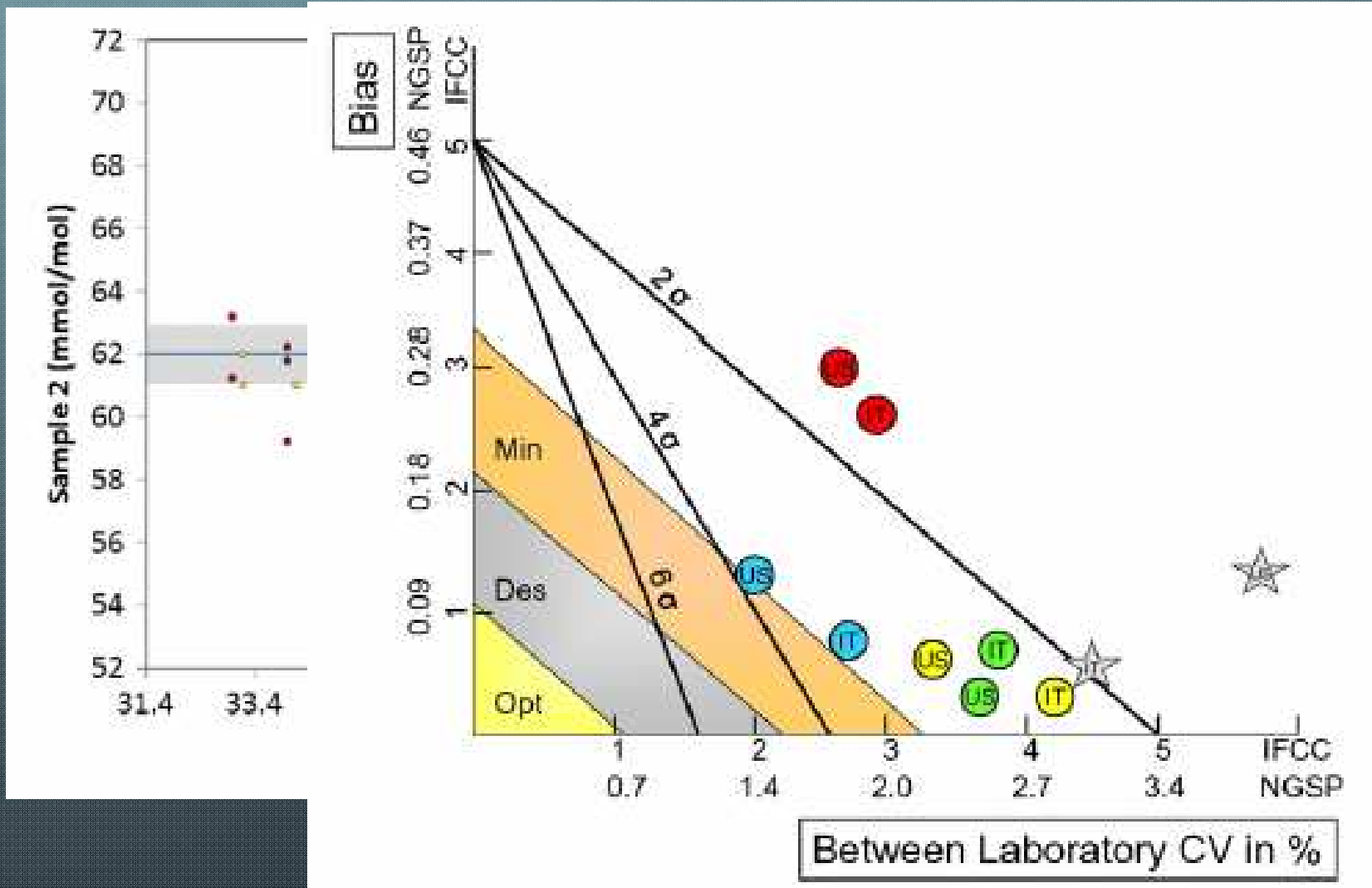
Riepilogo x Metodo HbA1c risultati numerici (> 7 Centri)

Metodo HbA1c	N.	Out	M.	C.V.	u <sub>r</sub>
Tosoh serie G8	94	2	63.49	2.2	0.2
Tosoh serie G7	29	0	64.07	2.7	0.4
Sebia Capillarys HbA1c	28	1	61.24	1.8	0.3
Menarini HA 8160	21	0	67.33	2.3	0.4
<b>Bio-Rad Variant II Dual kit</b>	<b>19</b>	<b>0</b>	<b>67.79</b>	<b>3.5</b>	<b>0.7</b>
Roche tina-quant su strum Cobas	15	0	65.77	4.7	1.0 <sup>a</sup>
Biorad Variant II Turbo	13	0	67.88	2.1	0.5 <sup>a</sup>
Bio-Rad D10 A1c	11	0	62.36	22.3	5.3 <sup>a</sup>
Beckman Synchron LX20/CX	8	0	69.08	3.8	1.2 <sup>a</sup>
Tosoh HLC-723 GX	8	0	62.75	5.9	1.6 <sup>a</sup>

<sup>a</sup> u<sub>r</sub> non trascurabile

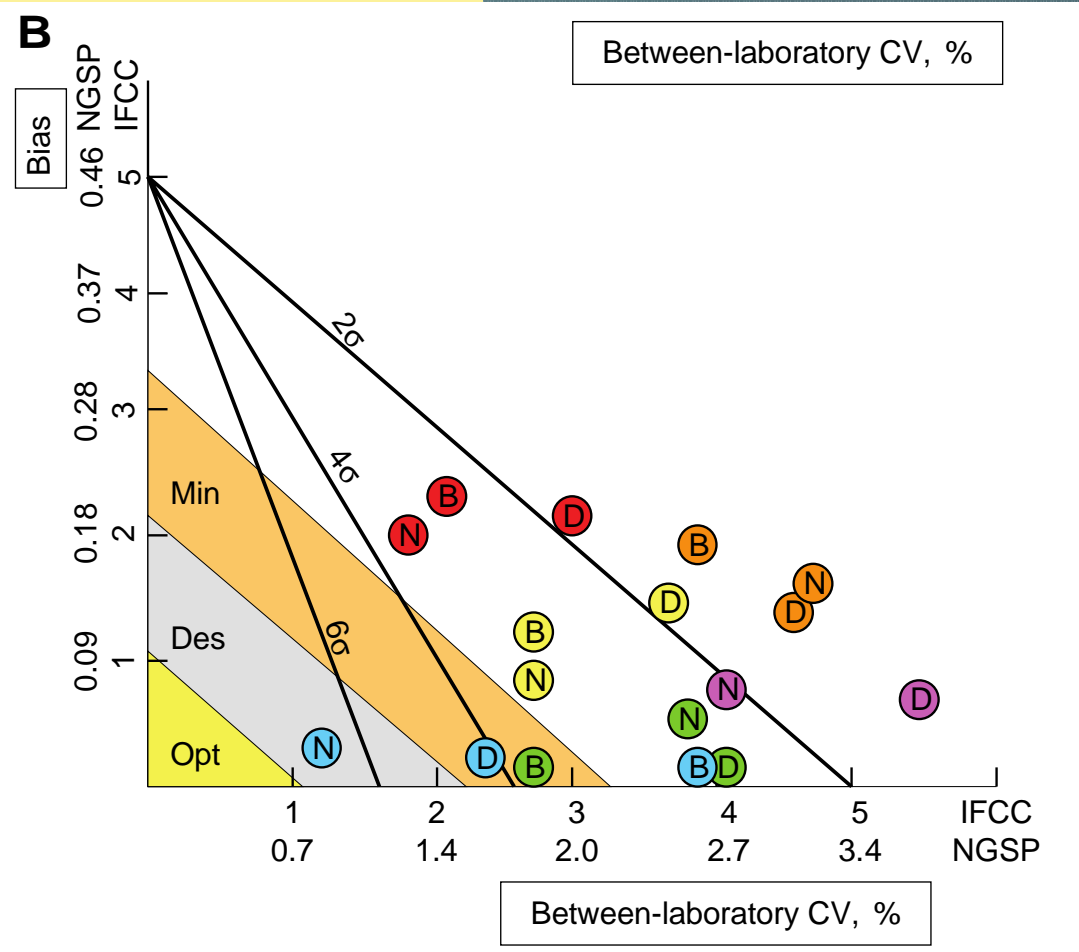
Tuo Metodo HbA1c  
Bio-Rad Variant II Dual kit





Mosca et al, Clin Chim Acta 2015;451:305

**HbA<sub>1c</sub>: EQA in Germany, Belgium and the Netherlands using fresh whole blood samples with target values assigned with the IFCC reference system**



**Figure 2:** The IFCC quality targets model HbA<sub>1c</sub> applied at country and manufacturer level.

# **Standardization of HbA<sub>1c</sub>: are all the pieces in place?**

- **Top of the chain: YES (IFCC Network)**
- **Middle: probably YES (information not uniform and difficult to be released)**
- **Bottom: difficult to draw objective evidences (depending on country and rules); more efforts needed to achieve standardization overall**

# What next?

- **Consensus on uncertainty**
- **Improving EQAS (EurA1c)**
- **Education and communication**

# aknowledgments

- **The IFCC Network laboratories**
- **Manufacturers**
- **Mauro Panteghini and CIRME staff**

## Thanks to the Network of HbA1c Reference Laboratories:

- Germany Prof. Hans Reinauer and Dr. Patricia Kaiser, INSTAND, Düsseldorf  
Dr. Uwe Kobold, Roche, Penzberg  
Dr. Franziscus Susanto, DDZ, Düsseldorf
- Italy Prof. Andrea Mosca, Dr. Renata Paleari and  
Prof. Donatella Caruso, CIRME, Milan
- Japan Dr. Tadao Hoshino, IBM, Kanagawa  
Dr. Masao Umemoto, SRC, Kawasaki  
Prof. Izumi Takei, SKMU, Tokyo, Japan
- The Netherlands Dr. Robbert Slingerland, IKW, Zwolle, The Netherlands  
Dr. Cas Weykamp, SKB, Winterswijk
- USA Dr. Hubert Vesper, CDC, Atlanta, GA  
Dr. Cindy Deng and Dr. Jim Albarella, Siemens MS, Norwood  
Dr. Randie Little, UMSM, Missouri