

Standardization of hemoglobin A_{1c} : myth or reality?

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20th IFCC-EFLM European Congress
of Clinical Chemistry and Laboratory Medicine
45th Congress of the Italian Society of
Clinical Biochemistry and Clinical Molecular Biology (SIBioC)



7th CIRME International Scientific Meeting
Metrological traceability and assay standardization
Stresa, Italy

POST-CONGRESS SATELLITE MEETINGS
May 24th, 2013

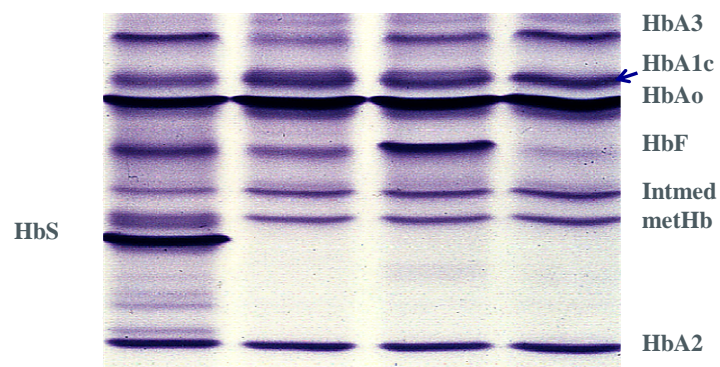
contents

- IFCC approach to the standardization of HbA_{1c}
 - IFCC Network
 - Manufacturers
 - Customers
- Conclusions

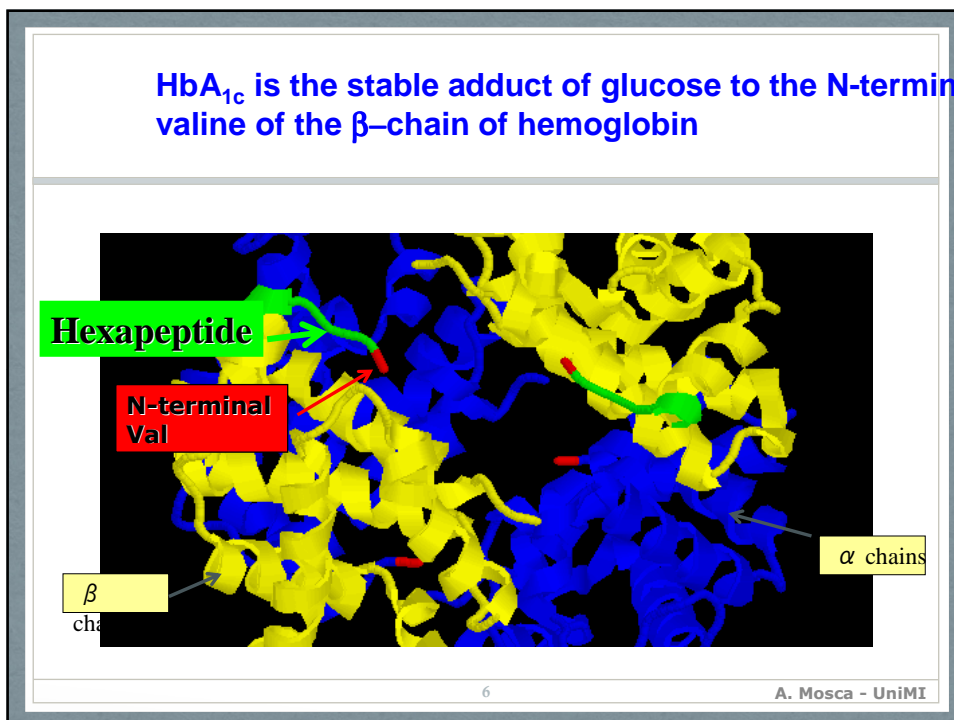
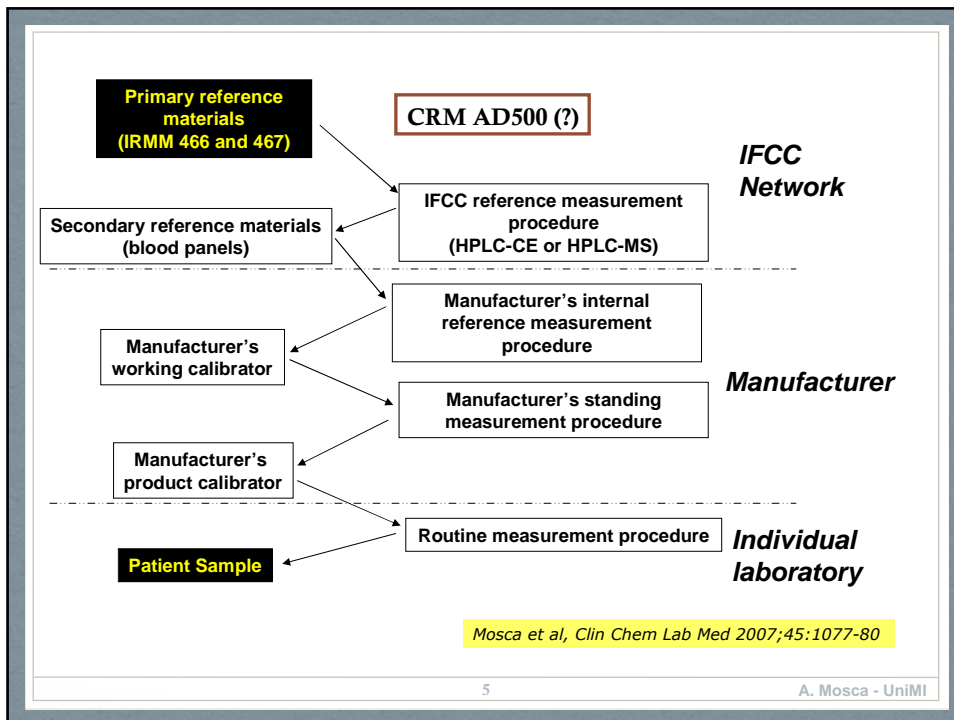
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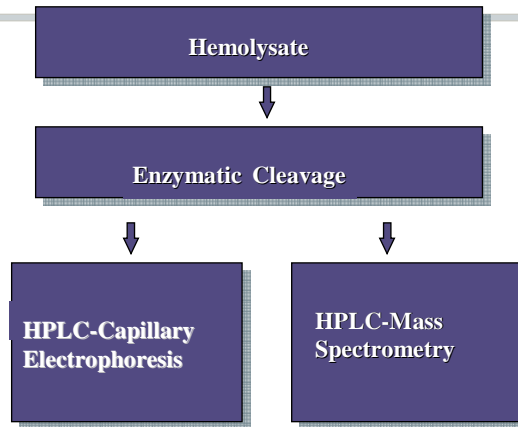
Electrofocusing of hemoglobins



courtesy Prof. J.O. Jeppson



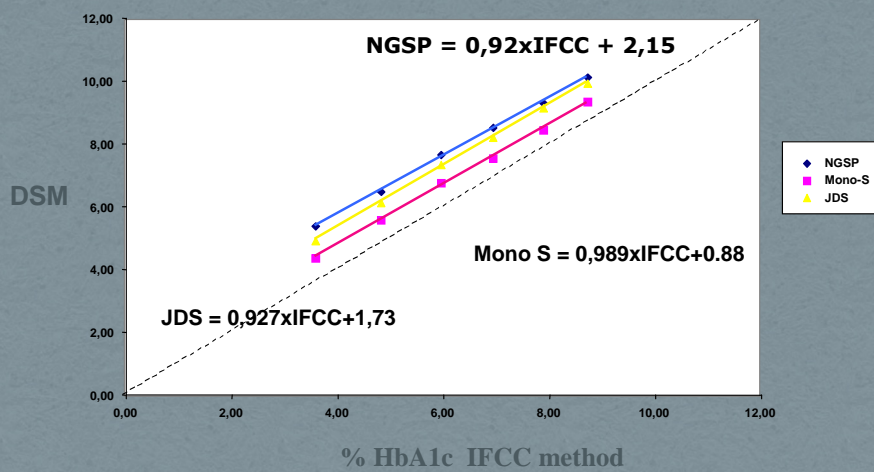
Flow chart for Reference Methods



Approved by IFCC 2001

7 Jeppsson et al, Clin Chem Lab Med 2002;40:78-89

Comparison between the IFCC reference method and three designated comparison methods



8 Hoelzel et al Clin Chem 2004;50:166-74

Clinical Chemistry

CLCH

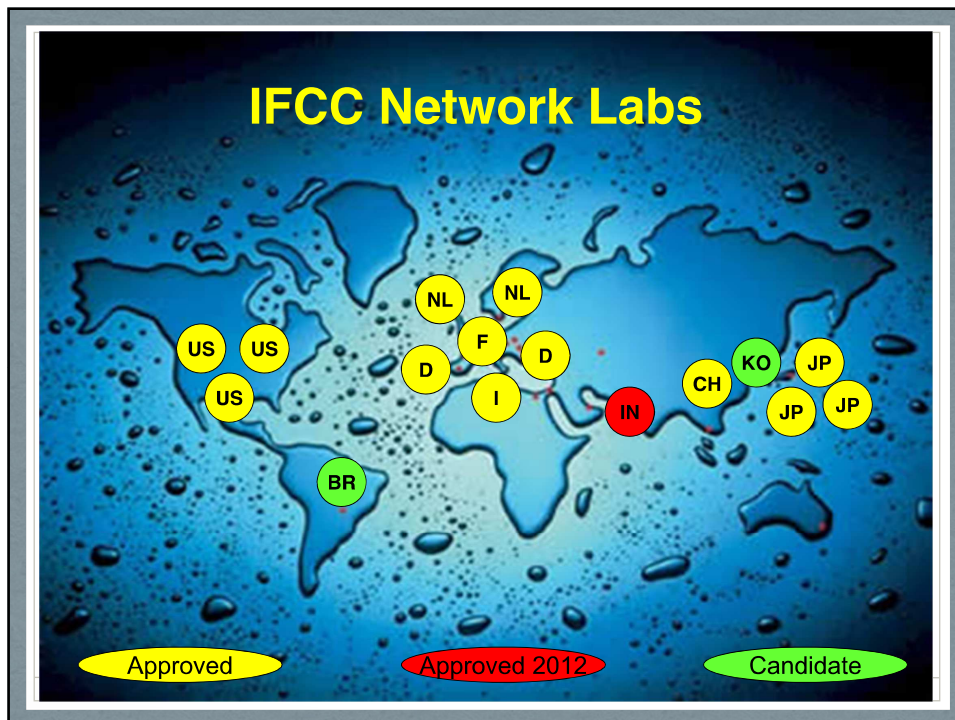
Clin Chem
2004; 50:
166-74

IFCC Reference System for Measurement of Hemoglobin A_{1c} in Human Blood and the National Standardization Schemes in the United States, Japan, and Sweden: A Method-Comparison Study

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

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A. Mosca - UniMI



The IFCC Reference Measurement System for HbA_{1c}: A 6-Year Progress Report

Cas Weykamp,^{1*} W Garry John,² Andrea Mosca,³ Tadao Hoshino,⁴ Randie Little,⁵ Jan-Olof Jeppsson,⁶ Ian Goodall,⁷ Kor Miedema,⁸ Gary Myers,⁹ Hans Reinauer,¹⁰ David B. Sacks,¹¹ Robbert Slingerland,⁸ and Carla Siebelder¹

BACKGROUND: The IFCC Reference Measurement System for hemoglobin (Hb)A_{1c} (IFCC-RM) has been developed within the framework of metrologic traceability and is embedded in a network of 14 reference laboratories. This paper describes the outcome of 12 intercomparison studies (periodic evaluations to control essential elements of the IFCC-RM).

METHODS: Each study included: unknown samples (to test individual network laboratories); known samples (controls); recently manufactured calibrators (to check calculated assigned value); stored calibrators (to test stability) and a calibration-set (to calibrate the IFCC-RM). The unknown samples are measured by use of the IFCC-RM and the designated comparison methods [DCMs; the National Glycohemoglobin Standardization Program (NGSP) in the US, Japanese Diabetes Society/Japanese Society for Clinical Chemistry (JDS/JSCC) in Japan, and Mono-S in Sweden] are used to investigate the stability of the Master Equation (ME), the relationship between IFCC-RM and DCMs.

RESULTS: A total of 105 IFCC-RM data sets were evaluated: 95 were approved, 5 were not, and for 5 no data were submitted. Trend analysis of the MEs, expressed as change in percentage HbA_{1c} per year, revealed 0.000% (NGSP, not significant), -0.030% (JDS/JSCC; significant) and -0.016% (Mono-S; not significant). Evaluation of long-term performance re-

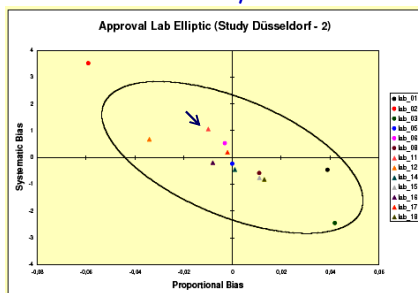
vealed no systematic change over time; 2 laboratories showed significant bias, 1 poor reproducibility. The mean HbA_{1c} determined by laboratories performing mass spectrometry (MS) was the same as the mean determined by laboratories using capillary electrophoresis (CE), but the reproducibility at laboratories using CE was better. One batch of new calibrators was not approved. All stored calibrators were stable.

CONCLUSION: A sound reference system is in place to ensure continuity and stability of the analytical anchor for HbA_{1c}.

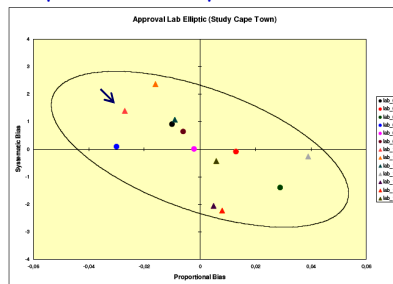
© 2008 American Association for Clinical Chemistry

Hemoglobin A_{1c} (HbA_{1c})^{1,2} is the most important marker for long-term assessment of the glyemic state in patients with diabetes (1). Goals for therapy are set at specific HbA_{1c} target values (2), and the importance of standardization of HbA_{1c} measurement has been well recognized, as reflected by the establishment of national designated comparison methods (DCMs); the National Glycohemoglobin Standardization Program (NGSP) in the US (3), the Japanese Diabetes Society/Japanese Society for Clinical Chemistry (JDS/JSCC) in Japan (4), and Mono-S in Sweden (5). A disadvantage of DCMs is that they are based on arbitrarily chosen analytical methods, with results in arbitrary units. International support is increasing for standardization of laboratory tests that requires a reference system based on the concept of metrologic traceability; the trace-

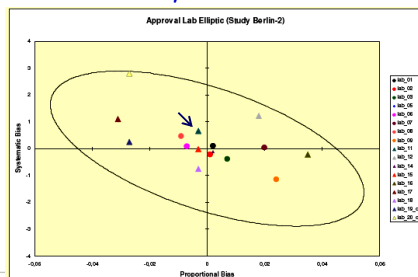
Düsseldorf 2 Study - 2008



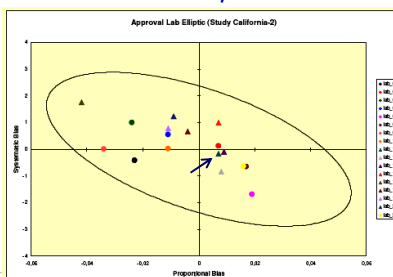
Cape Town Study - 2010

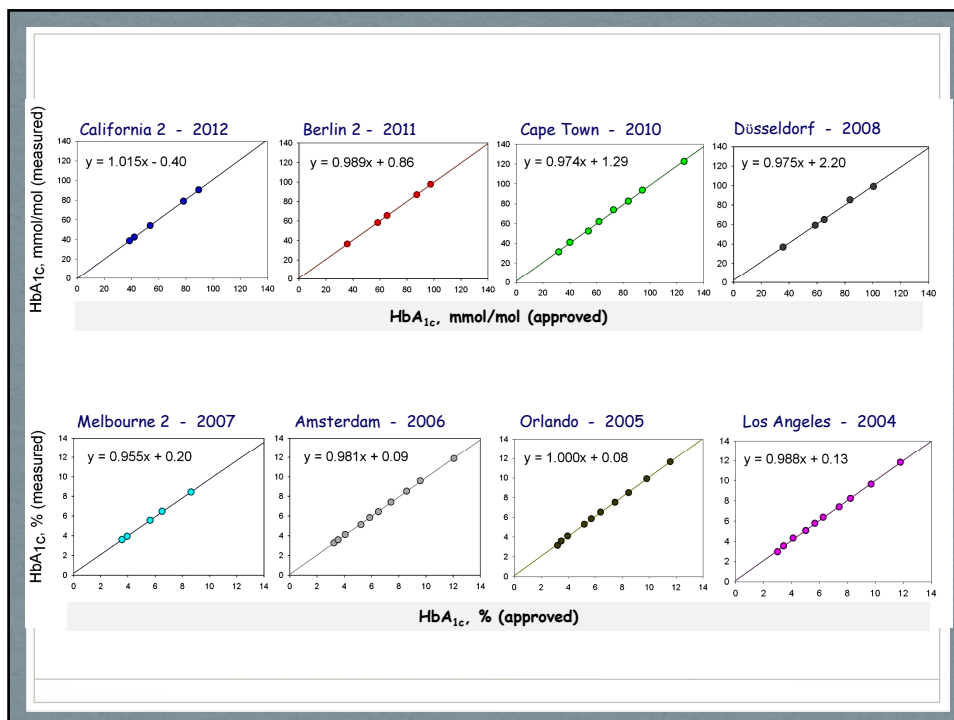


Berlin 2 Study - 2011



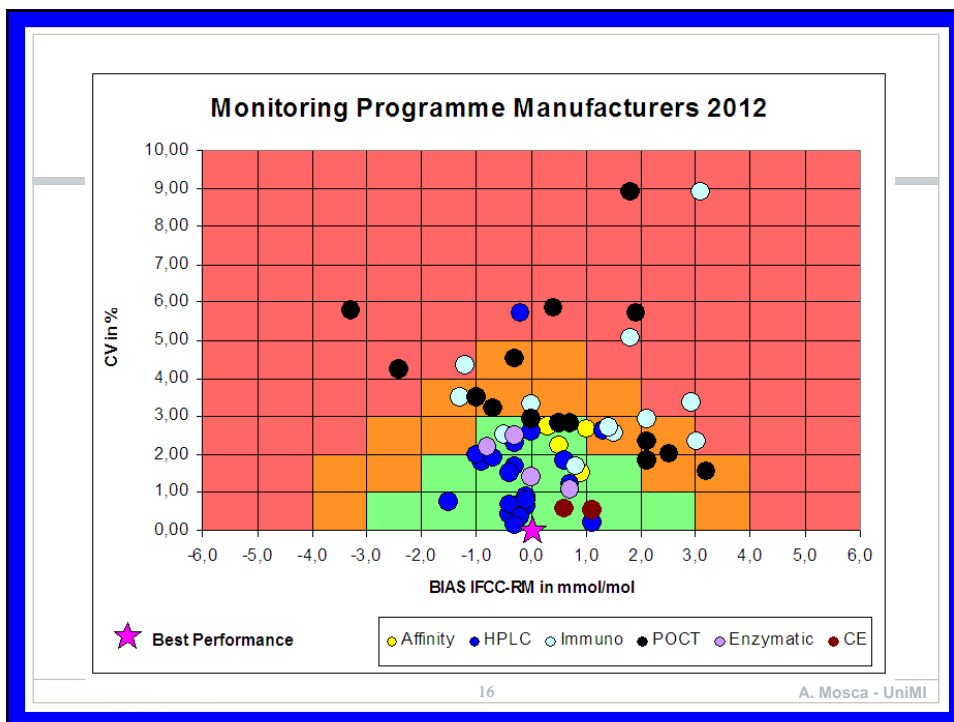
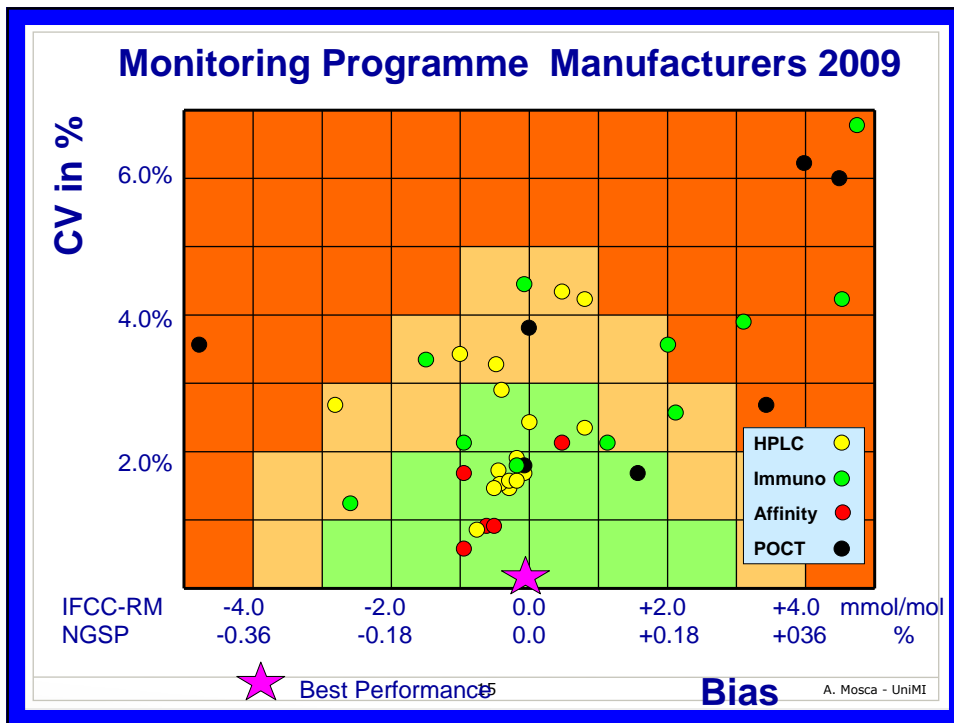
California 2 Study - 2012





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- IFCC approach to the standardization of HbA_{1c}
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Certificate

Traceability of Manufacturers to the IFCC Reference Measurement Procedure for HbA_{1c}

This certifies that [redacted] using [redacted] uses calibrators supplied by the IFCC Network to get traceable to the IFCC Reference Measurement Procedure and participates in the Monitoring Programme to demonstrate traceability. In the Monitoring Programme of 2009 the following performance was seen:

Deviation from IFCC-target	at 30 mmol HbA _{1c} /mol Hb :	1.7
	at 60 mmol HbA _{1c} /mol Hb :	0.0
	at 90 mmol HbA _{1c} /mol Hb :	-1.8
Reproducibility, coefficient of variation		2.40
Linearity, correlation coefficient		0.9970

Date of issue: 18 December 2009

Certification expires: 31 December 2010



IFCC Network Coordinator

contents

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Analytical goals

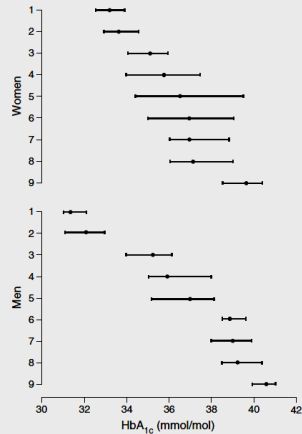


Fig. 1. Individual parametric mean and absolute range of HbA_{1c} values in studied subjects.

Table 2
Mean values, estimated average variance components and indices derived from data on biological variation of HbA_{1c}

Group	HbA _{1c} , mmol/mol	CV _A , %	CV _I , %	CV _G , %	II	CD, %	n
All	36.3	2.4	2.5	7.1	0.35	9.5	2
Men	36.5		1.9	8.9	0.21		
Women	36.1		3.2	5.1	0.62		

CV_A, CV_I, CV_G, II, CD and n as explained in Table 1.

Table 3
Analytical goals for HbA_{1c} 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

Braga et al, Clin Chim Acta 2011;411:1606-10

Table 1. Biological variation in Hb A_{1c} and estimated analytical goals related to the NGSP and IFCC measurement systems, as expressed in the Hb A_{1c} concentration unit of measure (percentage and millimoles per mole, respectively) and as a percentage of the Hb A_{1c} measured.

Parameter	NGSP system		IFCC system	
	Unit of measure, %	Percentage	Unit of measure, mmol/mol	Percentage
Biological variation				
Mean Hb A _{1c}	4.90		30.0	
Intraindividual variation	0.08 (as SD)	1.6%	0.88 (as SD)	2.9%
Interindividual variation	0.20 (as SD)	4.1%	2.20 (as SD)	7.3%
Reference interval (95% central interval)	4.50–5.30	92%–108%	25.6–34.4	85%–115%
Analytical goals (biological variation)^a				
Imprecision	0.04	0.8%	0.44	1.5%
Bias	0.05	1.1%	0.59	2.0%
Total error	0.12	2.4%	1.32	4.4%
Analytical goals (outcome based)^b				
Imprecision	0.15	2.0%	1.6	2.8%
Total error	0.50	6.7%	5.0	8.6%

^a Calculated according to Fraser et al. (5).

^b Calculated according to Mosca et al. (4).

Weykamp et al, Clin Chem 2011;57(8)

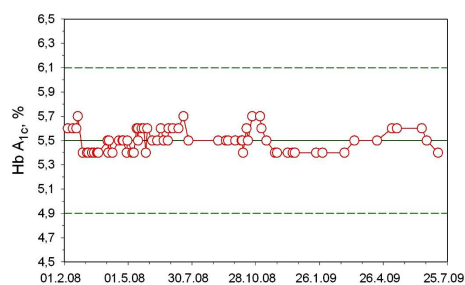
Essential steps for achieving the standardization of HbA_{1c}

Table 1 Essential steps in order to provide HbA_{1c} - IFCC standardized results.

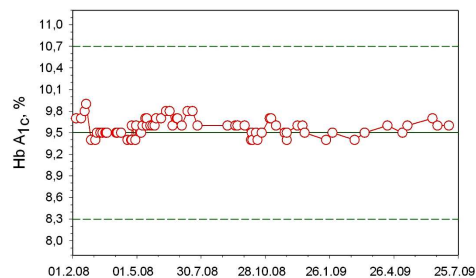
actions	tools
Choice of the method	Evaluate the IFCC certificate (ask the manufacturer)
Calibration	Enter the IFCC target values provided by the manufacturer (ask the manufacturer for traceability to the IFCC reference system)
Reporting the HbA _{1c} result	Use the mmol/mol units (eventually transform afterwards in % units) Report decisional limits (not the reference intervals)
Monitoring the long-term imprecision	Internal Quality Control with two levels materials Calculate the CVs per month (or over a longer time frame)
Evaluating the trueness	Regular participation to EQAS exercises (commutable materials, IFCC target values assigned by the IFCC reference measurement procedures)

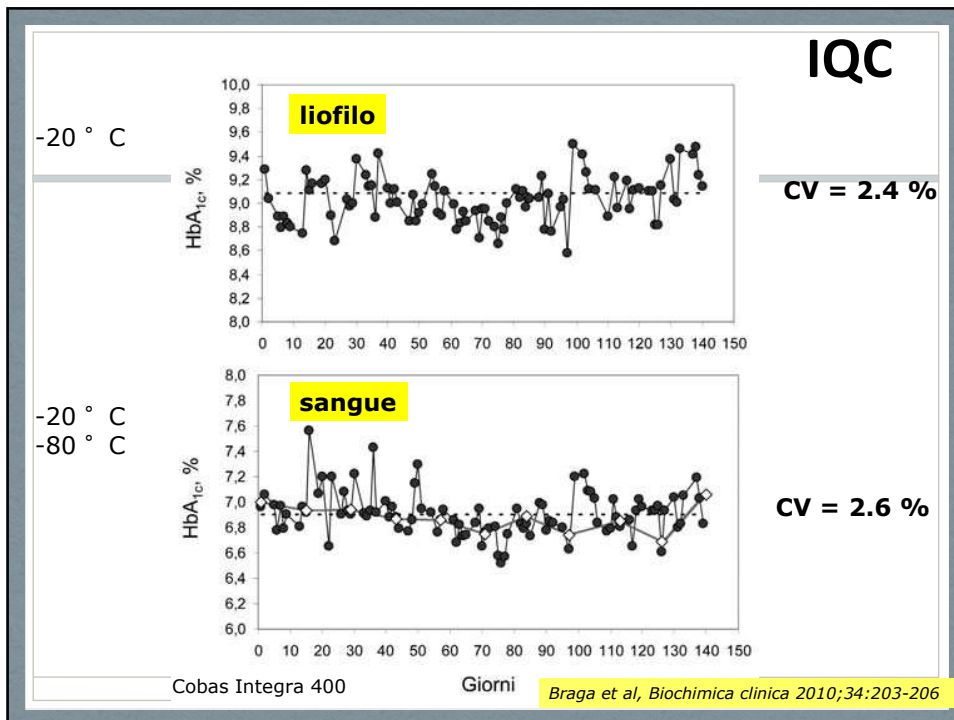
Mosca et al, Clin Chem Lab Med 2013, in press

Internal Quality Control



*Bio-Rad Variant II, Dual Kit
Lyphochek Diabetes Control
Lot. 33730*





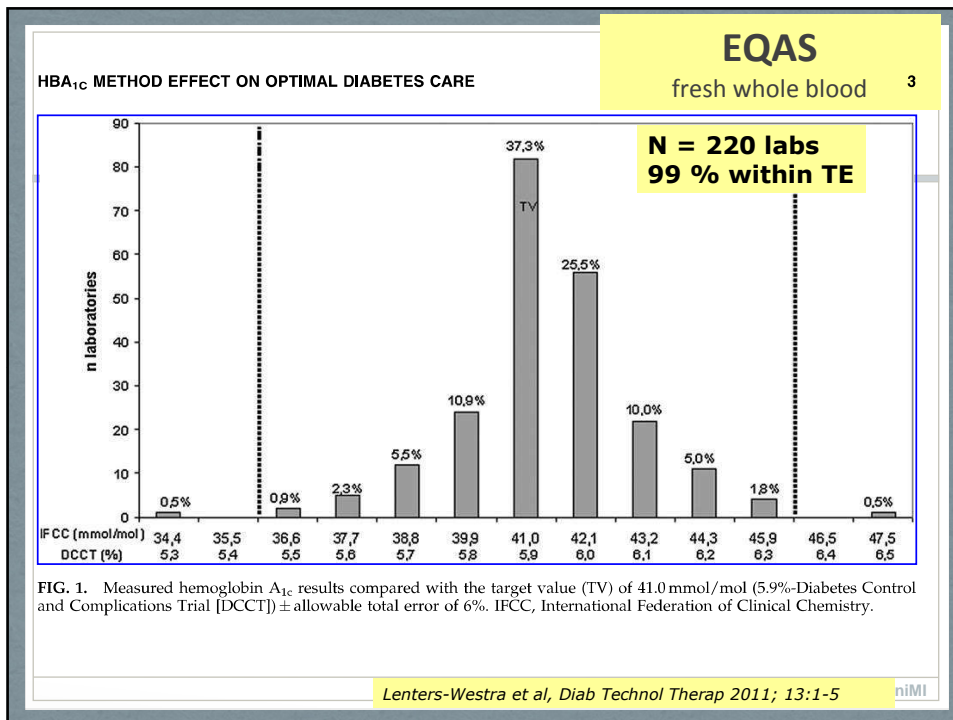
IQC

data from the manufa

Intervals too large!!!

Units	Level 1 - 33831		Level 2 - 33832		
	Mean	Range	Mean	Range	
Abbott ARCHITECT cSystems (NGSP)	%	5.00	4.00 – 6.00	9.48	7.59 – 11.4
Bayer ATCNOW+ (NGSP)	%	5.20	4.10 – 6.20	9.80	7.80 – 11.8
Beckman Coulter AU 400 / 600 / 640 / 680 / 2700 / 5400 (NGSP)	%	5.22	4.18 – 6.26	9.85	7.88 – 11.8
Beckman Coulter CX Series (NGSP)	%	5		5	
Beckman Coulter Synchron LX / UniCel DxC Series (NGSP)	%	5.22	4.17 – 6.26	9.57	7.65 – 11.5
Bio-Rad D-10 Hemoglobin A1C 220-0101 (NGSP)	%	5.30	4.70 – 5.90	10.2	9.00 – 11.4
Bio-Rad D-10 Dual Program 220-0201 (NGSP)	%	4.90	4.30 – 5.50	10.0	8.80 – 11.2
Bio-Rad In2i System A1C Test Cartridges 281-0001/281-0001EX (NGSP)	%	5.10	4.20 – 6.00	9.80	8.50 – 11.1
Bio-Rad VARIANT Hb A1C Program 270-0003 (NGSP)	%	5.30	4.70 – 5.90	10.0	8.80 – 11.2
Bio-Rad VARIANT II Hemoglobin A1C Program 270-2101NU (NGSP)	%	5.30	4.70 – 5.90	9.90	8.70 – 11.1
Bio-Rad VARIANT II TURBO HbA1C Kit 2.0 270-245S (NGSP)	%	5.40	4.80 – 6.00	10.1	8.90 – 11.3
Bio-Rad VARIANT II TURBO Hemoglobin A1C Program 270-241S (NGSP)	%	5.10	4.20 – 6.00	9.90	8.70 – 11.1
Ortho Vitros Series / Vitros Fusion 5,1 FS (Wet, Microt) (NGSP)	%	5.17	4.13 – 6.20	9.64	7.71 – 11.6
Pointe Scientific Inc. (NGSP)	%	4.80	3.70 – 5.90	10.2	8.10 – 12.2
Roche cobas INTEGRA (NGSP)	%	5.33	4.27 – 6.40	9.79	7.83 – 11.7
Roche cobas INTEGRA - TinaQuant A1c (NGSP)	%	5.35	4.28 – 6.42	9.75	7.80 – 11.7
Roche Hitachi cobas c Systems (NGSP)	%	▲		▲	
SiemensADIVA Chemistry Systems / A1c REF 06654744 (NGSP)	%	5.62	4.50 – 6.74	10.2	8.17 – 12.3
SiemensADIVA Chemistry Systems / HbA1c REF 00350395 / B01-4797-01 (NGSP)	%	5.40	4.32 – 6.48	9.70	7.76 – 11.6
Siemens DCA Series (NGSP)	%	5.48	4.38 – 6.57	10.2	8.13 – 12.2
Siemens Dimension Series HA1C REF DF105 (NGSP)	%	5.56	4.45 – 6.68	9.69	7.75 – 11.6
Siemens Dimension Series HB1C REF DF105A (NGSP)	%	5.40	4.32 – 6.48	10.0	8.02 – 12.0
Siemens Dimension VISTA Systems HA1C REF K3105 (NGSP)	%	5.20	4.16 – 6.24	9.93	7.95 – 11.9
Siemens Dimension VISTA Systems HA1C REF K3105A (NGSP)	%	5.13	4.10 – 6.16	9.65	7.72 – 11.6
TOSH G7 Automated HPLC Analyzer (NGSP)	%	5.20	4.80 – 5.60	10.0	9.30 – 10.7
TOSH G8 Automated HPLC Analyzer (NGSP)	%	5.20	4.80 – 5.60	10.0	9.30 – 10.7

ca - UniMI



EQAS
lyophilized materials

**12 samples in duplicate
(blind over one year), every 2 weeks**

ERL International - HbA_{1c} - annual report
University of Milano - Scien. Biomed. Techn. - Dr. Andrea Mosca

[back to define annual report](#)

year	2012	
reference group	all labs	
instruments	G8-10 - TOSOH G8 IFCC mmol/mol	

issue	your instrument(s)	all labs
number of labs	60	469
accuracy		
deviation from IFCC at 30 mmol/mol IFCC level	0 mmol/mol	0 mmol/mol
at 60 mmol/mol IFCC level	2 mmol/mol	1 mmol/mol
at 90 mmol/mol IFCC level	4 mmol/mol	3 mmol/mol
reproducibility		
coefficient of variation	1.7%	2.3%
linearity		
correlation coefficient	0.9978	0.9961
interpretation		
deviation from IFCC	excellent	excellent
reproducibility	excellent	excellent
linearity	excellent	excellent



Centro di Riferimento Sicurezza di Qualità
Valutazione esterna di qualità
HbA1c-Ciclo 2012



Regione Lombardia
Osservatorio dei servizi sanitari territoriali
e politiche di programmazione e controllo

EQAS lyophilized materials

Analita: **HbA1c (IFCC)** mmol/mol
Risultato atteso: **43**

	N.	Out	Media	C.V.	S.D.	Med.na
Tutti	277	7	45,68	5,9	2,7	46,00
Tuo Metodi HbA1c	32	1	46,52	4,2	2,0	47,00

Campione **4** (Scad. 04/05/2012)
Tuo risultato **47,0**

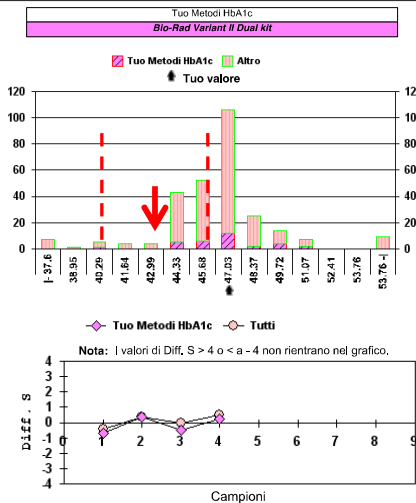
	Diff. S	Diff. %
Tutti	0,49	2,89
Tuo Metodi HbA1c	0,24	1,03

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

○ = Interno X = Esterno rispetto ai limiti dichiarati

N. risultati numerici **277**
N. risultati semiquantitativi/qualitativi
N. laboratori che non eseguono l'esame **56**

Riepilogo x Metodi HbA1c risultati numerici (> 7 Centri)					
Metodi HbA1c	N.	Out	M.	C.V.	
Toson serie G8	79	1	46,19	2,7	
Toson serie G7	43	2	45,71	6,6	
Bio-Rad Variant II Dual kit	32	1	46,52	4,2	
Miemma HA 3150	32	0	44,39	2,6	
Bio-Rad D10 A1c	15	0	47,07	4,8	
Roche Tina-quant su album Cobas	8	0	39,35	14,8	



TE = 6.0 %

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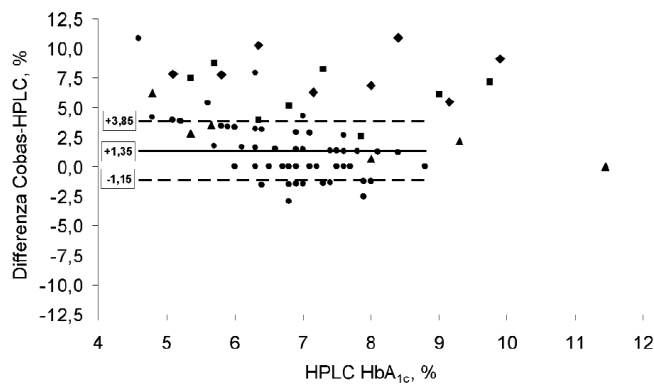
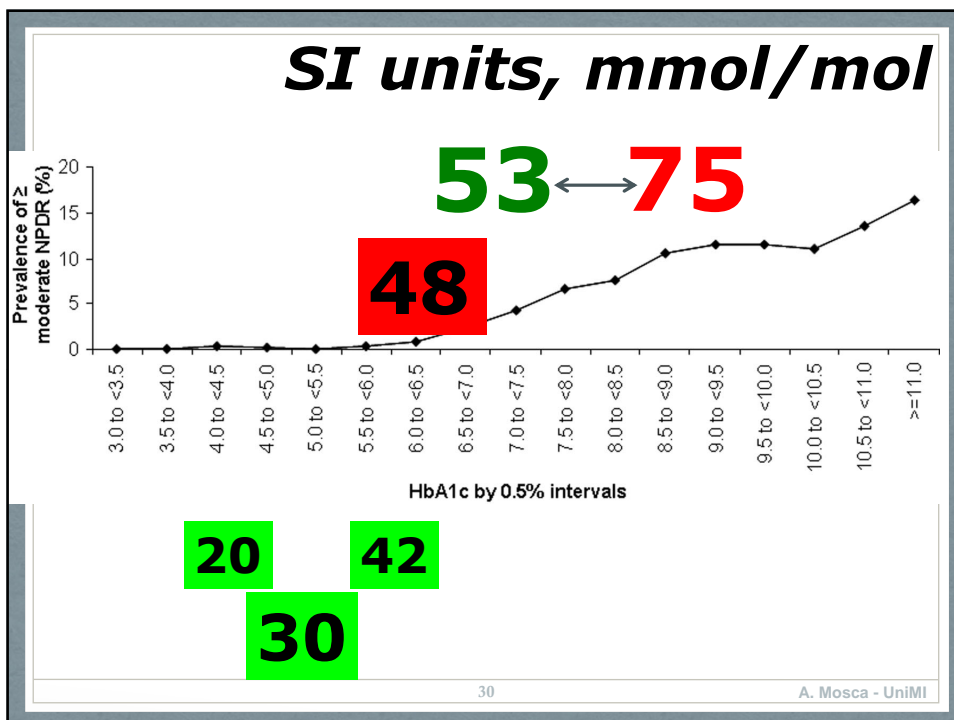
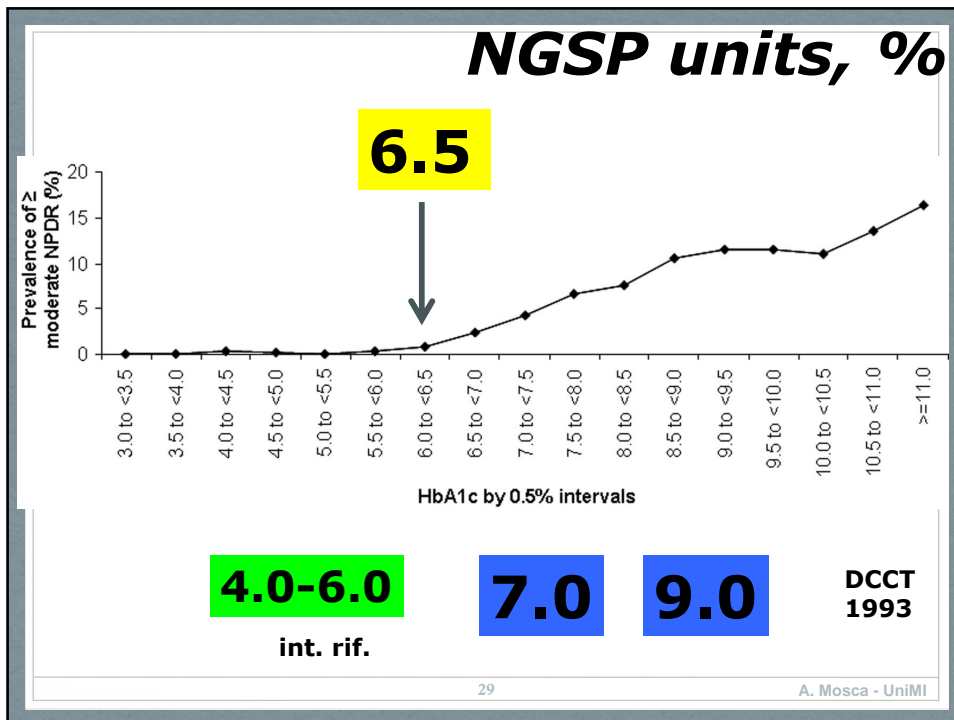


Figura 2
Risultati della commutabilità dei materiali per VEQ valutati.
I punti rappresentano lo scostamento percentuale tra i metodi per i campioni di sangue EDTA (WB) analizzati (n=58), mentre lo scostamento tra metodi per i materiali di controllo è riportato come quadrati (VEQ 2005), rombi (VEQ 2006) e triangoli (materiali IECW). La linea continua rappresenta lo scostamento medio percentuale tra Cobas Integra e HPLC sui 58 campioni WB, mentre le linee tratteggiate corrispondono ai limiti di accettabilità superiore ed inferiore utilizzati per definire la commutabilità dei materiali VEQ. I materiali commutabili sono quelli che mostrano risultati di scostamento compresi in tali limiti.

Valutazione della commutabilità per il sistema analitico Cobas Integra dei materiali di controllo utilizzati nella VEQ per l'emoglobina glicata*

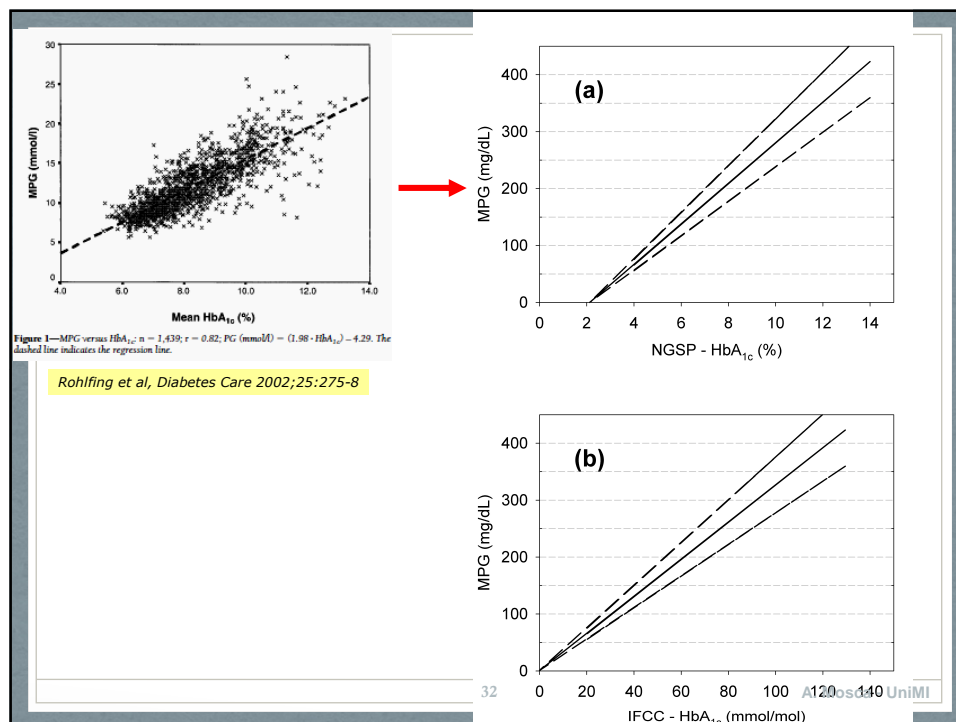


Why using IFCC standardized IFCC?

- Improved reliability and metrological traceability
→ Abandoning tests with poor performance
- Measurement units aligned to the S.I. system
- Avoiding the complications of using two different measurement units (different reference intervals, different decisional limits, different analytical goals)
→ Simpler report
- Expanding the physiopathological range
→ Greater attention to the result
- Better relationship to the physiopathological meaning

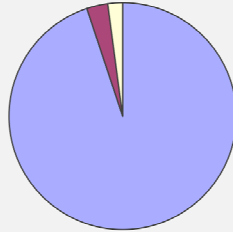
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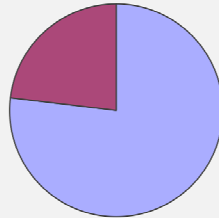
Survey on HbA_{1c} – Italy (1/2)
(data from 237 laboratories, 16-5-2013)

Utilizzate un metodo standardizzato IFCC?



si
no
non lo so

Avete valutato il certificato di riferibilità IFCC del produttore della strumentazione che avete in uso?



si
no

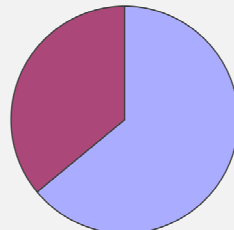
Survey on HbA_{1c} – Italy (2/2)
(data from 237 laboratories, 16-5-2013)

Avete iniziato a riportare le unità di misura IFCC (mmol/mol) per la refertazione dell'HbA_{1c}?



si
no

Se si le abbinate anche alle unità in %?



si
no

Conclusions

- A complete reference system is available for HbA_{1c}
- Laboratory professionals have to carefully evaluate the methods (IFCC certificates), to calibrate them and to run periodical IQC and EQAS exercises
- EQAS results are difficult to be compared each other (matrix effect, target values, programmes, criteria)
- Manufacturers should help into developing more adequate control materials
- Scientific Societies should constantly stimulate and monitor the process

Standardization of hemoglobin A_{1c}: myth or reality?



AND/OR

Aknowledgments

Renata Paleari (*University of Milano, Milano, IT*)
(HPLC analysis, data elaboration)

Cas Weykamp (*Qeen Beatrix H, Winterswijk, NL*)
(IFCC Network HbA_{1c})

Patricia Kaiser, Hans Reinauer (*INSTAND eV, Duesseldorf, DE*)
(ID-MS reference measurement procedure)