Standardizzazione della determinazione dell'emoglobina glicata

Diagnostica delle emoglobinopatie: tra clinica e laboratorio.
Verona, 25 Gennaio 2008

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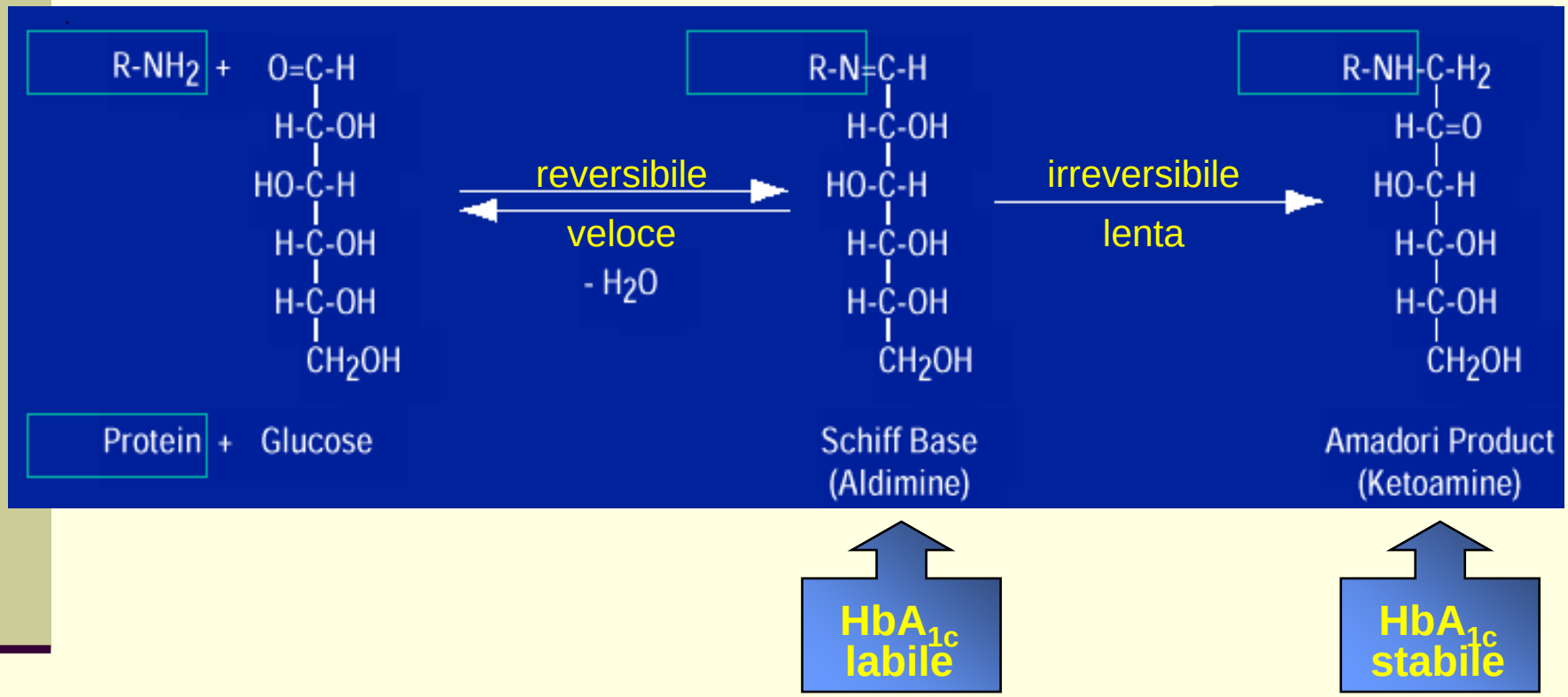
HbA_{1c}: reazione di glicazione



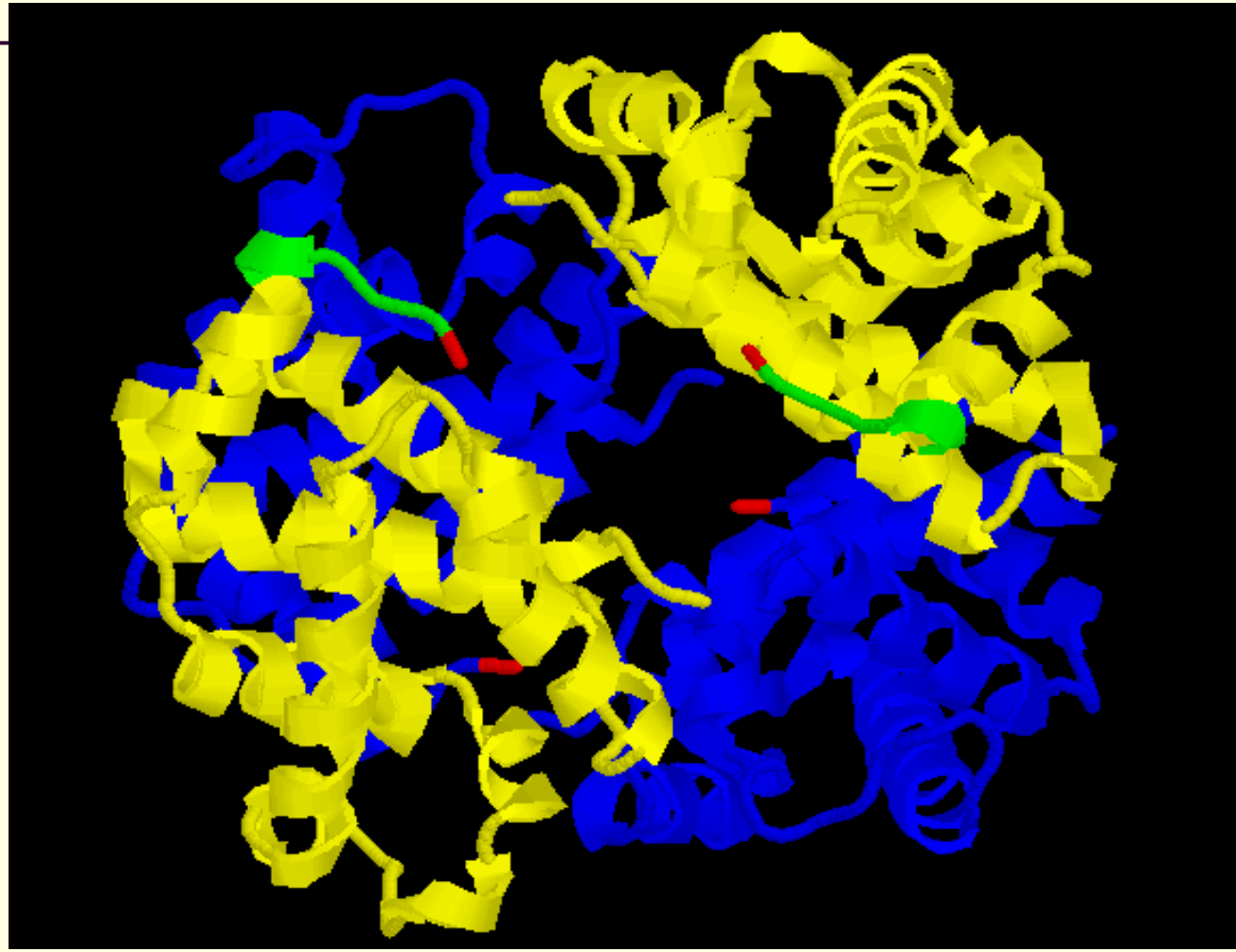
Reazione non enzimatica di condensazione tra il gruppo aldeidico del glucosio e il gruppo amminico N-terminale delle catene β della Hb.

Major Glycation sites of Hemoglobin

β-Chains: **Yellow**
α-Chains: **Blue**

N-terminal Valine: **Red**

Hexapeptide **Red**/**Green**



Variabilità inter-individuale

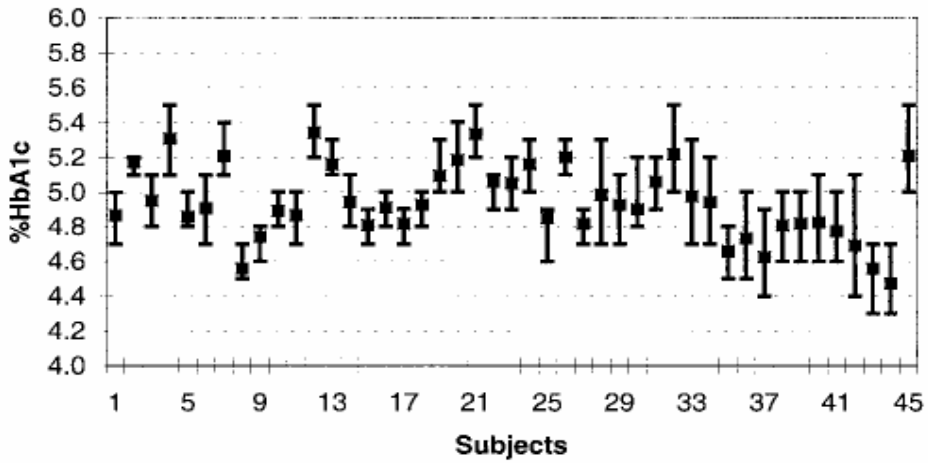


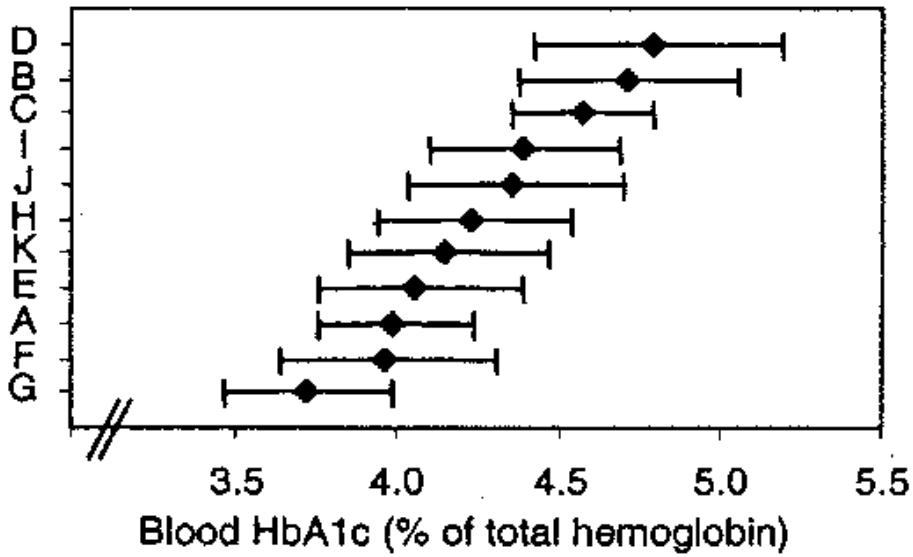
Fig. 1. Mean, minimum, and maximum GHb for study participants. *HbAlc,* hemoglobin A_{1c}.

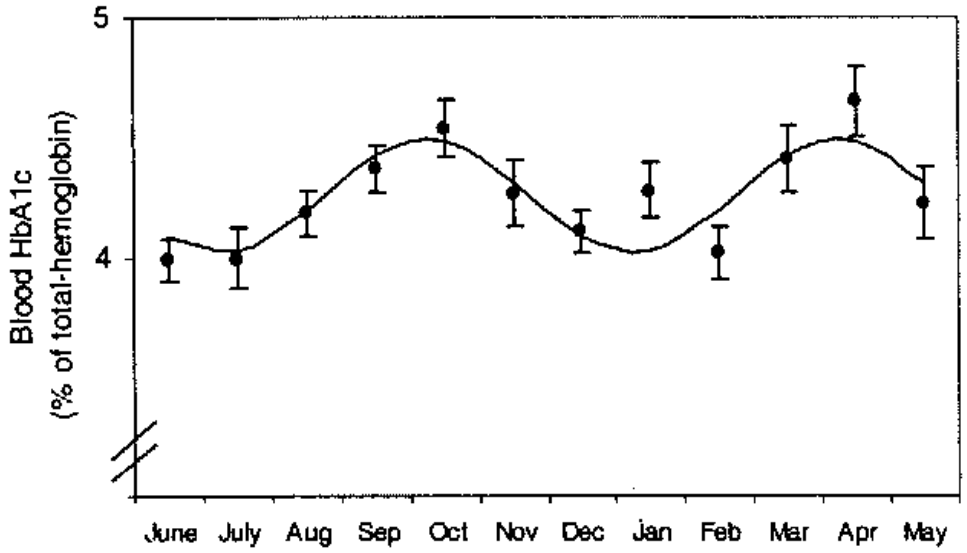
Table 1. Variance components for GHb and FPG.

Variance component	GHb, %	FPG, mmol/L
Between-subject S_g (CV_g)	0.20 (4.0%)	0.31 (5.8%)
Within-subject S_i (CV_i)[a]	0.08 (1.7%)	0.30 (5.7%)
Analytic S_a (CV_a)		
Between day	0.11 (2.3%)	0.09 (1.7%)
Within day[b]	0.07 (1.5%)	0.04 (0.8%)

[a] Also includes within-day analytical variation.
[b] Estimated from quality-control data.

Rohlfing et al, Clin Chem 2002





Biological variability
n = 11 F, 1 yr

Garde et al
Clin Chem 2000

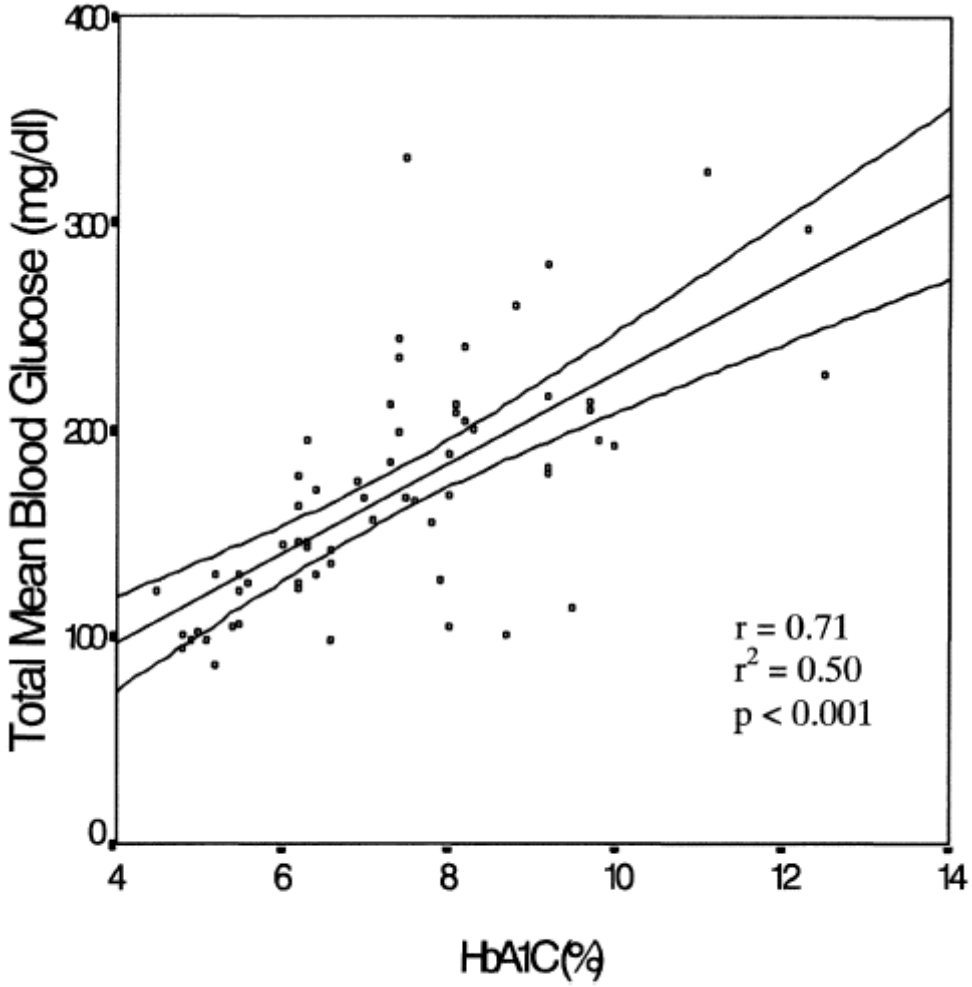


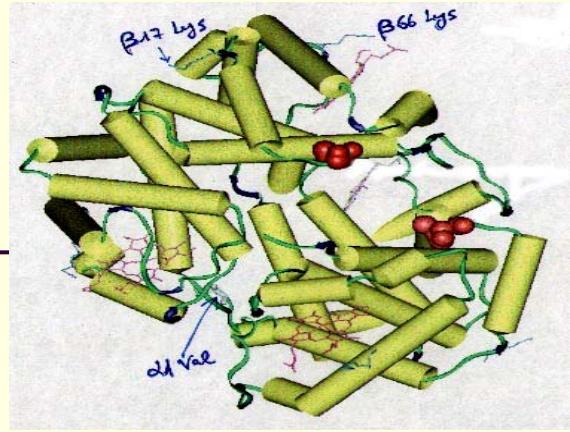
Fig. 1. Correlation of the total mean blood glucose with the HbA_{1C}. The regression line is indicated together with the 95% confidence interval.

MBG di 60 giorni prima

Merino Torres et al, Clin Chim Acta 2003

Metodiche analitiche attuali per la misura della Hb A_{1c}

βN1-deoxyfructosyl-Hb



Principio	*Tecniche analitiche*
Carica elettrica	**crom. scambio ionico (HPLC, minicolonnine, batch)**
Glucosio	**crom. di affinità (HPLC, minicolonnine, POCT)**
Epitopi	**immunochimiche (autom. anal., POCT)**
Proteolisi spec.	**HPLC – MS; HPLC – CE**

Traguardi analitici per l'HbA_{1c}

CV_b = circa 1 %
CV_w = 3,9 - 7,9 % = circa 5 % → **ET_a = 6,2 %**
CV_a = 2,5 %

Ricos et al. [Scand J Clin Lab Invest 1999]

TE < 1.65I + B ($\alpha \leq 0.05$) or
TE < 2.33I + B ($\alpha < 0.01$) [I =imprecision, B =bias].

For HbA1c the TE is 8 – 10%.

from methods reporting total GHB cannot be directly compared to NGSP Reference values. The NGSP target or reference values are based on replicate analyses using four NGSP certified secondary reference methods.

- What's New
- Background
- Protocol
- How to Obtain Certification
- Certified Methods/Labs UPDATED 10/05
- Steering Committee Members
- Laboratory Network Members
- ADA Recommendations
- CAP GH2 Data UPDATED 5/05
- IFCC Standardization of HbA1c
- The Relationship between GHB and Blood Glucose
- GHB Assay Interferences UPDATED 6/04
- Online Quarterly Monitoring Data Entry for Level 1 Labs

Related Links

2005 GH2-A (fresh pooled samples)

* = NGSP certified at the time of the survey

		GH2-01		GH2-02		GH2-03	
NGSP Reference Value[t]		7.4		12.0		7.4	
	no. labs	Median	%CV	Median	%CV	Median	%CV
Methods reporting HbA1c (or equivalent)							
& Abbott Aeroset	5	7.5	-	13.0	-	7.4	-
& Abbott Architect	8	7.1	-	11.8	-	7.1	-
* Bayer Advia	18	7.1	6.6	11.0	9.7	7.0	6.6
* Bayer DCA 2000	174	7.3	3.1	11.7	3.0	7.2	2.9
* Beckman Synchron System	279	7.0	4.8	12.1	5.1	7.0	4.5
* Bio-Rad D-10	75	7.7	2.4	12.7	2.1	7.7	2.2
* Bio-Rad Diastat	29	7.3	4.6	12.3	4.6	7.3	5.0
* Bio-Rad Variant A1c	32	7.4	3.0	11.8	3.8	7.4	3.1
* Bio-Rad Variant II A1c	298	7.6	3.1	12.4	3.0	7.6	3.2
* Bio-Rad Variant II Turbo A1c	17	7.6	3.1	12.4	3.3	7.6	2.6
* Dade Behring Dimension	419	7.6	3.4	11.8	2.8	7.5	3.3
* Metrika A1cNOW	12	7.2	7.8	11.9	5.1	7.2	7.7
* Olympus AU system	15	7.3	6.0	12.1	7.8	7.3	6.6
* Primus HPLC (affinity)	22	7.3	2.8	12.4	2.2	7.3	2.8
* Primus Nycocard	5	7.6	-	11.7	-	7.6	-
* Roche Cobas Integra	266	7.6	3.5	12.6	4.3	7.7	3.7
* Roche/Hitachi (Tina Quant II)	81	7.0	3.7	11.9	4.6	7.0	3.7
* Tosoh A1c 2.2 Plus	212	7.8	2.6	12.9	2.6	7.8	2.8
* Tosoh G7 Auto HPLC	169	7.6	1.9	12.5	2.0	7.6	1.8
$Methods reporting Total GHB							
Bio-Rad Variant	13	8.5	3.7	15.8	3.2	8.6	3.9
Primus	8	9.6	-	18.2	-	9.6	-

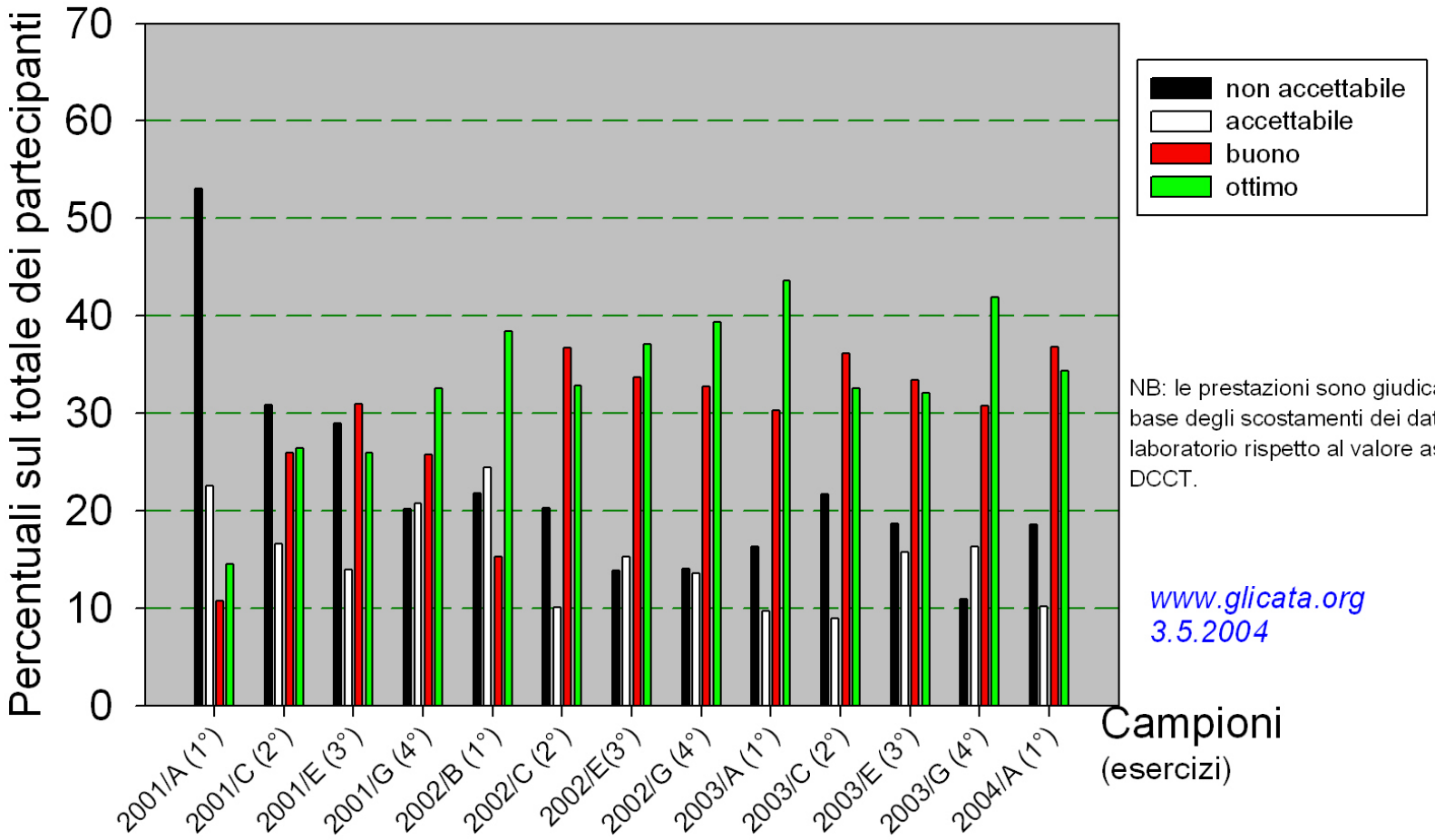
CAP 2005 (n = 2157 lab)

- Accuratezza:
  - Certificazione NGSP: >99 %
  - 80 % dei partecipanti, bias < 0,2-0,3 % di $HbA1_c$ (bassa conc.), < 0,5 % $HbA1_c$ (alta conc.)
- Imprecisione:
  - > 95 % laboratori ha riportato uno scostamento non superiore allo 0,5 % di $HbA1_c$ tra le due misure replicate

VEQ Hb A_{1c} in Italia (1/2)

- ✓ Partecipazione volontaria, sotto patrocinio SIBioC, SIMeL, AIPaC, AMD, SID
- ✓ Comitato scientifico (1 – 2 rappresentanti per Società), in collaborazione con CRB (Castelfranco Veneto)
- ✓ 1999-2002, circa 250 partecipanti

Distribuzioni delle prestazioni analitiche, 2001-2004



NB: le prestazioni sono giudicate sulla base degli scostamenti dei dati di laboratorio rispetto al valore assegnato DCCT.

www.glicata.org
3.5.2004

Standardization - USA

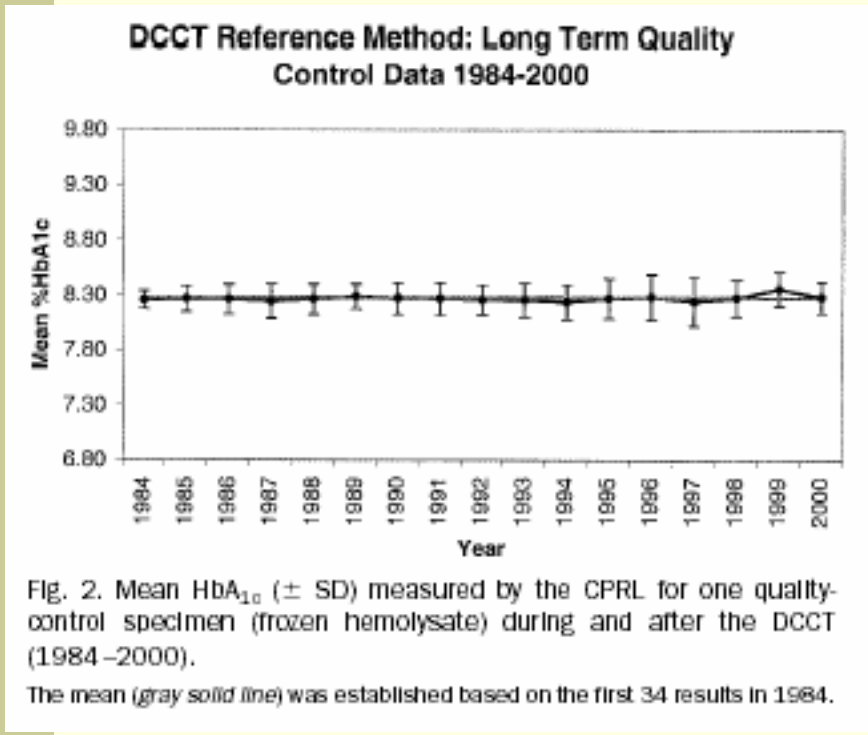


Fig. 2. Mean HbA_{1c} (± SD) measured by the CPRL for one quality-control specimen (frozen hemolysate) during and after the DCCT (1984–2000).

The mean (*gray solid line*) was established based on the first 34 results in 1984.

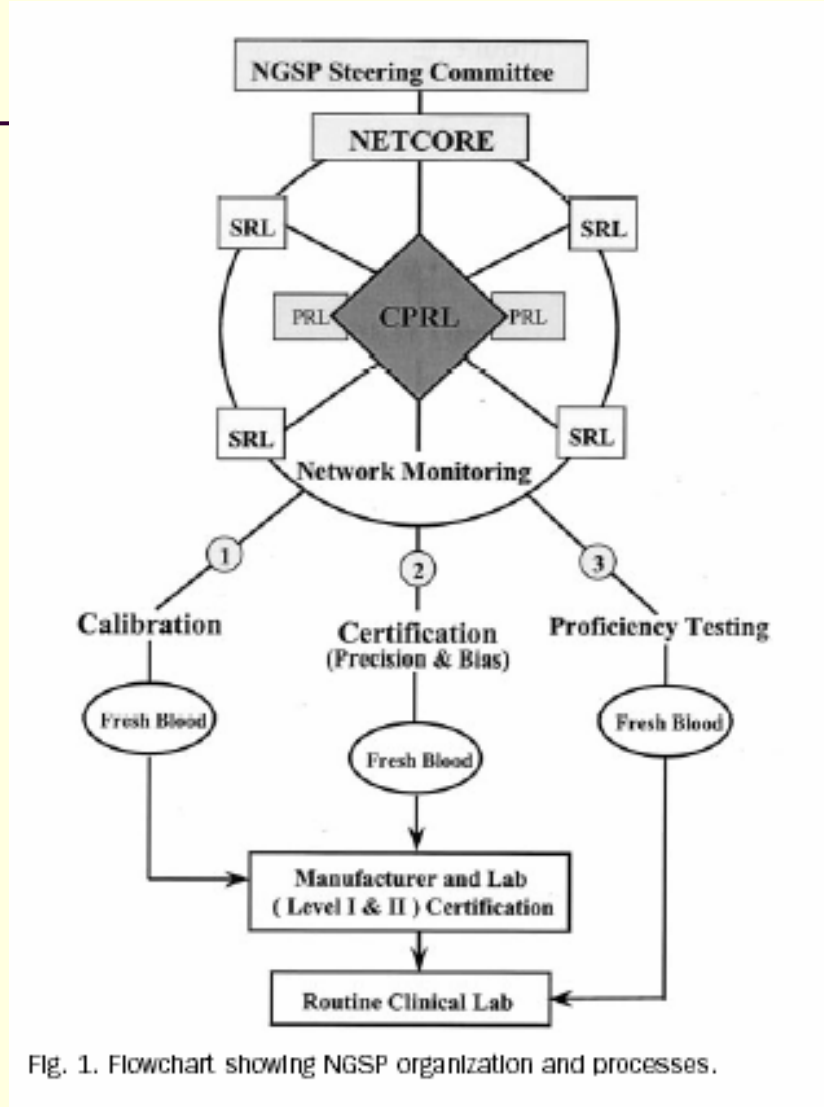


Fig. 1. Flowchart showing NGSP organization and processes.

R. Little et al
Clin Chem 2001;47:1985-1992

AACC subcommittee 1993
-> NGSP 1996

What about the DCM's

- All arbitrarily based on HPLC ion-exchange
- HbA_{1c} is a peak in a chromatogram
- Due to interferences, all these methods define its own 'HbA_{1c}' and differ in result
- All DCM's are unspecific; possible contamination of the HbA_{1c} peak , while not all HbA_{1c} elutes under the one peak
- Providers of modern commercial HbA_{1c} assays add 20 50 % to the original results to report 'NGSP-values'

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)

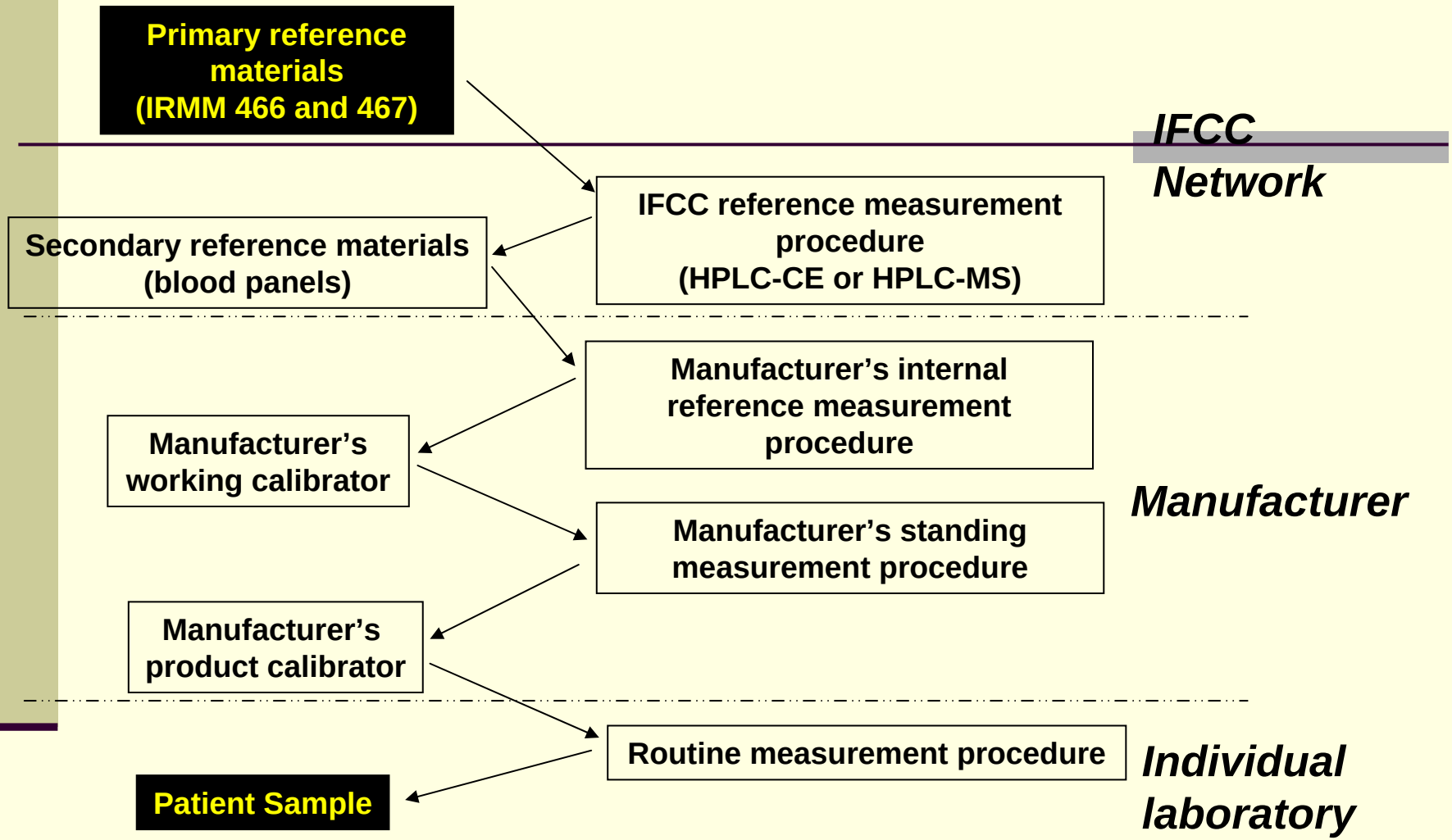
How to fulfill these essential requirements?

Through the availability of:

→ Reference materials

→ Reference methods

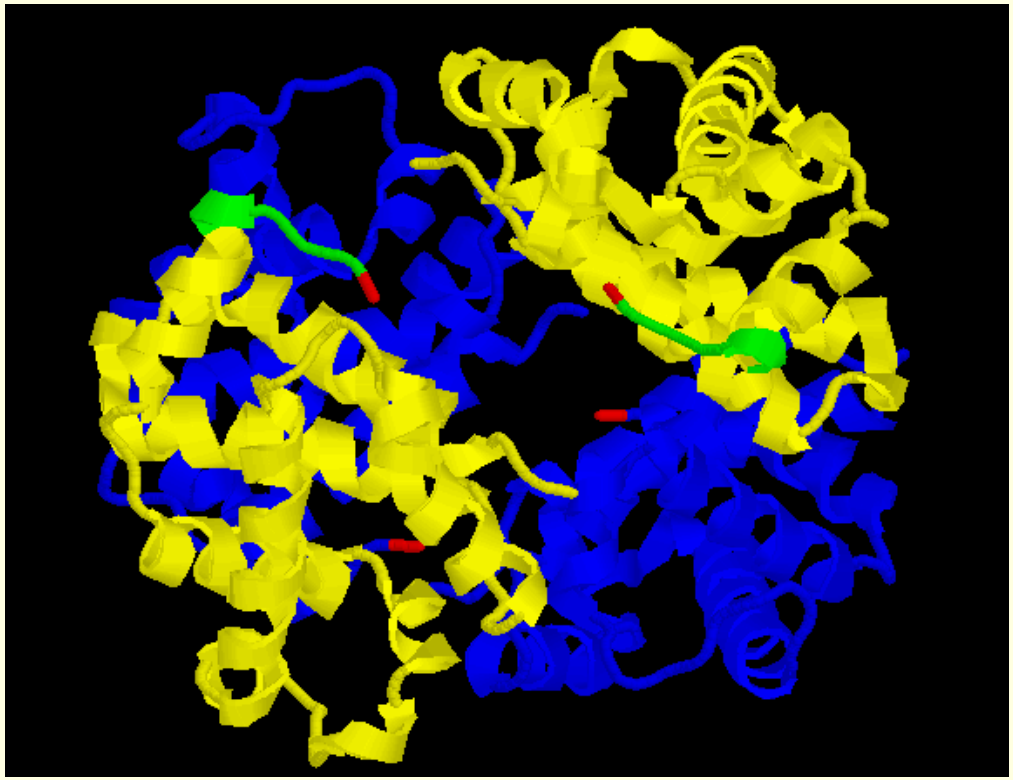
→ Reference laboratory services

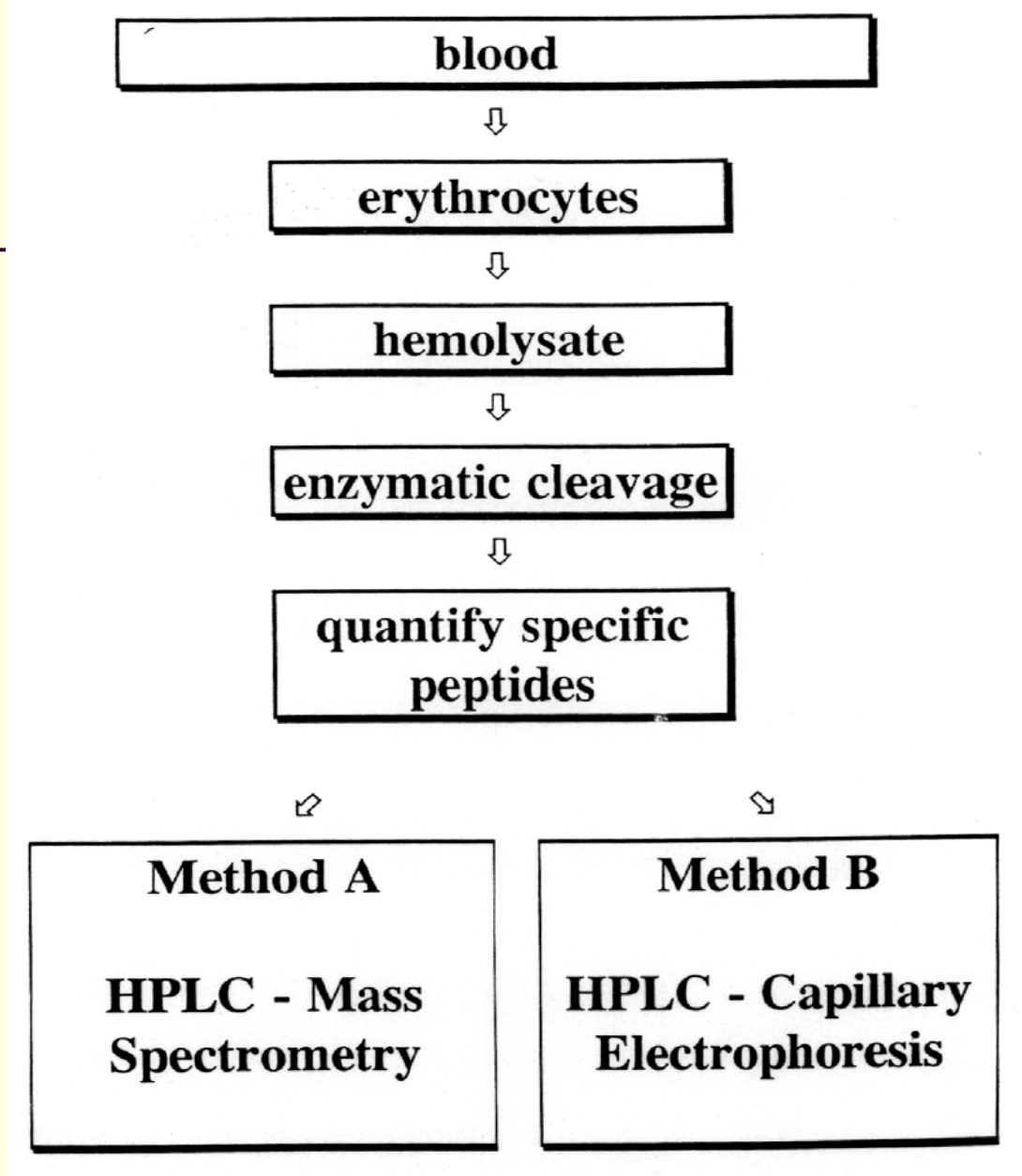

Primary reference materials (IRMM 466 and 467)
IFCC Network
IFCC reference measurement procedure (HPLC-CE or HPLC-MS)
Secondary reference materials (blood panels)
Manufacturer's internal reference measurement procedure
Manufacturer's working calibrator
Manufacturer
Manufacturer's standing measurement procedure
Manufacturer's product calibrator
Routine measurement procedure
Individual laboratory
Patient Sample

IFCC Reference System for HbA_{1c}

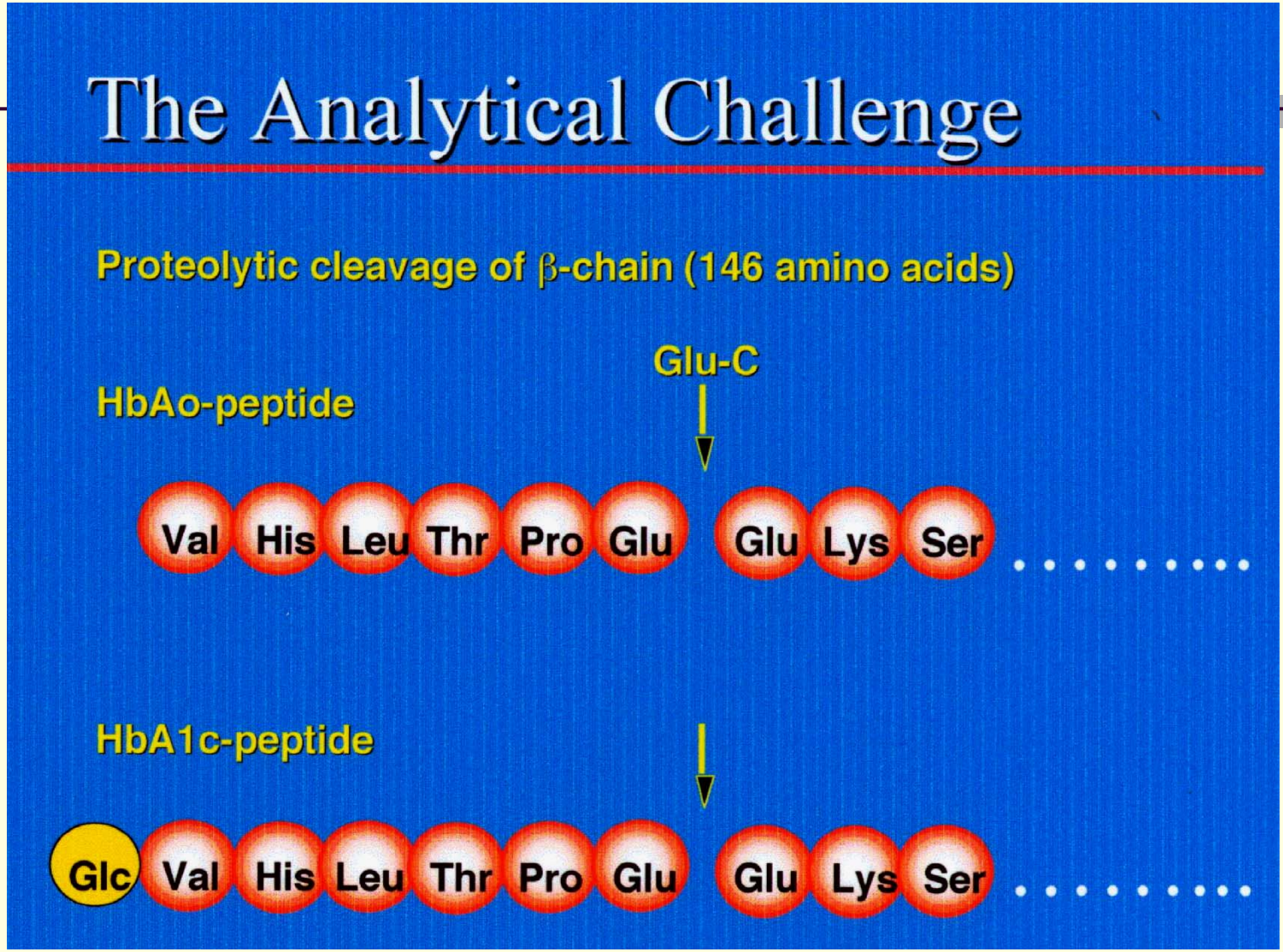
* Definition of the analyte
* Preparation of pure HbA_0 and HbA_{1c}
* Development of reference method
* Installation of a Reference Lab Network
• Preparation of secondary ref. Material

Hb A_{1c}, βN1-deoxyfructosyl-Hb

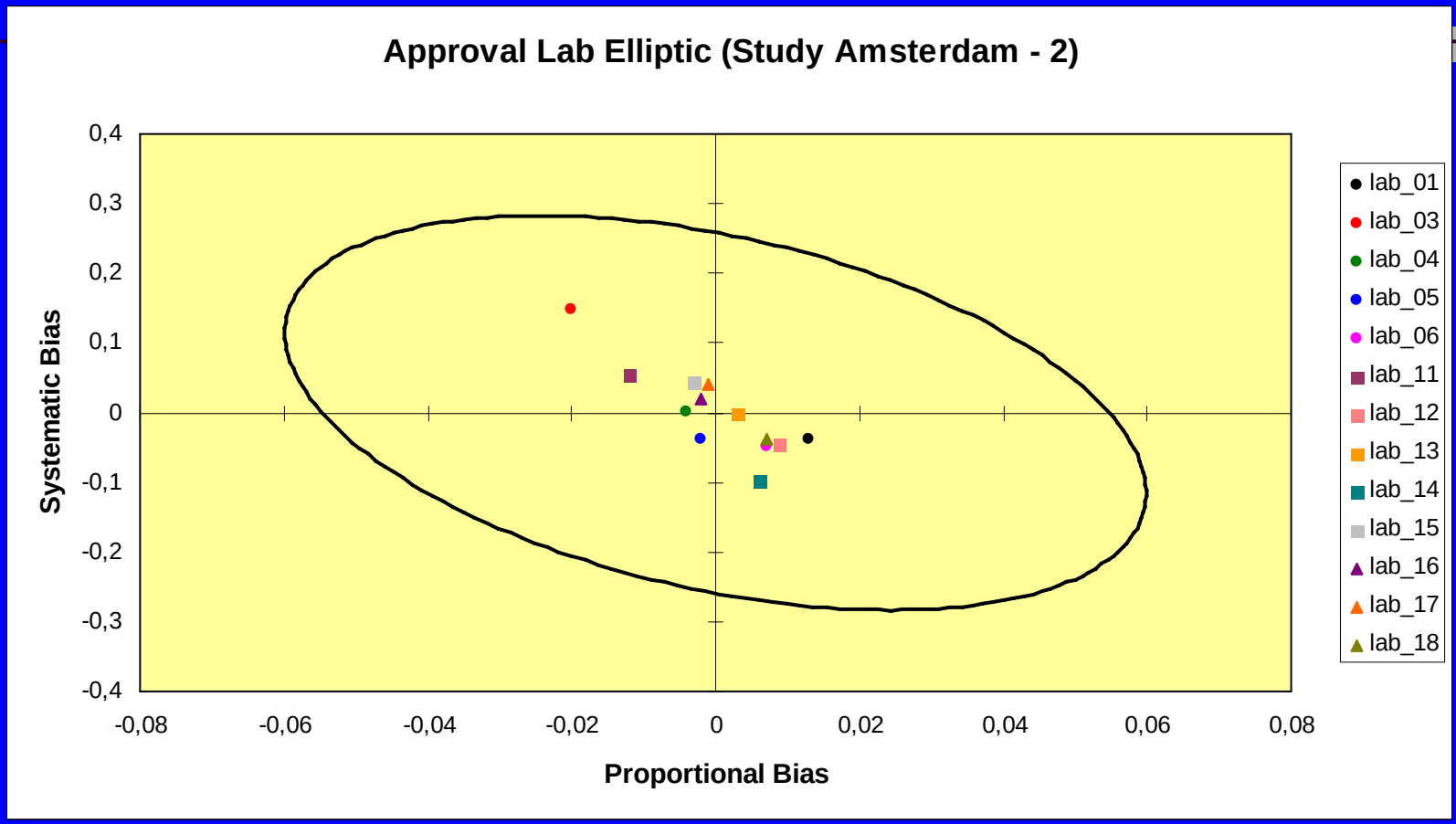



blood
erythrocytes
hemolysate
enzymatic cleavage
quantify specific peptides
Method A
HPLC - Mass Spectrometry
Method B
HPLC - Capillary Electrophoresis

IFCC reference method HbA_{1c}

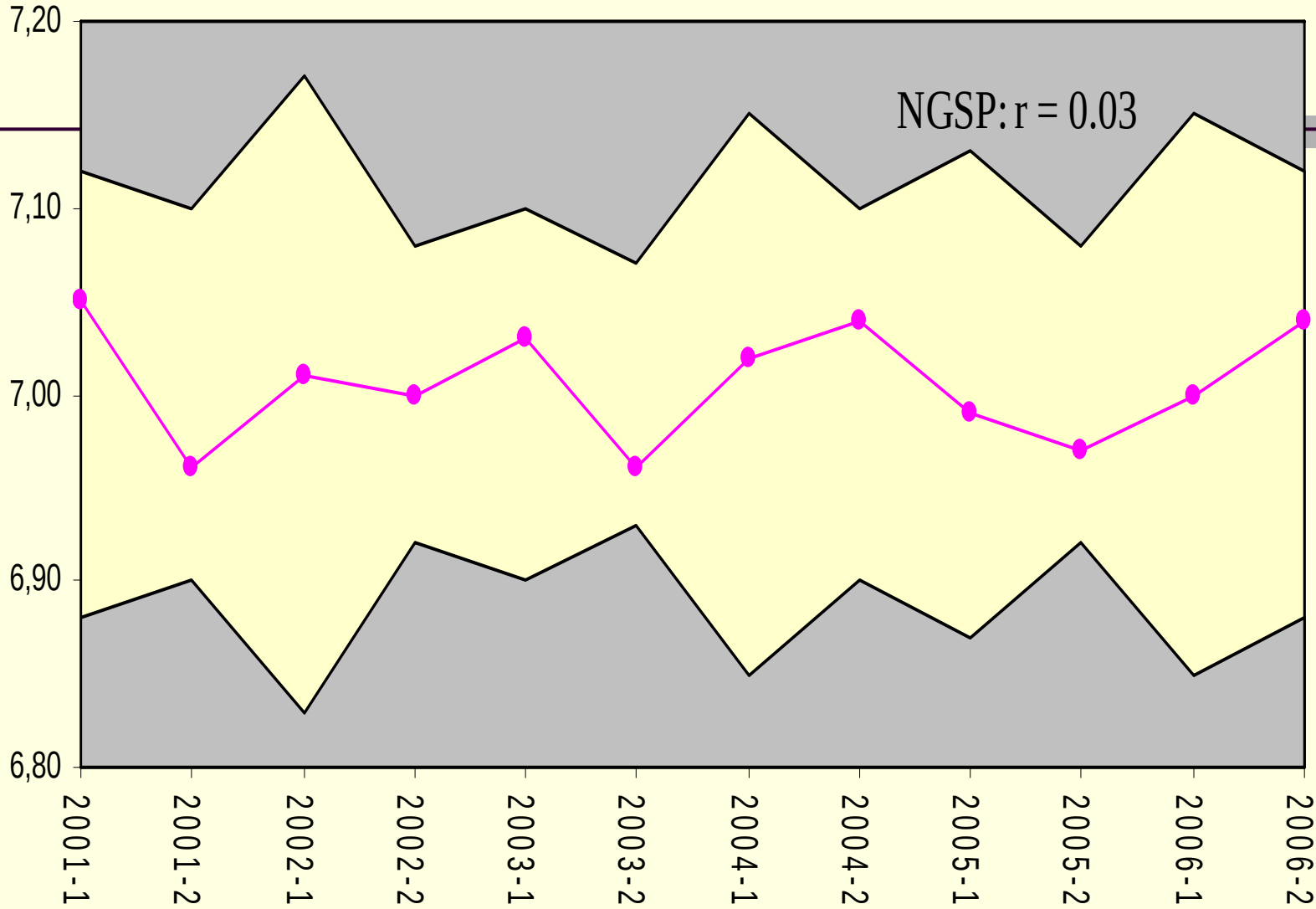


Amsterdam 2 Study



Excellent Performance of all Network Labs

Shewhart Chart NGSP outcome in %HbA1c at IFCC-RM = 53 mmol/mol



X-axis: The subsequent studies in 6 years
Y-axis: NGSP percentage HbA1c
Grey Zone: Area with significant difference from published ME

IFCC SD WG-SHbA1c

* **Definition of the analyte**
* **Preparation of pure HbA_0 and HbA_{1c}**
* **Development of reference method**
* **Installation of a Reference Lab Network**
- **Preparation of secondary ref. Material**
- **Implementing the reference system**

$HbA_{1c}(NGSP) = 0.9148\ HbA_{1c}\ (IFCC) + 2.15$

$HbA_{1c}(IFCC) = 1.093\ HbA_{1c}(NGSP) - 2.35$

Hoelzel et al, Clin Chem 2004

Condizione clinica	*IFCC*	*NGSP*
Limite superiore non-diabetici	4.3 %	6.1 %
ADA target diabete tipo 1	5.3 %	7.0 %
Cattivo controllo glicometabolico	8.6 %	10.0 %

Comparison of the DCCT- HbA1c, the IFCC- HbA1c and mean blood glucose levels in type 1 and 2 diabetes patients in stable glycaemic control and in healthy subjects: Redefining long term glycaemic control

Dutch Working Group*

J.C. Kuenen, S. Simsek, K. Miedema **, P. Kostense, M. Diamant, E.M.W. Eekhof, R.J. Heine

International Working group:

Robert Heine, Philip Home, David Sacks, Ed Horton, Robert Rizza, Jorn Nerup, David Nathan

Research protocol, 3 mayl 2005, Final

Visit		1	2	3	4	5	6	
	- 26 w	-6W run in	0w	4W	8W	12W	16W	
Informed Consent		x						
Incl./Excl. Criteria		x						
Demography		x						
Insulin Therapy		x						
Patient History		x						
Physical Exam.		x						
Vital Signs		x						
Concomitant medication and new medical event		X	x	x	x	x	x	
DCCT-$HbA_{1c\ Zwolle/central}$			x	X	X	X	X	
Secondary IFCC-$HbA_{1c\ Zwolle/central}$*			x	X	X	X	X	
HbA1c local lab	X	x	x					
Hb/Ht/RBC/		x		x	x	x	x	
CRP/WCC/Platelets/reticulocytes, Creat/ureum bilirubin, ASAT,ALAT,AF, gGT, LDH		x						
CGMS			x	X	x	x		
HemoCue: 8 points SMBG			x	X	x	x		
Lifescan: 7 points SMBG, 3 days a week			x	x	x	x	x	
Study information, HemoCue training and lifescan meter training		X						

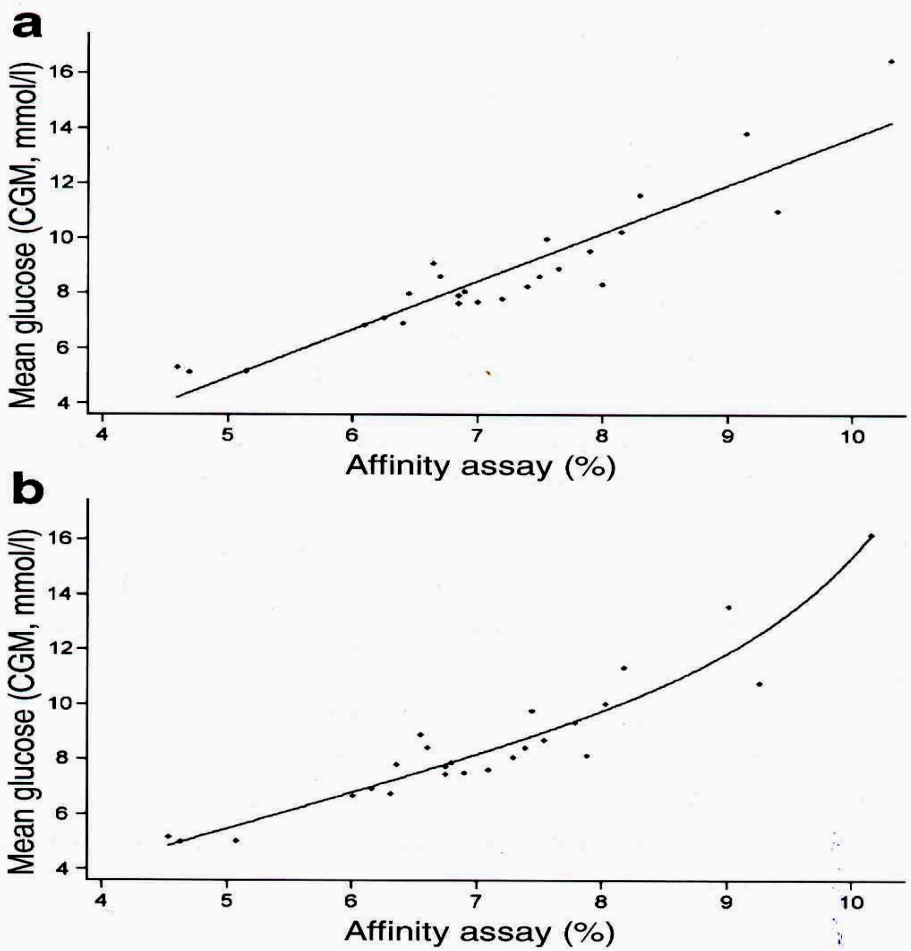


Fig. 1 Relationship between HbA_{1c} at month 3 and mean glucose level calculated from CGM during 12 previous weeks according to (**a**) linear regression mean CGM=HbA_{1c}×1.75–3.81 (r=0.89, p<0.001), and (**b**) the exponential mean CGM=1.28HbA_{1c}+0.000136exp (HbA_{1c})–0.92 (r=0.89, p<0.001). Continuous line, fitted values; diamonds, observed values

Nathan DM *et al*. Diabetologia 50:2245-53, 2007

ADAG

Estimated Average Glucose - eAG

HbA_{1c}(%)	DCCT*	eAG+
5	5.6	5.4
6	7.5	7.0
7	9.4	8.6
8	11.4	10.1
9	13.3	11.7
10	15.3	13.3
11	17.2	14.9
12	19.2	16.5

* Based on DCCT data- 7 point plasma glucose profile measured every 3 months.

+Linear regression eAG= 1.583 x Hb1c- 2.52

International ADAG Study: limitations

- Small sized ethnic groups
- No data in
  - Children
  - Renal impairment
  - Pregnant women
- Acceptance criteria too wide?
- What about changes in glycemic control?

Consensus Statement on the Worldwide Standardization of the Hemoglobin A1C Measurement

The American Diabetes Association, European Association for the Study of Diabetes, International Federation of Clinical Chemistry and Laboratory Medicine, and the International Diabetes Federation

- 1. The HbA_{1c} results should be standardized worldwide, including the reference system and results reporting.
- 2. The IFCC reference system for HbA_{1c} represents the only valid anchor to implement standardization of the measurement.
- 3. The HbA_{1c} assay results are to be reported worldwide in IFCC unit (mmol/mol) *and* derived NGSP unit (%), using the IFCC-NGSP master equation.
- 4. If the ongoing "average plasma glucose study" fulfills its *a priori* specified criteria, an HbA_{1c}-derived average glucose (ADAG) value will also be reported as an interpretation of the HbA_{1c} result.
- 5. Glycemic goals appearing in clinical guidelines should be expressed in IFCC units, derived NGSP units, and as ADAG.

Diabetes Care 2007;30:2399

Advantages

- The use of a completely different unit (mmol/mol instead of %) will avoid confusion when recalculating old HbA_{1c} targets to the new IFCC standardized values if clinical laboratories wish to implement HbA_{1c} results in SI units.
- A positive impact of changing of scale of reported HbA_{1c} results is expected, allowing clinicians and diabetic patients to better understand the marker changes (currently they may perceive small changes in percentage values – although linked to large health effects – as unimportant).
- Supposed increased potential for future use of HbA_{1c} as diagnostic tool.

Table 1 Suggested units and target values for HbA1c when measured with methods traceable to the IFCC reference system. A comparison with the current figures is also given.

	Current[a]	IFCC traceable methods
Reference interval (non-diabetics)	4–6%	20–42 mmol/mol
Target for treatment in diabetics[b]	<7%	<53 mmol/mol
Change of therapy in diabetics[b]	>8%	>64 mmol/mol

[a]Refer to methods aligned to the US National Glycohemoglobin Standardization Program. [b]As recommended by the American Diabetes Association.

Conclusioni – HbA_{1c} (1/2)

- Utilizzare metodi di provata riproducibilità (CVa < 2 %)
- Non vanno trascurati i processi di QA ed il miglioramento continuo della qualità

- 12 dicembre 2007: meeting IFCC-Manufacturers
  - entro 31.12.2009: riferibilità IFCC
  - dal 1.1.2011: esito test in unità IFCC e NGSP (nuovi strumenti)
  - HbA_{1c} (non A1c)
  - eAG: dopo fine studio ADAG; non compito dei produttori
  - VEQ: materiali commutabili, titolo IFCC, giudizio su scostamento da ET (non dal consenso)

HbA1c Assigned IFCC RM 53 mmol/mol

Derived Numbers From IFCC RM

NGSP = 7.00% HbA1c*

eAG = 154 mg/dL Glucose**

eAG = 8.6 mmol/L Glucose**

* According to Clin Chem 2004;50:166-174

** According to Presentation ADAG Study at EASD Meeting, Amsterdam 18 September 2007 (Provisional Results)

Conclusioni – HbA_{1c} (2/2)

Preparazione all'implementazione della standardizzazione globale:

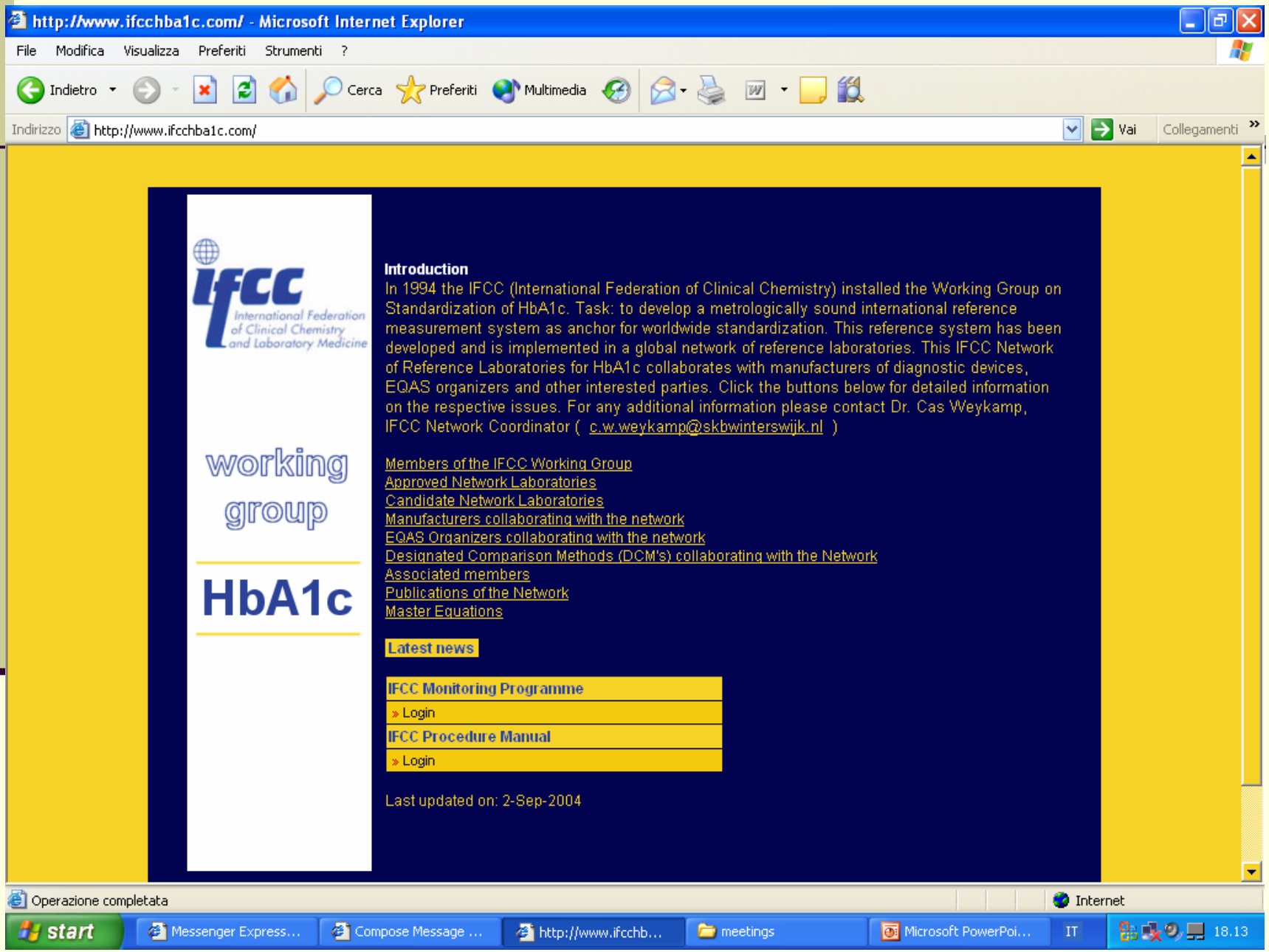
argomenti

- **definire tempistica e modalità refertazione**
- **terminolgia**
- **interfacciamento ai sistemi informatici dei laboratori**
- **goals analitici**
- **campagna informativa**

soggetti

- **società scientifiche**
- **enti governativi**
- **organizzatori VEQ**
- **...**

www.ifcchba1c.net



The IFCC Reference Measurement System for HbA1c: A 6-Year Progress Report

Cas Weykamp, W Garry John, Andrea Mosca, Tadao Hoshino, Randie Little, Jan-Olof Jeppsson, Ian Goodall, Kor Miedema, Gary Myers, Hans Reinauer, David B. Sacks, Robbert Slingerland, Carla Siebelder

Clinical Chemistry, in press

HbA1c: Monitoring of the relation betwee the IFCC reference method and the Designated Comparison Methods in US, Japan and Sweden.

Andrea Geistanger, Sabine Arends, Tadao Hoshino, Jan-Olof Jeppsson, Randie Little, Carla Siebelder, Cas Weykamp

Clinical Chemistry and Laboratory Medicine , to be submitted