

Impact of Reference Measurement Systems on Clinical Evidence : HbA_{1c} and Diabetes

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JCTLM Members and Stakeholders Meetings at BIPM
December 4th, 2013

Why is HbA_{1c} important in diabetes mellitus ?

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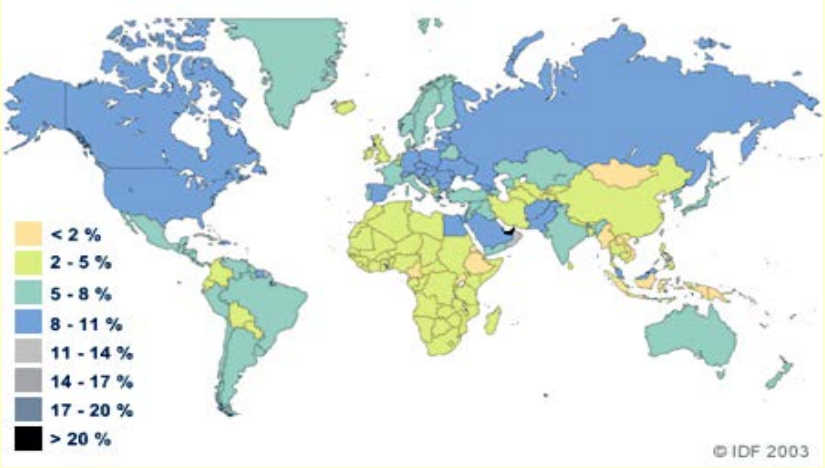
Diabetes Mellitus : a "non infectious epidemic disease"

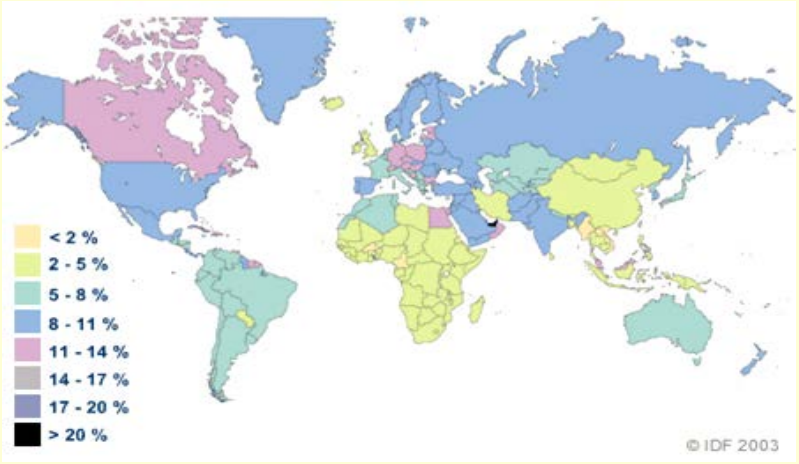


Prevalence in 2012

371 million patients

Estimation in 2030

552 million patients (+51%)



- Challenge : to prevent or delay severe degenerative long-term complications
- Necessity : optimal metabolic control (direct link between glycemic control and occurrence of complications)

→ **HbA_{1c} : a major tool**

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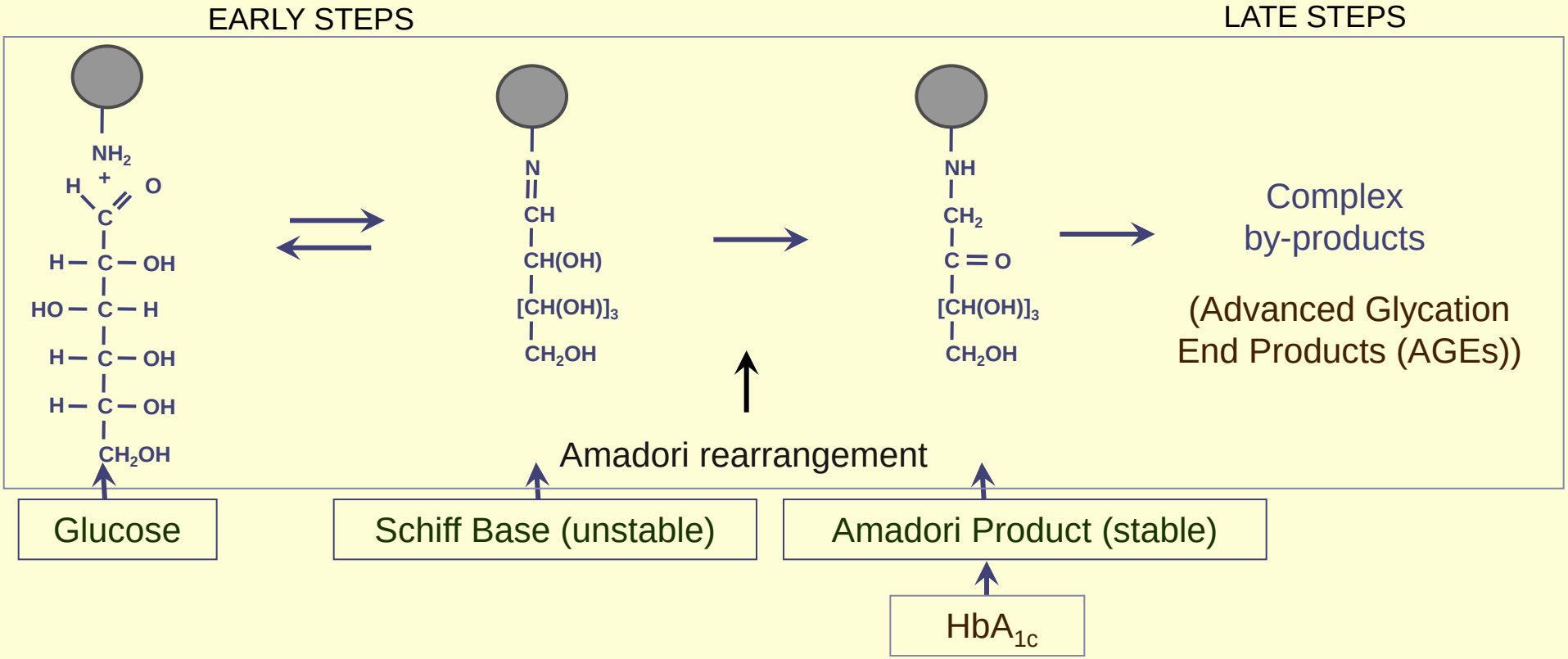
HbA_{1c} : a glycated protein

Nonenzymatic glycation :

- Spontaneous binding of sugars (glucose) and by-products on aminogroups of proteins
- Cumulative and irreversible process related to red blood cell lifespan (120 days) and glucose concentration



- Structural and functional protein alterations : participation in molecular protein ageing and involved in pathology

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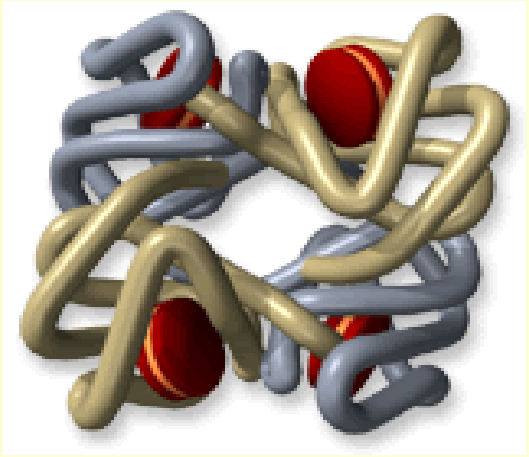
Nonenzymatic glycation of HbA

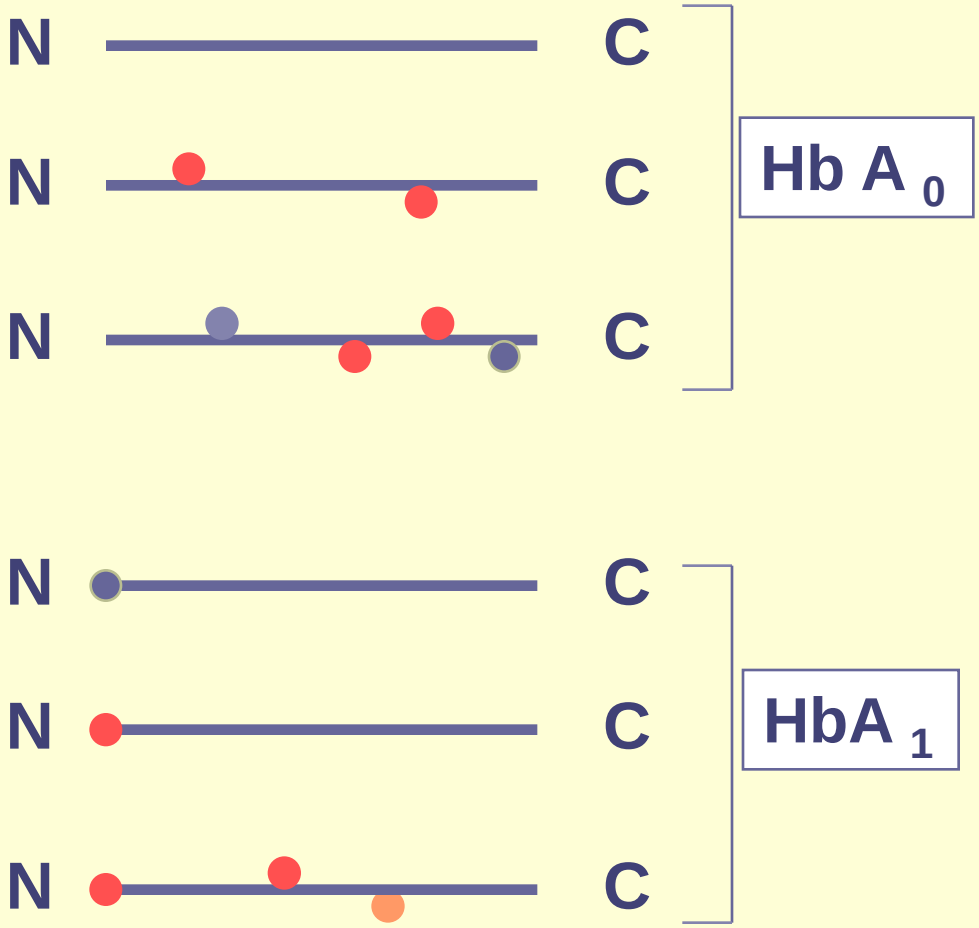
Hb A ($\alpha_2\beta_2$) α = 141 aminoacids
β = 146 aminoacids



Preferential glycation sites

- **β - Val - 1**
- **α - Lys - 16**
- **β - Lys - 66**
- **β - Lys - 17**
- **α - Val - 1**
- **α - Lys - 7**
- **β - Lys - 120**

Heterogeneity of glycated HbA



HbA_{1a1} : fructose 1,6-bisphosphate
HbA_{1a2} : glucose-6-phosphate
HbA_{1b} : pyruvate
HbA_{1c} : glucose

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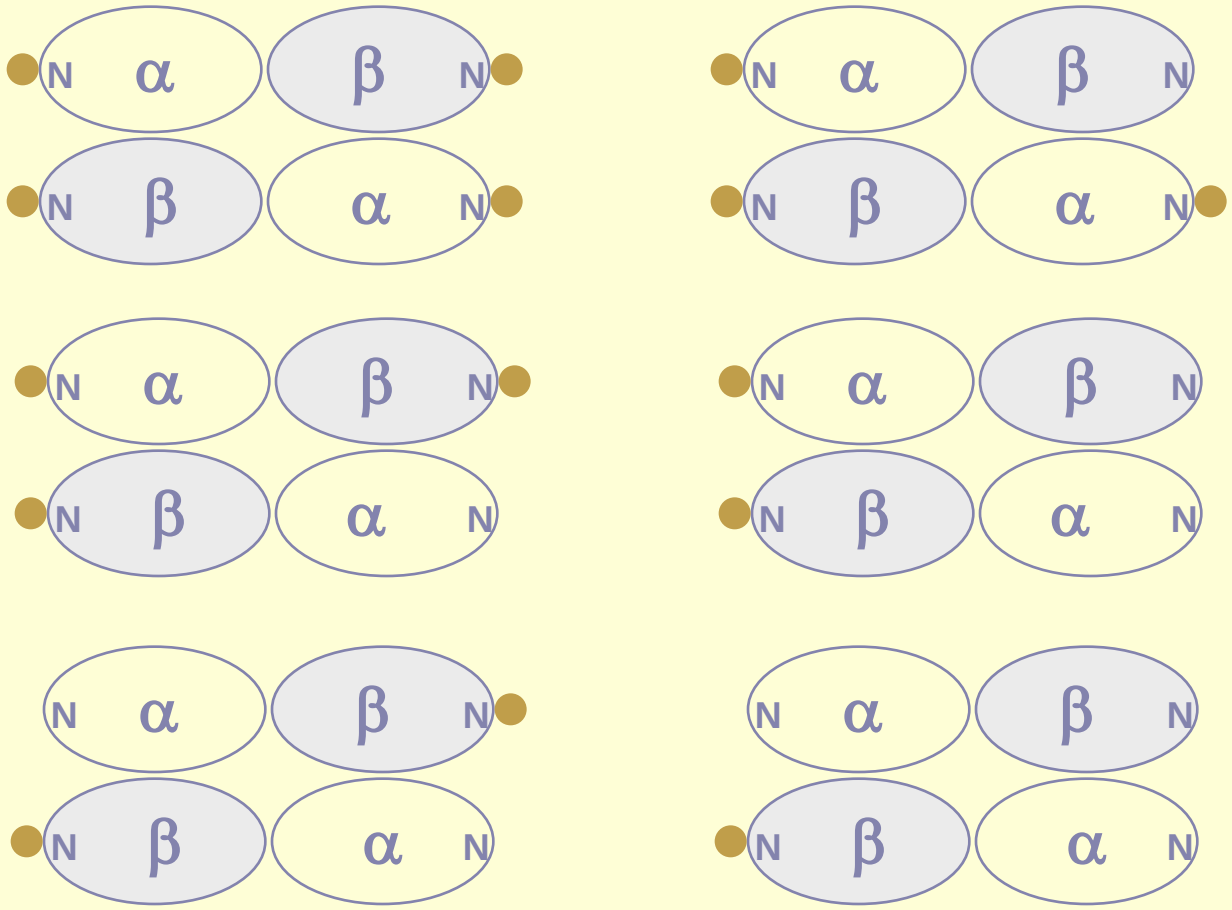
The different theoritical forms of HbA_{1c}



Necessity of a strict definition for standardization purpose

HbA_{1c} : the gold standard of diabetic survey

- Relationship HbA_{1c} / degenerative complications of diabetes mellitus (DCCT and UKPDS large-scale studies)

→ HbA_{1c} : retrospective and cumulative index of glycemic balance (4-8 weeks before sample)

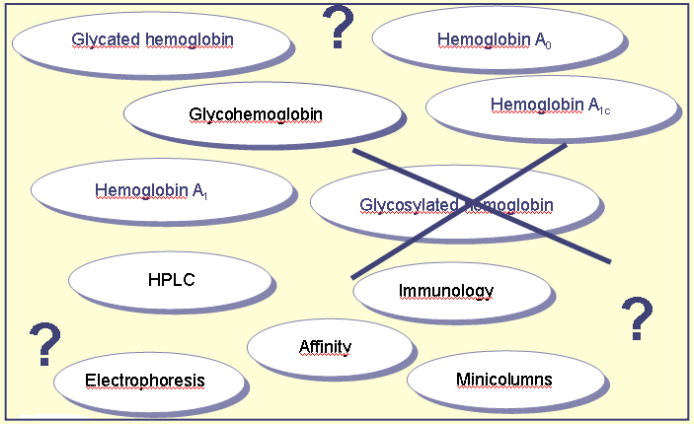
- Reference values and therapeutic targets
  - ✓ Reference Range : 4 - 6% of total Hb
  - ✓ Good Glycemic Control : < 6.5% (T2D)
    < 7.0% (T1D)
  - ✓ Poor Glycemic Control : > 8.0%

Note : HbA_{1c} values were established with reference to the NGSP standardization program (USA/international, non specific "reference method")

HbA_{1c} use before standardization

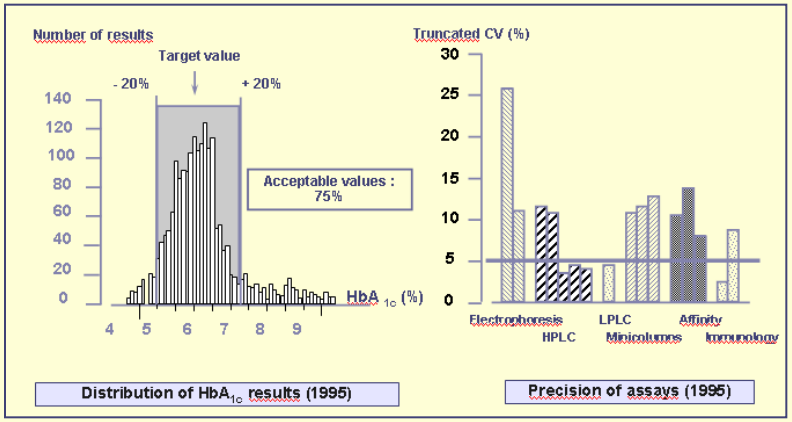
Disorders in terminology and concepts

Glycated hemoglobin language : another tower of Babel



Variable quality of methods

First quality control assessment in France (1995)



The conditions of the international standardization of HbA_{1c} assays

- Prerequisite : Selection of robust field methods

Clinical Chemistry 57:2
205–214 (2011)

Reviews

Status of Hemoglobin A_{1c} Measurement and Goals for Improvement: From Chaos to Order for Improving Diabetes Care

Randie R. Little,[1*] Curt L. Rohlfing,[1] and David B. Sacks[2,3*] for the National Glycohemoglobin Standardization Program (NGSP) Steering Committee

REVIEW ARTICLE

Measurement of Hemoglobin A_{1c}

A new twist on the path to harmony

DAVID B. SACKS, MB, CHB, FRCPATH

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journal homepage: www.elsevier.com/locate/clinchim

Invited critical review

The long and winding road to optimal HbA1c measurement

Randie R. Little *, Curt L. Rohlfing

Department of Pathology and Anatomical Sciences, University of Missouri School of Medicine, One Hospital Dr., Columbia, MO, United States

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December 4th, 2013

The conditions of the international standardization of HbA_{1c} assays

- Prerequisite : Selection of robust field methods, achieved by using intensive proficiency testing and quality assurance schemes

- Rationale : The NGSP standardization program previously used in most clinical studies (for establishing clinically meaningful values)
  - ✓ was based on a non specific reference method for HbA_{1c} assay (ion-exchange chromatography)
  - ✓ although having international activities, was only one national program (USA) among other standardization programs (Japan-Sweden)
  - ✓ could not garantee long-term traceability (valid permanent anchor)

The standardization process

- **International standardization (achieved by IFCC)**
- Aim : - Definition of the Hb species measured and of the measurand (HbA_{1c} or glycated Hb ?)
  - Definition and validation of RMP
- 1990s–2000s : IFCC Working Group on HbA_{1c} standardization
- 2002 : The definitive IFCC Reference Method

- **Glycated species measured** : HbA_{1c}
  - ✓ Clearly defined biochemical structure
  - ✓ Nonenzymatic binding of glucose
  - ✓ N-terminal extremity of HbA ($\alpha_2\beta_2$) β chains
  - ✓ Amadori rearrangement (glucose → deoxyfructose)
  - ✓ HbA_{1c} = N-(1-deoxyfructose-1-yl) β chain of hemoglobin

= DOF-hemoglobin (DOF-Hb)

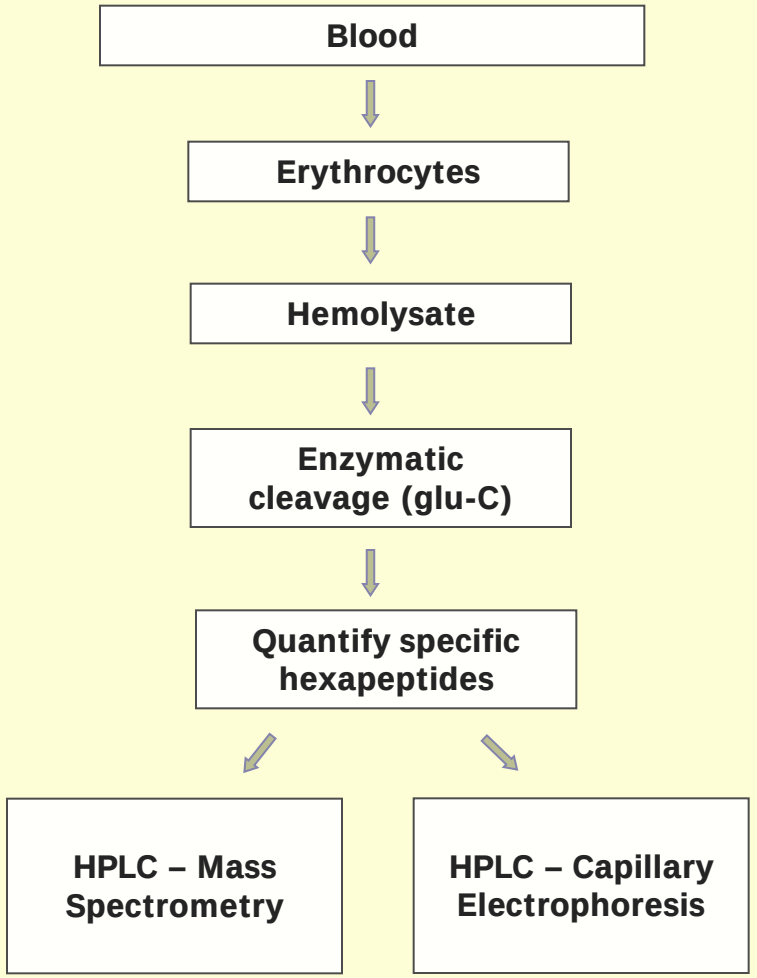
- **Primary reference materials** : Purified HbA_0 and HbA_{1c}

Global IFCC standardization

- Reference method (IFCC) :

  HPLC/MS or HPLC/CE

- Measurand : β-N-terminal hexapeptide [(glycated *vs* non glycated (mmol HbA_{1c}/mol HbA_0 + HbA_{1c})]



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

Effect on standardization on long-term stability

- RMP maintained by an **international IFCC network of approved laboratories : a valid anchor** (especially for calibration of field methods by manufacturers)
- More than 10 years of experience

Missions of the IFCC Network HbA_{1c}

- Guarantee continuity of the IFCC Reference Measurement Procedure (IFCC-RMP)
- Make HbA_{1c} assays wordwide traceable to the IFCC-RMP

IFCC Network Labs HbA_{1c} in 2013

16 approved laboratories

Asia

- China: Shanghai
  Prof. Ju Yi
- China: Beijing
  Prof. Wenxiang Chen
- Japan: ReCCS
  Dr. Violeta Raneva,
- Japan: Tokyo
  Prof. Izumi Takei
- Japan: Kanagawa
  Dr. Tadao Hoshino
  Dr. Yashihiro Hishinuma
- India: Calcutta
  Dr Bhaskar Bhattacharya
- South Korea: CDC
  Dr Junghan Song

Europe

- Italy: Universita di Milano
  Prof Andrea Mosca
- Netherlands: Isala Klinieken
  Dr. Robbert Slingerland
- **Netherlands: Queen Beatrix Hospital**
  Dr. Cas Weykamp, Coordinator
- Germany: Roche
  Dr. Angela Puhlmann
  Dr Roland Thiele
- Germany: INSTAND e.v
  Dr. Patricia Kaiser
- France: Reims
  Prof. Philippe Gillery

America

- USA: CDC
  Dr. Maria Ospina
- USA: Siemens, Norwood, MA
  Dr. Yuanfang Deng
- USA: Univ. Columbia, MO
  Prof. Randie Little

+ 2 candidate laboratories

www.ifcchba1c.net

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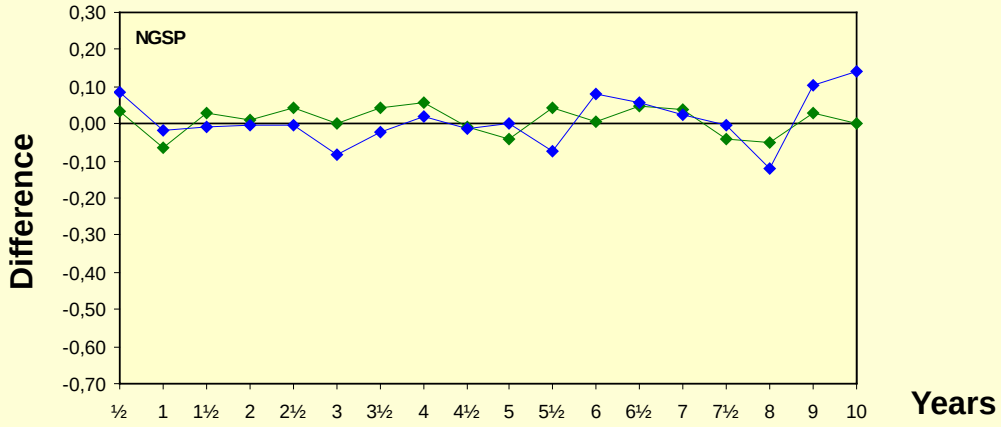
Stability

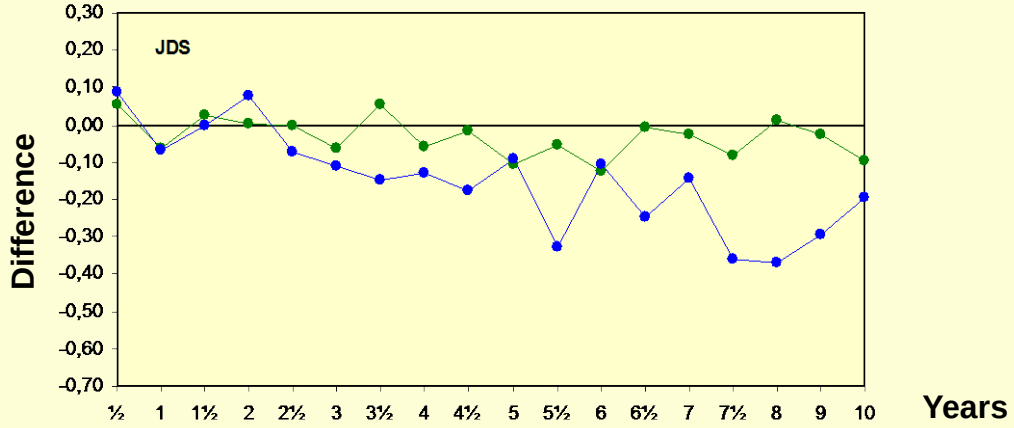
Master Equation IFCC – NGSP over 10 years

Green = Low HbA_{1c} level **Blue = High HbA_{1c} level**



Master Equation IFCC – JDS/JSCC over 10 years

Green = Low HbA_{1c} level **Blue = High HbA_{1c} level**



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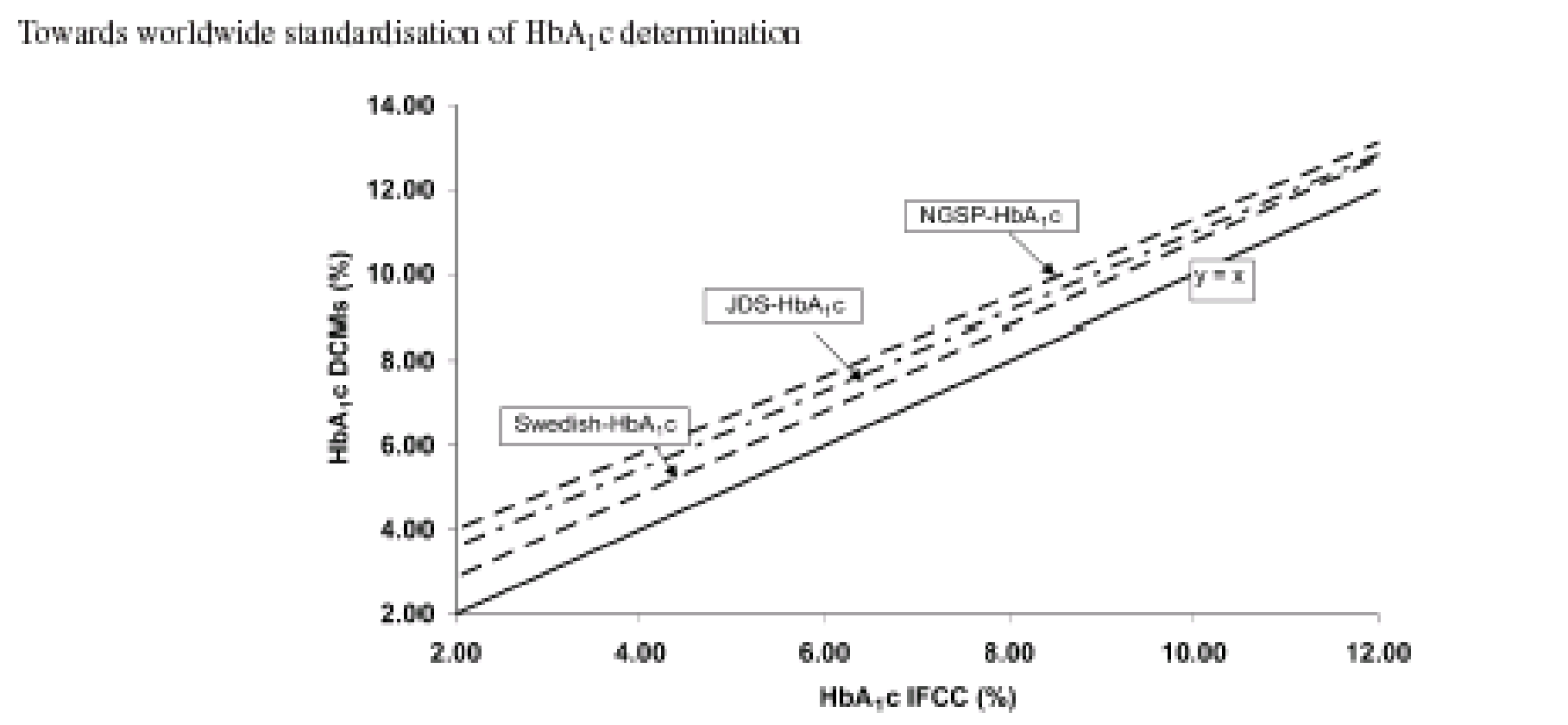
C. Weykamp et al

Master Equation IFCC vs other standardization programs



NGSP/DCCT HbA_{1c} = (0.915 x IFCC HbA_{1c}) + 2.15

Hoelzel et al., Clin. Chem., 2004, 50:166-174

Effect on result expression and reporting of units

IFCC reference method for HbA_{1c} is more specific than NGSP reference procedure and thus provides lower values in % (e.g. : 4 - 6% NGSP correspond to 2.0 - 4.2% IFCC)

- Keep the previous units (NGSP / DCCT) ?
  - ✓ Pro : well known and clinically meaningful values
  - ✓ Con : not "true" value
- Change to IFCC units in % ?
  - ✓ Pro : "true" values
  - ✓ Con : risk of destabilisation and of clinical unefficiency (⇨ not realistic)
- Use another expression mode ?
  - ✓ Other units for HbA_{1c} : mmol HbA_{1c}/mol Hb ("IFCC units" or "SI units") ?

Consensus ADA/EASD/IDF/IFCC (May 2007, updated 2013)

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

1. HbA_{1c} results should be standardized worldwide, including the reference system and results reporting
2. The new IFCC reference system for HbA_{1c} represents the only valid anchor to implement standardization of the measurement
3. HbA_{1c} results are to be reported worldwide in IFCC units (mmol/mol) and derived NGSP units (%), using the IFCC-NGSP master equation
4. - HbA1c conversion tables easily accessible to the diabetologic community
   - Report in both SI and NGSP/DCCT units in scientific journals
   - HbA_{1c} is the reportable term (A_{1c} may be used in guidelines and educational materials)

Consensus Committe, Diabetes Care, 2007, 30:2399-2400
R Hanas, WG John, Clin Chem Lab Med, 2013, 51, 1041-1042

IFCC standardization of HbA_{1c} assays : What remains to be done ?

⇨ **Strategy of implementation** of the new international standardization (**IFCC Integrated Project**) : reporting of units different according to the countries : dual reporting (*e.g*. France), SI reporting only (*e.g*. UK, Italy) …. or NGSP reporting only (*e.g*. USA, Canada)

⇨ **Validation of new numbers** by large-scale clinical studies

		mmol HbA_{1c} /mol Hb
✓ Reference Range	: 4 - 6% of total Hb	20 - 42
✓ Good Glycemic Control	: < 6.5% (T2D)	< 48
	< 7% (T1D)	< 53
✓ Poor Glycemic Control	: > 8%	< 64

Standardization : Effect of result expression on patient outcome

A first experience with percentages

Psychological Impact of Changing the Scale of Reported HbA_{1c} Results Affects Metabolic Control

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DIABETES CARE, VOLUME 25, NUMBER 11, NOVEMBER 2002

Aim : To evaluate the effect on a diabetic patient population of raising the reference scale up to the DCCT level in 1992 and then down to the Swedish National Standard in 1997.

Lab situation

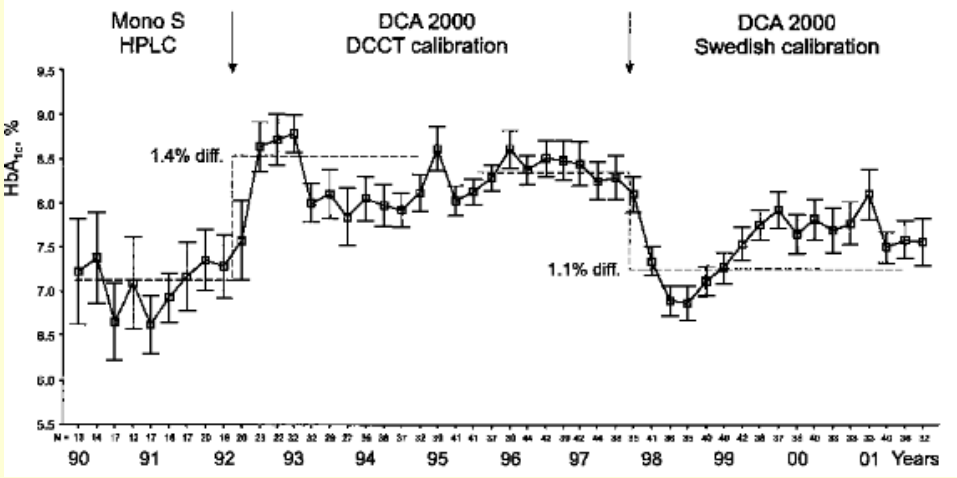
Before 1992 : Samples sent to central lab (Mono S HPLC method, Pharmacia, **reference range 3.0 - 4.6%**)

1992 : POCT use of DCA 2000, Bayer, **reference range 4.1 - 5.7%** DCCT reference

1997 : DCA 2000 calibration adjusted to the Swedish National Standard (**reference range 3.1 - 4.6%**)

Patients

- ✓Diabetes onset at least 3 years after the change in 1997
- ✓ Follow-up for at least 2 years after the change
- ✓ 49 children and adolescents (born 1971 to 1985)
- ✓ Intensive insuline therapy



1992 : - Expected : 1.4% higher
- Observed : after 9 - 12 months, mean HbA_{1c} value decreased $\approx$ 0.5% (i.e. glycemic control improved)

1997 : - Expected : 1.1% lower
- Observed : after transient decrease, mean HbA_{1c} values increased again (i.e. glycemic control deteriorated)

Conclusions

- Psychological impact of absolute numbers very high when small changes are made to reference levels.
- Be careful with changes of units ("IFCC perspective").

Clinical Chemistry 59:10
1457–1460 (2013)

Endocrinology and Metabolism

Glycemic Control in the 12 Months following a Change to SI Hemoglobin A_{1c} Reporting Units

Eric S. Kilpatrick,[1*] Alan S. Rigby,[2] Stephen L. Atkin,[3] and Julian H. Barth[4]

UK experience

- June 1st, 2009 : dual reporting (% DCCT and SI units)
- October 1st, 2011 : results reported solely in SI units (mmol/mol)
- 12 months evaluation

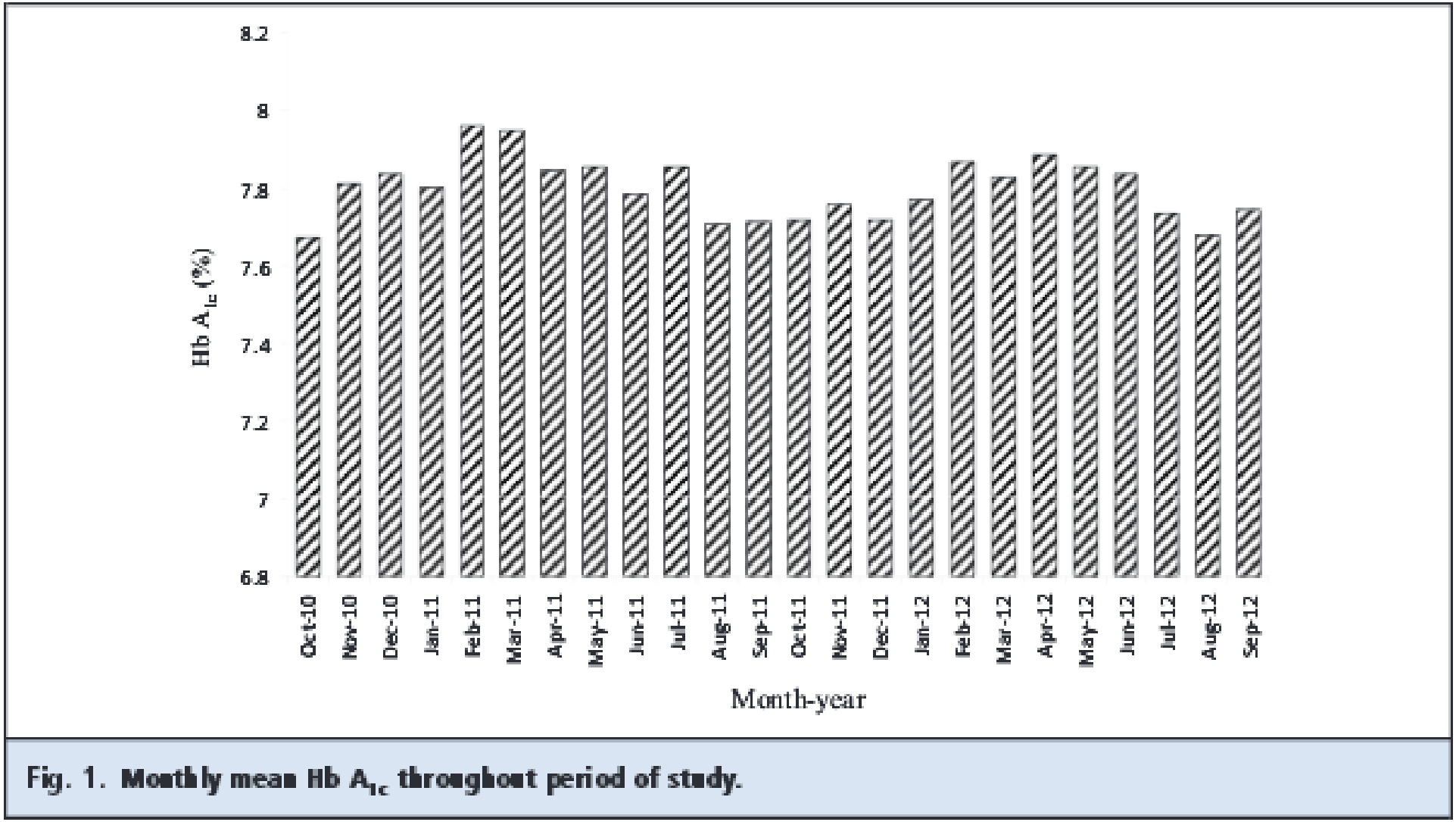


Fig. 1. Monthly mean Hb A_{1c} throughout period of study.

Table 1 : HbA_{1c} before and after change to reporting HbA_{1c} in SI units alone.[a]

	Year before unit change 2010-2011	Year after unit change 2011-2012	*P*
All samples			
n	21 880	22 841	
HbA_{1c}			
%	**7.5** (6.6, 8.7)	**7.5** (6.5, 8.7)	0.34
mmol/mol	**58** (49, 72)	**58** (48, 72)	
HbA_{1c} initially > 8% (64 mol/mol)[b]			
n			
HbA_{1c} change			
%	**- 0.2** (-0.9, 0.3)	**- 0.2** (-0.8, 0.3)	0.44
mmol/mol	**- 2** (10.3)	**- 2** (9.3)	
Days between HbA_{1c} samples	**99** (64, 147)	**98** (64, 147)	0.45

[a] All data expressed as median (25th, 75th) centiles.

[b] Change in HbA_{1c} represents the difference between 2 successive DCCT/SI values (before unit change) and 2 successive SI-only values (after unit change) in samples with initial values > 8% (64 mmol/mol)

Use of SI units

- No influence of unit change on quality of glycemic control
- To be confirmed on a longer period

Clinical Chemistry 59:10
1427–1429 (2013)

Editorials

Reporting Hemoglobin A_{1c}: Do the Units Matter?

David B. Sacks[1*]

Standardization : Effect on analytical goals

The Analytical Goals for Hemoglobin A_{1c} Measurement in IFCC Units and National Glycohemoglobin Standardization Program Units Are Different

Cas W. Weykamp[2*]
Andrea Mosca[3]
Philippe Gillery[4]
Mauro Panteghini[3]

Clinical Chemistry 57:8 (2011) 1205

Result expression and analytical goals

- **Could the change of units for reporting HbA_{1c} results impact analytical goals ?**
- Example : Repeatability study

	NGSP (%)	IFCC (mmol/mol)
HbA_{1c}	6.8	51
	6.5	48
	7.2	55
	7.0	53
Mean	6.88	51.8
SD	0.30	3.0
CV	4.3%	5.8%

Why this difference ?

Analogy with temperature

Unit variation equivalent to 1°C

- Celsius (°C, Europe)
- Fahrenheit (°F, USA) (°F = 1.8°C + 32)
- Kelvin (°K, official unit) (°K = °C + 273)

		Variation	Variation in percentage		
Celsius	37°C	1°C	1/37 x 100	=	2.7%
Fahrenheit	99°F	1,8°F	1.8/99 x 100	=	1.8%
Kelvin	310°K	1°K	1/310 x 100	=	0.3%

Could the conclusion be : « Temperature variation is lower in scientists and higher in Europeans » ?

Why this difference ?

- In both case, the conversion equation from one unit system to another is y = ax + b, where b (y intercept) is not equal to zero.
- Variation across metrologic systems cannot be compared in terms of relative percentages when b is different from zero.
- A higher y-intercept value has a greater impact (°F = 1,8°C + 32 and °K = °C +273)
- In clinical chemistry, it means that the specificity of both systems is different
- Case of HbA_{1c}

  . Master equation : NGSP/DCCT = (0.0915 IFCC + 2,15)

  . b (2.15%) represents the difference of specificity between the two methods

(NB: HbA_{1c} peak in ion-exchange chromatography is not pure)

Result expression and analytical goals

- Result expression mode (IFCC or NGSP units) modifies analytical goals, even when crude results are the same.
- Target values, estimated performance, CVs are concerned.

- Different expressions,different goals

Weykamp et al., Clin. Chem., 2011, 57, 1204-1205

Conclusions

- The international standardization of HbA_{1c} assays has brought
  - ✓ a valid anchor for all methods
  - ✓ a long term stability
  - ✓ significant changes in unit use and result reporting that necessitate a global strategy of implementation

- This strategy allows the optimal use of a valuable biological test in a important context of public health (and of new indications of HbA_{1c} assay : *e.g.* diagnosis of diabetes mellitus)

Thank you for your attention !

Pr Philippe Gillery
December 4th, 2013