## Il Punto

# Il punto sulla standardizzazione dell'emoglobina glicata

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**Riassunto**

**Nel corso degli ultimi due anni la standardizzazione dell'emoglobina glicata è oggetto di intensi dibattiti in vista della sua implementazione a livello mondiale. Nella presente relazione vengono presentati due recenti documenti internazionali sull'argomento e si delineano quelli che saranno gli ultimi passi da compiere prima di poter finalmente implementare in Italia il sistema di riferimento internazionale per l'$HbA_{1c}$.**

**Summary**

**Report on the standardization of glycated hemoglobin**

In the past two years the topic of the standardization of glycated hemoglobin has been extensively debated, in view of its global implementation. Within this report we comment on two recent documents, and we highlight the next steps to be taken before implementing the international reference system for $HbA_{1c}$ in Italy.
*Key-words:* $HbA_{1c}$, average blood glucose, diabetes.

La misura dell'emoglobina glicata ($HbA_{1c}$) nel sangue è da più di vent'anni il *gold-standard* per la valutazione del controllo glicemico nei soggetti diabetici. Infatti, dal momento della pubblicazione dei risultati dello studio del Diabetes Complications Control Trial (DCCT), sappiamo che livelli elevati dell'$HbA_{1c}$ aumentano il rischio delle complicanze micro-vascolari (ed anche macro-vascolari) e che quindi i clinici usano questo parametro come target per guidare le scelte terapeutiche[1]. La misura dell'$HbA_{1c}$ è anche molto importante per la valutazione del controllo glico-metabolico nei diabetici di tipo 2, e molti cominciano ad utilizzare questo parametro anche nella fase di diagnosi ed in una recente rassegna ho fornito varie informazioni sugli aspetti pre-analitici ed analitici che sono correlati all'utilizzo di questo parametro[2].

Come noto, perché il dato dell'$HbA_{1c}$ sia utilizzabile, occorre che le misure siano standardizzate, ed in quest'ottica dal 1995 la Federazione Internazionale di Chimica Clinica (IFCC) ha promosso le attività di un gruppo di lavoro che affrontasse il problema (IFCC WG-$HbA_{1c}$). Dopo circa 13 anni siamo giunti al punto di avere un metodo ufficiale di riferimento[3], di aver prodotto e resi disponibili due materiali primari di riferimento[4], di aver implementato una rete internazionale di laboratori di riferimento[5], di avere coinvolto tutte le aziende diagnostiche nell'allineamento al sistema di riferimento, e di essere sulla strada per sviluppare e rendere disponibili materiali secondari di riferimento, in collaborazione con l'ente europeo che ha già in deposito i materiali primari e che renderà disponibili anche i materiali secondari, cioè l'Institute for Reference Methods and Materials (IRMM).

La fase che ora dobbiamo affrontare riguarda l'implementazione, a livello degli utilizzatori finali (laboratori di analisi, centri per la cura del diabete, etc.), di questo nuovo sistema di riferimento. Questo numero della rivista pubblica, a tal fine, due importanti docu-

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menti che fanno luce su quest'ultima fase.

Il primo dei due[6] riporta in maniera concisa le decisioni prese a maggio del 2007 da un gruppo di persone rappresentative delle principali Società Scientifiche direttamente coinvolte nella tematica, quali l'American Diabetes Association (ADA), l'European Association for the Study of Diabetes (EASD), l'International Diabetes Federation (IDF) e l'IFCC. In quell'incontro si sono prese posizioni precise sulla standardizzazione, sulle unità di misura da adottare e sull'eventualità di riportare, accanto al dato dell'$HbA_{1c}$, un valore di glicemia media stimata sulla base dei risultati di uno studio clinico allora in corso ($A_{1c}$-Derived Average Glucose, ADAG).

Il secondo documento[7] riporta le conclusioni raggiunte a Milano in un successivo incontro tenutosi presso la sede IFCC con i rappresentati delle aziende diagnostiche. In tale incontro si sono fatti ulteriori progressi, che possono essere così riassunti:

a) Tutti i produttori di diagnostici si allineeranno al sistema di riferimento IFCC entro il 31 dicembre 2009.
b) Il nome del test sarà "$HbA_{1c}$" (non "$A_{1c}$", come già in voga soprattutto negli USA).
c) Tutti gli strumenti che saranno messi sul mercato dopo il primo gennaio 2011 (data facile da ricordare: 1-1-11) esprimeranno il risultato della misura in unità IFCC (mmol/mol) ed in unità derivate, allineate al sistema di riferimento americano del National Glycohemoglobin Standardization Program (NGSP), cioè in unità %.
d) I sistemi di misura non implementeranno, unitamente al risultato della misura dell'$HbA_{1c}$, il dato della glicemia media stimata, calcolabile sulla base dei risultati dello studio ADAG, lasciando ai professionisti di laboratorio la possibilità di refertare anche questo dato interfacciandosi con i sistemi informatici di laboratorio.
e) I materiali di controllo che dovranno essere usati nelle Valutazioni Esterne di Qualità (VEQ) dovranno essere commutabili ed avere un valore di $HbA_{1c}$ assegnato mediante il metodo di riferimento IFCC. Nei programmi di VEQ dovrà anche essere chiaramente definito il limite per l'errore totale ammissibile.
f) Il gruppo di studio IFCC sarà a disposizione dei produttori di diagnostici per aiutarli nella fase di allineamento al sistema di riferimento IFCC.

Per completezza comunichiamo che lo studio ADAG si è concluso, e che i risultati della prima elaborazione globale sono stati pubblicati[8]. C'è già intensa discussione sui risultati di questo studio, sulle sue limitazioni, e non intendiamo affrontare in questa sede una discussione su questo argomento. Infine, è stata da poco pubblicata una ulteriore puntualizzazione sulla terminologia e le unità di misura[9].

Cosa rimane quindi da fare a questo punto? A nostro avviso occorre ora prendere decisioni a livello nazionale con tutti coloro che hanno competenza nella discussione (*stakeholders*) e che, oltre che i professionisti di laboratorio ed i diabetologi, includono sicuramente i medici di base, gli operatori sanitari, gli endocrinologi, i pediatri, i rappresentanti dei pazienti, solo per menzionarne alcuni. E' quindi con questo spirito che nei prossimi mesi inizierà i lavori un gruppo di lavoro nazionale, con esperti nominati da 10 Associazioni e Società rappresentative delle categorie sopra menzionate, per arrivare ad un documento di consenso che possa fare chiarezza sulle prossime azioni e chiudere finalmente questa tematica. Il processo non sarà breve e qualunque siano le conclusioni che tale gruppo raggiungerà, occorrerà poi una valida campagna di informazione per implementare finalmente la standardizzazione di questo importante parametro di laboratorio.

## Bibliografia

1. DCCT Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993; 329:977-86.
2. Mosca A. La misura dell'emoglobina glicata nel sangue umano, attualità e prospettive. Biochimica clinica 2008; 32:27-35.
3. Jeppsson JO, Kobold U, Barr J, Finke A, Hoelzel W, Hoshino T, et al. Approved IFCC reference method for the measurement of HbA1c in human blood. Clin Chem Lab Med 2002; 40:78-89.
4. Finke A, Kobold U, Hoelzel W, Weykamp C, Miedema K, Jeppsson JO. Preparation of a candidate primary reference material for the international standardisation of HbA1c determinations. Clin Chem Lab Med 1998; 36:299-308.
5. Weykamp C, John WG, Mosca A, Hoshino T, Little R, Jeppsson JO, et al. The IFCC Reference Measurement System for HbA1c: A 6-Year Progress Report. Clin Chem 2008; 54:240-8.
6. Consensus statement. Consensus Statement on the Worldwide Standardization of the Hemoglobin A1C Measurement. Diabetes Care 2007; 30:2399-400.
7. Report. Implementation of standardization of HbA1c Measurement. Clin Chem Lab Med 2008; 46:573-4.
8. Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D, Heine RJ for the A1c-Derived Average Glucose Study Group. Translating the A1C Assay Into Estimated Average Glucose Values. Diabetes Care 2008; 31:1-6.
9. John WG, Nordin G, Panteghini M. What's in a name? Standardisation of HbA1c: a response. Clin Chem Lab Med 2008; 46:1326-7.

Reviews/Commentaries/ADA Statements
CONSENSUS STATEMENT

# 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

CONSENSUS COMMITTEE*

The hemoglobin A1C (A1C) assay has become the gold-standard measurement of chronic glycemia for over two decades. Anchored in the knowledge that elevated A1C values increase the likelihood of the microvascular complications of diabetes (and perhaps macrovascular complications as well), clinicians have used A1C test results to guide treatment decisions, and the assay has become the cornerstone for the assessment of diabetes care.

The clinical world has assumed that the A1C assay reflects average glycemia over the preceding few months. However, the data supporting that premise are not exceptionally robust (1–5); glucose concentrations were not measured frequently enough to compute a true "average." To gain a better understanding of the relationship between A1C and average blood glucose, an international study has been initiated to document this relationship, using frequent capillary measurements and continuous glucose monitoring. The results of this study will be known around September 2007. Although some clinicians are already providing patients with their "average blood glucose," by simply converting the current A1C test results (6) to a term more relevant to the values obtained from patient self-monitoring, the results of the study will hopefully provide a more accurate conversion algorithm.

Based on the work of the National Glycohemoglobin Standardization Program (NGSP) in the U.S. and other similar programs in other parts of the world, the current A1C assay has been harmonized on reference methods that measure a mixture of glycated hemoglobins (7–9). However, to achieve a more uniform standardization of A1C measurements, it is desirable to have a reference method that measures only a well-defined analyte. Accordingly, after several years of work, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) developed a new reference method that specifically measures the concentration of only one molecular species of glycated A1C (10,11). Results by the new reference method have also been compared with the results obtained by current methodologies (12), and the relation between the assays can be expressed by simple regression equations ("master equations"). Of note, the new reference method is only used to standardize the A1C assay and cannot be used by clinical laboratories in their measurement of A1C.

In keeping with the measurement of other analytes, the IFCC has also suggested that the test results be provided in scientifically correct units, i.e., mmol/mol (13). The impact of both changes proposed by the IFCC would be to significantly change the numeric results provided to clinicians. For example, an A1C value of 5% would become ~33 mmol/mol and an 8% would be ~65 mmol/mol.

### What are the implications of the above activities?

The advent of a new reference method to standardize the A1C results, along with the anticipated documentation that the assay does indeed indicate average blood glucose, has led to a variety of proposed changes in the reporting of A1C test results worldwide. To reach an agreement on a course of action, a meeting was held in Milan, Italy, on 4 May 2007, at which a consensus agreement emerged. The following statements have been approved by the American Diabetes Association, the European Association for the Study of Diabetes, the International Diabetes Federation, and the IFCC:

1. A1C test results should be standardized worldwide, including the reference system and results reporting.
2. The new IFCC reference system for A1C represents the only valid anchor to implement standardization of the measurement.
3. A1C results are to be reported worldwide in IFCC units (mmol/mol) and derived NGSP units (%), using the IFCC-NGSP master equation.
4. If the ongoing "average plasma glucose study" fulfills its a priori–specified criteria, an A1C-derived average glucose (ADAG) value calculated from the A1C result will also be reported as an interpretation of the A1C results.
5. Glycemic goals appearing in clinical guidelines should be expressed in

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*A list of the Consensus Committee members can be found in the APPENDIX.

This article is being simultaneously published in 2007 in *Diabetes Care* and *Diabetologia* by the American Diabetes Association and the European Association for the Study of Diabetes. 

**Abbreviations:** ADAG, A1C-derived average glucose; IFCC, International Federation of Clinical Chemistry and Laboratory Medicine; NGSP, National Glycohemoglobin Standardization Program.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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*Consensus Statement*

IFCC units, derived NGSP units, and as ADAG.

All the organizations agreeing with this consensus statement propose that these recommendations be implemented globally as soon as possible. We believe this agreement will further contribute to the worldwide comparability of A1C results, paralleling the progress of scientific knowledge related to the analytical and biochemical features of A1C testing. Expressing test results in scientifically correct units along with a clinically relevant interpretation of those results is not an uncommon practice (e.g., creatinine and estimated glomerular filtration rate). Consequently, clinicians will have the opportunity to convey the concept of chronic glycemia in terms and units most suitable to the patients under their care.

## APPENDIX

### Consensus Committee

For the IFCC: Jocelyn Hicks, PhD; Mathias Muller, MD; Mauro Panteghini, MD, PhD; and Garry John, PhD. For the American Diabetes Association: Larry Deeb, MD; John Buse, MD, PhD; David M. Nathan, MD; and Richard Kahn, PhD. For the European Association for the Study of Diabetes: Ele Ferrannini, MD, and Robert Heine, MD. For the International Diabetes Federation: Martin Silink, MD, and Jean-Claude Mbanya, MD.

**References**

1. Koenig BS, Peterson CM, Kilo C, Cerami A, Williamson JR: Hemoglobin A1C as an indicator of the degree of glucose intolerance in diabetes. *Diabetes* 25:230–232, 1976
2. Svendsen PA, Lauritzen T, Soegaard U, Nerup J: Glycosylated hemoglobin and steady-state mean blood glucose concentration in type I (insulin-dependent) diabetes. *Diabetologia* 23:403–405, 1982
3. Nathan DM, Singer DE, Hurxthal K, Goodson JD: The clinical information value of the glycosylated hemoglobin assay. *N Engl J Med* 310:341–346, 1984
4. Rohlfing CL, Wiedmeyer HM, Little RR, England JD, Tennill A, Goldstein DE: Defining the relationship between plasma glucose and $HbA_{1c}$: analysis of glucose profiles and $HbA_{1c}$ in the Diabetes Control and Complications Trial. *Diabetes Care* 25:275–278, 2002
5. Murata GH, Hoffman RM, Duckworth WC, Wendel CS, Shah JH: Contributions of weekly mean blood glucose values to hemoglobin in A1C in insulin-treated type 2 diabetes: the diabetes outcomes in veterans study (DOVES). *Am J Med Sci* 327:319–323, 2004
6. American Diabetes Association: Standards of medical care–2007. *Diabetes Care* 30 (Suppl. 1):S10, 2007
7. Goldstein DE, Little R, Lorenz RA, Malone JI, Nathan DM, Peterson CM, Sacks DB: Tests of glycemia in diabetes. *Diabetes Care* 27:1761–1773, 2004
8. Little RR, Rohlfing CL, Wiedmeyer HM, Myers GL, Sacks DB, Goldstein DE: The National Glycohemoglobin Standardization Program (NGSP): a five-year progress report. *Clin Chem* 47:1985–1992, 2001
9. Mosca A, Paleari R: Standardization schemes for hemoglobin A1c determination. In *Monitoring Glycaemic Control in the Diabetic Patient*. John WG, Ed. London, Harcourt Health Communications, Mosby International, 2001, p. 137–150
10. Kobold U, Jeppsson J, Dulffer T, Finke A, Hoelzel W, Miedema K: Candidate reference methods for hemoglobin A1C based on peptide mapping. *Clin Chem* 43:1944–1951, 1997
11. Jeppsson JO, Kobold U, Barr J, Finke A, Hoelzel W, Hoshino T, Miedema K, Mosca A, Mauri P, Paroni R, Thienpont L, Umemoto M, Weykamp C; International Federation of Clinical Chemistry and Laboratory Medicine (IFCC): Approved IFCC reference method for the measurement of HbA1c in human blood. *Clin Chem Lab Med* 40:78–89, 2002
12. Hoelzel W, Weykamp C, Jeppsson JO, Miedema K, Barr JR, Goodall I, Hoshino T, John WG, Kobold U, Little R, Mosca A, Mauri P, Paroni R, Susanto F, Takei I, Thienpont L, Umemoto M, Wiedmeyer HM; IFCC Working Group on HbA1c Standardization: IFCC reference system for measurement of hemoglobin A1C in human blood and the national standardization schemes in the United States, Japan, and Sweden: a method-comparison study. *Clin Chem* 50:166–174, 2004
13. Nordin G, Dybkaer R: Recommendation for term and measurement unit for "HbA1c." *Clin Chem Lab Med.* In press

# Implementation of standardization of $HbA_{1c}$ measurement

## Summary of the meeting with manufacturers held in Milan, IT - Dec 12, 2007

The IFCC Working Group on Standardisation of $HbA_{1c}$ (WG-$HbA_{1c}$) has been working for a number of years towards global standardisation of the $HbA_{1c}$ assay results. The infrastructure to achieve this objective is now in place; a reference method has been developed and a network of reference laboratories has been established. The IFCC has also been actively discussing reporting of $HbA_{1c}$ results with the clinical diabetes associations (ADA, EASD, and IDF). An outcome of these discussions was the publication of a Consensus Statement (Diabetes Care 2007;30:2399-400); this statement recommends reporting $HbA_{1c}$ results in mmol/mol (SI units) and derived NGSP units (%), using the IFCC-NGSP master equation. There is also the option of reporting an "interpretation" of the $HbA_{1c}$ result as "estimated average glucose" (eAG).

These proposals will not only have a significant impact on laboratory medicine and clinical diabetology, but also on industry. Thus, the IFCC welcome the opportunity of discussing these changes with manufacturers of $HbA_{1c}$ assays, inviting companies to attend this meeting where full information was exchanged.

The network facilities for traceability of manufacturers' assays to the IFCC reference system for $HbA_{1c}$, i.e. the way of giving values to the calibrators, the relationship ("master equation") between IFCC and NGSP values, the stability of this master equation, and the uncertainty issue, were illustrated. Tools, supplied by the IFCC network to the manufacturers, are now in place in order to achieve, check and prove traceability, as well to convert IFCC to NGSP numbers according with the ADA/EASD/IDF/IFCC Consensus Statement.

A general discussion, aimed to define the requirements for implementing IFCC and NGSP numbers for central laboratory instruments and point-of-care systems, the requirements and agreement for change, and a time frame for implementation, was then initiated. Several in the audience commented about the above issues and the following topics were specifically discussed and answered:

a) Q.: Who has to be contacted in order to participate to the activities of the IFCC network devoted to the manufacturers?
   A.: Any manufacturer wishing to prove traceability to the IFCC reference system has to contact Dr Cas Weykamp, the IFCC network coordinator.
b) Q.: How to implement the IFCC reference measurement procedure and to become member of the IFCC network of reference laboratories?
   A.: The reference measurement procedure is described with technical details in a standard operating procedure, available from the IFCC WG-

HbA$_{1c}$ website (www.ifcchba1c.net). Alternatively, documentation can be asked to the WG-HbA$_{1c}$ secretary Prof Andrea Mosca. The network actually includes 15 laboratories worldwide located.

c) Q.: How to run split sample comparison with the reference laboratories?
A.: The Joint Committee for Traceability in Laboratory Medicine (JCTLM) is listing the reference laboratory services available to run split sample exercises (http://www.bipm.org/en/committees/jc/jctlm/).

d) Q.: To what extent of accuracy (trueness and imprecision) the method developed by the manufacturers have to comply?
A.: It is a task of the IFCC WG-HbA$_{1c}$ to define the goals for imprecision, bias and total error to be then fulfilled by the manufacturers and every laboratory measuring HbA$_{1c}$ in the clinical field. This will be one of the WG's priorities for 2008.

**Meeting outcomes**

At the end of the discussion it was agreed that:

1) All manufacturers should implement worldwide the traceability to the IFCC reference system for HbA$_{1c}$. In the European Union (EU) the implementation of calibration traceability in laboratory medicine to higher-order standards is already mandatory. The EU directive 98/79/EC on in vitro diagnostic (IVD) medical devices explicitly requires manufacturers to ensure and document metrological traceability of their products.
2) The deadline for implementing traceability to the IFCC reference system is December 31$^{st}$, 2009 for all the instruments in current use.
3) The name (abbreviation) of the test in the laboratory reports and in the clinical setting should be "HbA$_{1c}$" (not "A1c").
4) All new instruments sold after January 1$^{st}$, 2011 will report (as a result of an HbA$_{1c}$ test) both SI (mmol/mol – no decimals) and NGSP derived units (percentage – one decimal), in agreement with the Consensus Statement.
5) The implementation of HbA$_{1c}$ results in terms of eAG will be discussed after the "A1c-derived average glucose" (ADAG) results of the ongoing clinical trial are published. Participants, however, agree that it is not an issue for analytical systems but, e.g., for laboratory information system (LIS) vendors (in analogy with the NKDEP/IFCC recommendation for implementation of the GFR estimating equation).
6) Introduction of External Quality Assessment (EQA) programs that use commutable control materials with target values assigned using the IFCC reference measurement procedure together with a clear definition of the clinically allowable total error of measurements is required. True value assignment to commutable EQA materials facilitates objective

evaluation of the performance of IVD devices, together with an accuracy-based (instead of the inferior consensus-based) grading of the competency of participating laboratories.

7) The IFCC WG-$HbA_{1c}$ is willing to review the proposed manufacturer's traceability chain. This does not mean that the IFCC WG will function in a regulatory role like a notified body or competent authority under the EU IVD directive. The WG-$HbA_{1c}$ can, however, provide an expert scientific opinion about the suitability of a manufacturer's proposed $HbA_{1c}$ traceability chain and offer some metrological advice and guidance if appropriate. This would allow manufacturers to engage in a dialog with the WG as appropriate to ensure that traceability for their $HbA_{1c}$ tests is acceptable.

*Persons attending the meeting*

*IFCC officers:*
Prof Mauro Panteghini, Chair of the IFCC Scientific Division (SD)
Dr Garry John, Chair of the IFCC SD WG on Standardization of HbA1c (WG-$HbA_{1c}$)
Prof Andrea Mosca, Secretary of the IFCC SD WG-$HbA_{1c}$
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David Ambruster, Lieselotte Lennartz (Abbott); Beate Saeger, Takeshi Takagi (Arkray); Cathinca Vargmo, Kjersti Grimsrud (Axies-Shield and Vogt); Ben Irvin (Bayer); Elisabetta Della Dea (Beckman Coulter); Tamara Davis, Gianni Bertoli, Laura Madia (Bio-Rad Laboratories); Chistiane Wernz, Alexandra Lein (Dia Sys Diagnostic Systems); Nick Mayor (Genzyme); Francesco Caggiano (Menarini); Bernd Vögt (Roche Diagnostics); Geneviève Hennache (Sebia); Takuya Yotani (Sekisui Chemical Co.); Mary Lou Gantzer (Siemens); Nancy Van Bijlen (Tosoh Bioscience).