THE HONG KONG COLLEGE OF PATHOLOGISTS
SCIENTIA ILLUMINAT MEDICINAM
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## Editorial note:

In this issue of Topical Update, Dr. Morris TAI discussed on the use and standardization of the HbA1c test, an important assay in the management of diabetes mellitus. We welcome any feedback or suggestions. Please direct them to Dr. Janice Lo (e-mail: janicelo@dh.gov.hk) of Education Committee, the Hong Kong College of Pathologists. Opinions expressed are those of the authors or named individuals, and are not necessarily those of the Hong Kong College of Pathologists.

# Global Standardization of HbA1c

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### Introduction

The prevalence of diabetes mellitus (DM) has been increasing in recent years and DM is now a global epidemic. Haemoglobin A1c (HbA1c) plays an important role in the management of DM as the vast majority of outcome studies on diabetic complications are based on it. The most famous of such studies, which demonstrated the relationship of HbA1c to diabetic complications, are the Diabetes Control and Complications Trial (DCCT) & the United Kingdom Prospective Diabetes Study (UKPDS). HbA1c is formed via a posttranslational nonenzymatic attachment of glucose to haemoglobin in an irreversible fashion. In strict chemical terms, the molecular structure of HbA1c is β-N-(1-deoxy)-fructosyl-haemoglobin and it serves as an indicator of glycaemic control over the preceding 2- to 3- month period.

There are a great number of analytical methods used in the measurement of HbA1c. More than 20 methods were in clinical use as reported in the year 2004. The heterogeneity of methodology eventually generated concerns about comparability and usability of HbA1c, especially when patients' data were to be compared with study results. The call for test standardization was therefore critical. Various standardization programmes have been carried out since the 1990s. The National Glycohaemoglobin Standardization Program (NGSP) and the International Federation of Clinical Chemistry (IFCC) are the two most important international standardization programmes while local ones such as Japan Diabetes Society/Japanese Society for Clinical Chemistry (JDS/JSCC) and Mono-S have been adopted in Japan and Sweden respectively.

## The standardization programmes

The NGSP was initiated by the American Association of Clinical Chemistry in July 1996 aiming at harmonization of HbA1c methods so that HbA1c results generated from different methods could be aligned to the ones employed in the DCCT & the UKPDS. Designated comparison methods, but not a primary reference method, is the standardization method used in NGSP, as well as JDS/JSCC and Mono-S.

The HbA1c standardization working group of IFCC was formed in 1994. They adopted a totally different approach. They prepared pure standards of Hb and HbA1c, which were subsequently digested with endopeptidase. The glycated and non-glycated N-terminal hexapeptides were then separated by reversed phase high-performance liquid chromatography (HPLC) followed by identification and quantification by capillary electrophoresis or electrospray ionization mass spectrometry (ESI-MS). This method is highly specific - only the compounds matching the eluent time in HPLC and mass spectrum in ESI-MS are detected as Hb and HbA1c. Because of the high specificity, the IFCC HbA1c values are lower than the NGSP values by about 2 %. Correlation studies demonstrated that NGSP and IFCC results are highly correlated and the results are interchangeable by a master equation (HbA1c-NGSP) = 0.915(HbA1c-IFCC)+2.15%. Equations converting IFCC values to either JDS- or Mono S-equivalents are also available.

Following these developments, a meeting was held in Milan 2007 and a consensus statement was published jointly by American Diabetes Association (ADA), European Association for the Study of Diabetes (EASD), IFCC and International Diabetes Federation (IDF). The five recommendations were:

1. HbA1c test results should be standardized worldwide, including the reference system and results reporting.
2. The new IFCC reference system for HbA1c represents the only valid anchor to implement standardization of the measurement.
3. HbA1c 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” fulfils 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. Glycaemic goals appearing in clinical guidelines should be expressed in IFCC units (i.e. mmol/mol), derived NGSP units (i.e. %), and as ADAG values (i.e. mg/dl or mmol/L).

Concerning point 4, the correlation study was finished and published in August 2008; the results support the notion between HbA1c levels and ADAG for both type 1 and type 2 DM.

## Impact on management of DM

The new standardized IFCC-HbA1c result is not trouble-free at all. It has been criticized that patients & health care professionals may be confused and be falsely reassured by the seemingly lower IFCC values (in %). The introduction of ADAG and the use of the unit mmol/mol for IFCC-HbA1c may solve this problem and certainly more time is required for the clinicians and patients getting used to the new reporting format. As NGSP methods were used in previous studies, comparison of new data with historical ones requires conversion by master equation and this creates substantial inconvenience.

Currently screening and diagnosis of DM rely on two tests, fasting plasma glucose and oral glucose tolerance test. The former suffers from inadequate sensitivities while the latter is cumbersome and is infrequently used in clinical settings. Fasting is required in both tests and repeated testing is necessary to establish the diagnosis. Ever since the invention of glycated haemoglobin, it has been suggested to be used as a tool for diagnosis of DM. In fact, the glycation of haemoglobin may be more accurately reflecting the pathogenesis of the complications associated with prolonged hyperglycaemia toxicity in which toxic advanced glycation end products are involved. Furthermore

fasting is not necessary if the test is done for screening and short-term life style changes do not affect the HbA1c level at all. However the use of HbA1c as screening and diagnosing tools was rejected in the current ADA recommendations, which were made a decade ago, largely because HbA1c was considered at that time to be inadequately standardized and insensitive. The resolution of the standardization issue has allowed the use of HbA1c for diagnosis of DM. An expert panel recently published new diagnostic guidelines, recommending an HbA1c screening cutoff of 6% as a threshold for close follow-up, and a diagnostic cutoff of 6.5%. This recommendation improves the investigation flow as diagnosis of DM can be made after a single blood collection for HbA1c and fasting glucose as the simultaneous glucose result may support the diagnosis of DM.

**Summary**

HbA1c is the cornerstone of diabetes care. It is widely used as a treatment goal and to predict the risk of development of complications in DM patients. Various standardization programmes have been carried out in the last 15 years and results obtained by different methods are interchangeable by master equations. It is agreed that the measurement should be standardized against the IFCC method, which detects the “true” HbA1c. NGSP values will not be abandoned as it is aligned to most large DM studies. ADAG values may provide more comprehensible results to patients. Future reports may contain more than one of these measured and derived values.

**References**


1. Consensus Committee. 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. Diabetes Care. 2007 Sep;30(9):2399-400.
2. Geistanger A, Arends S, Berding C, Hoshino T, Jeppsson JO, Little R, Siebelder C, Weykamp C; IFCC Working Group on Standardization of Hemoglobin A1c. Statistical methods for monitoring the relationship between the IFCC reference measurement procedure for hemoglobin A1c and the designated comparison methods in the United States, Japan, and Sweden. Clin Chem. 2008 Aug;54(8):1379-85.
3. Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D, Heine RJ; A1c-Derived Average Glucose Study Group. Translating the A1c assay into estimated average glucose values. Diabetes Care. 2008 Aug;31(8):1473-8.
4. Saudek CD, Herman WH, Sacks DB, Bergenstal RM, Edelman D, Davidson MB. A new look at screening and diagnosing diabetes mellitus. J Clin Endocrinol Metab. 2008 Jul;93(7):2447-53.
5. Weykamp C, John WG, Mosca A, Hoshino T, Little R, Jeppsson JO, Goodall I, Miedema K, Myers G, Reinauer H, Sacks DB, Slingerland R, Siebelder C. The IFCC Reference Measurement System for HbA1c: a 6-year progress report. Clin Chem. 2008 Feb;54(2):240-8.