**Global standardisation of HbA1c**

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**Abstract**

HbA1c is used for assessing glycaemic control in patients with diabetes. It is also used for treatment goals and as a target for therapeutic intervention. The Direct Control and Complications Trial in the USA showed that HbA1c can be used to predict the risk of complications. Hence, it is important for HbA1c assays to be standardised. The National Glycohemoglobin Standardization Program (NGSP) in the USA was formed in 1996 so that HbA1c results from different laboratories would be comparable to those reported in the DCCT study. There were also HbA1c standardisation programmes in Sweden and Japan. These three standardisation programmes are, in fact, direct comparison methods (DCMs), and yield different HbA1c results. In 1994, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) established a Working Group on Standardisation of HbA1c. This working group has developed a global HbA1c reference system with very much improved intra-assay and inter-assay coefficients of variation. Recommendations have been made to report HbA1c results as IFCC-HbA1c values in SI units (mmol HbA1c/mol Hb) and NGSP-HbA1c (%) as well as estimated average glucose (eAG), once a tight relationship has been shown to exist between eAG and HbA1c.

**Key words:** Standardisation of HbA1c, estimated average glucose, International Federation of Clinical Chemistry and Laboratory Medicine, National Glycohemoglobin Standardization Program

**INTRODUCTION**

HbA1c is the gold standard for assessment of glycaemic control in patients with diabetes, reflecting the average blood glucose over the preceding two to three months. It is used for treatment goals and as a target for therapeutic intervention. The Direct Control and Complications Trial (DCCT) in the USA, published in 1993, showed that HbA1c can be used to predict the risk of development of complications in patients with diabetes.\(^\text{1}\) Hence, alignment of HbA1c results to that of the DCCT has become clinically important so that HbA1c can be used to predict the risk of various complications. The HbA1c value measured in Malaysia or anywhere in the world should yield similar values to that of the DCCT.

**STANDARISATION OF HbA1c METHODS IN USA, SWEDEN AND JAPAN**

In 1993, the American Association for Clinical Chemistry (AACC) formed a Subcommittee on Glycohemoglobin Standardization as the methods in existence for the measurement of HbA1c all yielded widely varying results. The National Glycohemoglobin Standardization Program (NGSP) was formed in July 1996 to standardise HbA1c methods so that HbA1c results from different laboratories would be comparable to those reported in the DCCT study.\(^\text{2}\) The NGSP standardisation of HbA1c methods has been adopted by many countries including the USA, Canada, Mexico, South America, most European Countries and most Asian Countries. HbA1c standardisation programmes were also established in Japan [Japanese Diabetes Society (JDS) in collaboration with the Japanese Society of Clinical Chemistry (JSCC)]\(^\text{3}\) and in Sweden (Mono S)\(^\text{4}\) in the mid to late 1990s. These three standardisation programmes improved the quality of HbA1c assays in clinical use. The methods used in these standardisation programmes are, however, not primary reference methods but are direct comparison methods (DCMs). These three DCMs yield different results.
IFCC REFERENCE SYSTEM FOR HbA1c

In 1994, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) established a working group on standardisation of HbA1c. This working group has met regularly since 1995 and has developed a global HbA1c reference system, prepared pure HbA and HbA1c as the primary reference material, developed two reference methods, installed a network of 13 reference laboratories in the USA, Europe and Japan, and prepared secondary reference material.5,6 HbA1c has been defined as β-N-valine glycated haemoglobin (β-N-(1-deoxy)-fructosyl-haemoglobin), which is the major glycation site of HbA1c. The reference methods are based on two-dimensional peptide mapping after proteolytic cleavage with endoprotease Glu-C, then separation of the resulting glycated and non-glycated N-terminal hexapeptides by reversed-phase high performance liquid chromatography, followed by quantification by mass spectrometry or by capillary electrophoresis (Figure 1).7 Both methods give identical results.

The precision of the methods used to measure HbA1c was substantially improved using the IFCC standardisation with mean intra-laboratory coefficients of variation (CVs) of 1.0 to 1.2% and inter-laboratory CVs of 1.4 to 1.9%.8

Considering the lack of specificity of the DCMs used in the standardisation programmes in USA (NGSP), Japan (JDS/JSCC) and Sweden (Mono S), it is not surprising that the HbA1c results generated by these DCMs are higher than the results produced by the IFCC reference method.

Relationship between the IFCC reference method and the direct comparison methods

Correlations between the IFCC reference method and the DCMs have yielded the following master equations which have been found to be very robust:

1. NGSP-HbA1c = 0.915(IFCC-HbA1c) + 2.15% \( (r^2 = 0.998) \)
2. JDS/JSCC-HbA1c = 0.927(IFCC-HbA1c) + 1.73% \( (r^2 = 0.997) \)
3. Swedish-HbA1c = 0.989(IFCC-HbA1c) + 0.88% \( (r^2 = 0.996) \)

Reasons for implementing the IFCC reference method

The In-Vitro Diagnostic (IVD) Directive concerning medical devices in Europe states that diagnostic manufacturers must guarantee the traceability of their routine measurements to reference methods and materials of higher metrological order. This implies that manufacturers must calibrate using IFCC methodology, being the reference method of higher metrological order. A second reason for change is to improve globally the quality of HbA1c assays.

Concerns by clinicians over the reporting of IFCC-HbA1c

IFCC-HbA1c values are lower than the NGSP-HbA1c values by about 2%. This has caused considerable disquiet amongst diabetes specialists who are very concerned that reporting of the lower IFCC-HbA1c values may lead to misinterpretation of the degree of glycaemic control and, thus, confuse both doctors and patients. The learning curve for both doctors and patients might be as long as three years. During this period there will be a worsening of glycaemic control resulting in adverse clinical outcomes for patients. Whilst the biochemist may wish to report IFCC-HbA1c values, diabetes specialists prefer the reporting of IFCC-HbA1c values as mean blood glucose (MBG) since this would be readily understood by both doctors and patients. The main concern which the IFCC and many biochemists have regarding the reporting of MBG is that an additional error would be introduced which could be substantial if there is considerable scatter about the linear regression between MBG and IFCC-HbA1c.
A meeting was held in London on 20 January 2004 amongst the International Diabetes Federation (IDF), European Society for the Study of Diabetes (EASD) and the American Diabetes Association (ADA) with the Chairman of the IFCC-HbA1c working group and a representative of NGSP. This International Diabetes Working Group, led by the IDF, agreed that the IFCC Reference Method should become the global reference standard and that all manufacturers should calibrate to the new method. DCCT-aligned (NGSP) HbA1c values would continue to be reported in the interim period. It was accepted at that meeting that MBG would be reported instead of HbA1c, provided that an international study to be conducted prospectively showed that there was a tight correlation between IFCC-HbA1c values and MBG. Public and professional education programmes about the new reporting units and system should be planned.

The IFCC, IDF, EASD and ADA met in Milan, Italy on 4 May 2007, chaired by the President of IFCC, Dr Jocelyn Hicks, and agreed the following.10,11

1. HbA1c results would be reported worldwide in IFCC units (mmol HbA1c/mol Hb), instead of % HbA1c in order to avoid reporting the lower HbA1c values which may be confusing to both clinicians and patients, and derived NGSP units (%) using the IFCC-NGSP master equation.

2. If the ongoing “average plasma glucose study” fulfills its specified criteria, an A1C-derived average glucose (ADAG) value calculated from the HbA1c result will also be reported.

3. Glycaemic goals appearing in clinical practice guidelines should be expressed in IFCC units, derived NGSP units and ADAG.

In September 2007, the interim results of the ADAG Study were presented at the EASD meeting in Amsterdam and several clinical societies supported the reporting of an estimated average glucose (eAG) instead of MBG or ADAG.

A meeting was held on 12 December 2007 in Milan, Italy between the IFCC and diagnostic companies. It was agreed at this meeting that all manufacturers should implement worldwide traceability to IFCC reference system for HbA1c. The deadline for implementing the IFCC reference system for all instruments in current use is 31 Dec 2009. The name of the test in laboratory reports and clinical setting would be HbA1c, and not, as used in the United States, A1C. All new instruments sold after 1 Jan 2011 would report HbA1c in both SI (mmol/mol – no decimals) and NGSP-derived units (percentage – one decimal place). The implementation of estimated average glucose (eAG) would be discussed after the ADAG clinical trial was published.

The following is a summary of a United Kingdom press release in April 2008 by the Association for Clinical Biochemistry and Diabetes UK regarding the reporting of HbA1c.

1. HbA1c should be standardised using the IFCC Reference Measurement procedure.

2. An extensive education programme should be developed urgently for all healthcare professionals and people with diabetes to help the understanding and interpretation of the new IFCC units.

3. HbA1c results should be reported in both IFCC units (mmol/mol) and derived NGSP units (%).

4. There is currently insufficient experimental evidence to support the introduction of eAG as the study was conducted on a predominantly white population.

5. Further research into the use of eAG in children, different ethnic groups and pregnancy is required.

**A1C-DERIVED AVERAGE GLUCOSE STUDY**

The ADAG study12 was published in August 2008. The study had recruited 507 subjects from ten international centres. There had initially been eleven centres but one centre withdrew from the study. The study populations comprised 268 subjects with type 1 diabetes, 159 subjects with type 2 diabetes and 80 non-diabetic subjects. HbA1c levels obtained at the end of three months and measured in a central laboratory were compared with the average glucose levels during the previous three months. The average glucose was calculated by combining weighted results from at least two days of continuous glucose monitoring performed four times, with seven-point daily self-monitoring of capillary glucose performed at least three days per week. Approximately 2,700 glucose values were obtained by each subject over three months. A
reasonably good linear regression was obtained between the eAG and HbA\textsubscript{1c} \((r^2 = 0.84, P = 0.0001)\). Table 1 shows the corresponding eAG concentrations in both mg/dL and mmol/L for any given NGSP-HbA\textsubscript{1c} value. Table 2 shows the corresponding IFCC-HbA\textsubscript{1c} values in SI units (mmol HbA\textsubscript{1c}/mol Hb) and eAG concentrations for any given NGSP-HbA\textsubscript{1c} value.

**CONCLUSION**

There is now general agreement that we should begin to report HbA\textsubscript{1c} values in both SI units (mmol/mol) and derived NGSP units (%). Whilst the ADA also favours the reporting of eAG based on the findings of the ADAG study, this should perhaps await further studies assessing the relationship between eAG and HbA\textsubscript{1c} in different ethnic groups, children and in pregnancy.

**REFERENCES**


