

# **HbA1c method performance: the great success story of global standardization.**

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## **Abstract**

Diagnosing and monitoring the treatment of people with diabetes is a global issue and uses considerable resources in laboratories and clinics worldwide. Hemoglobin A1c (HbA1c) has been the mainstay of monitoring glycaemic control in people with diabetes for many years and more recently it has been advocated as a diagnostic tool for type 2 diabetes (T2DM). Good analytical performance is key to the successful use of any laboratory test but is critical when considering using the test to diagnose disease, especially when the potential number of diagnoses could exceed 500 million people. Very small variations in bias or increased imprecision could lead to either a missed diagnosis or over diagnosis of the disease and given the scale of the global disease burden this may mean erroneous categorization of potentially millions of people. Fundamental to good performance of diagnostic testing is standardization, with defined reference material and measurement procedures. In this review we discuss the historical steps to first harmonize HbA1c testing, followed by the global standardization efforts and provide an update as to the current situation and future goals for HbA1c testing.

## **Introduction**

The global burden of diabetes, and its complications, is increasing rapidly and it is estimated that by 2049, 629 million people will have the disease (1). Approximately one in two of those with diabetes do not know they have the disease. It is essential that there is a low cost, reliable and robust tool for diagnosing and monitoring disease progression. Haemoglobin A1c (HbA1c) has long been accepted as a monitoring tool for diabetes but more recently has also been advocated as a diagnostic tool for type 2 diabetes (T2DM) (2-4).

The Diabetes Control and Complications Trial (DCCT) study in people with type 1 diabetes mellitus, which was published in 1993, clearly demonstrated that the risk of the development and progression of micro-vascular complications was closely related to the degree of glycaemic control (5). Long term follow up studies showed that macro vascular complications were also correlated with increasing HbA1c levels (6). At the same time as the DCCT study the UKPDS trial addressed improving glycaemic control

in patients with T2DM and similarly demonstrated that improved control, evidenced by lower HbA1c values, resulted in improved patient outcomes (7). Since these studies were completed numerous large scale studies have added to our understanding of diabetes treatments and management but HbA1c remains at the heart of new treatment regimes (8-16). Treatment targets have moved away from those initially determined by those early studies and are now based on multiple factors such as age of the person, duration of disease, co-morbidities and likely compliance with therapy (17).

In 2011 the WHO advocated the use of HbA1c for the diagnosis of diabetes at a value of 48mmol/mol (6.5%), in line with American Diabetes Association (ADA) recommendations. The WHO guidance categorically states that “provided that stringent quality assurance tests are in place and assays are standardised to criteria aligned to the international reference values” (3). It is essential therefore that manufacturers of HbA1c methods, used for diagnosis of diabetes, are able to demonstrate alignment to the IFCC reference measurement procedure and perform well in quality assurance schemes with accuracy based targets (18-25). In this review we detail the current state of the art of HbA1c measurement and standardization, and highlight the key roles play by different organisations and laboratory users in the determination of high quality and accurate HbA1c values to ensure optimal patient care.

## **What is HbA1c?**

HbA1c is formed when glucose binds to the N-terminal valine of the beta chain of an haemoglobin molecule. The glycation process is non-enzymatic, therefore it is only dependent on time, glucose concentration and haemoglobin levels. Red blood cells are generally thought to circulate for around 106 days with a variation of  $\pm 20\%$  (26) and so HbA1c represents the average glucose concentration that the Hb is exposed to over a period of around 106 days. The distribution of red blood cells with time means that the HbA1c is a greater reflection of the preceding 30-60 days. This integration of glucose exposure over time makes HbA1c a valuable marker in assessing glycaemic control, over a longer period of time than can be assessed using glucose which only indicates glycaemia at a single time point.

## **Method principles for the measurement of HbA1c**

There are currently more than 100 HbA1c assays available on the market all of which are based on one of two principles: separation based on charge differences and separation based on structural differences, which occur when glucose binds to Hb. The former principle is used in ion-exchange chromatography and electrophoresis-based assays, whilst the latter principle is used in immunoassays, assays based on boronate affinity chromatography and enzymatic methods.

### ***Methods based on charge differences***

Methods based on charge differences depend on the extra negative charge that occurs when glucose is attached to the N-terminal valine of the HbA  $\beta$ -chain. Examples of such methods are cation-exchange chromatography and electrophoresis. Cation-exchange chromatography is a process that allows the separation of a mixture of proteins based on the charge properties of the molecules in the mixture. Charged haemoglobins and other haemoglobin components are eluted at varying times depending upon the net charge of the molecule in relation to a gradient of increasing ionic strength buffers passed through a cation-exchange column.

Capillary electrophoresis uses the principle of liquid-flow capillary electrophoresis in free solution. With this technique, charged molecules are separated by their electrophoretic mobility in an alkaline buffer at a specific pH. The separation of the different haemoglobin fractions takes place in silica capillary tubes and the migration is performed at high voltage (e.g. 9800 volts) with temperature control using a Peltier device. The haemoglobins are detected at an absorption wavelength of 414 nm at the cathodic end of the capillary using an optical detector (27).

### ***Methods based on structural differences***

Affinity separation is based on the covalent binding of cis-diols of glucose in glycated haemoglobin to a boronate matrix. Non-glycated haemoglobin does not bind to the boronate matrix and is eluted directly from the column. Glycated haemoglobin initially binds to the column and is released when buffers with a higher affinity for the boronate binding sites is applied, thus displacing the bound glycated haemoglobin. The resulting chromatogram shows two peaks, a non-glycated peak and a glycated peak (28).

Immunoassays utilise specific anti-HbA1c antibodies that recognize the first 3, 4 or 5 amino acids and the glucose attached to the N-terminal of the  $\beta$ -chain of the haemoglobin molecule. Total haemoglobin is measured separately using a bichromatic assay and the ratios of the two components are calculated. Assay designs differ substantially from each other, ranging from immunoturbidimetry to latex agglutination inhibition methods (using monoclonal antibodies) (29).

The enzymatic method principle consists of two separate steps: measurement of glycated dipeptide, obtained by enzymatic cleavage, and measurement of total hemoglobin (30). The glycated dipeptide is measured by adding fructosyl peptide oxidase to produce hydrogen peroxide, which reacts with a coloured agent in the presence of peroxidase. The change in absorbance is measured to calculate HbA1c concentration. Hemoglobin is transformed to stable met-hemoglobin and the concentration of hemoglobin is determined by measuring absorbance. The ratios of the two components are then calculated.

### **Advantages and disadvantages of different method principles**

Each of the different method principles have specific advantages and disadvantages and method choice will depend on clinical and analytical factors. Ion exchange methods are associated with good performance but are often prone to interference from haemoglobin variants which may yield falsely lower or higher HbA1c results. To identify interference from haemoglobin variants, every chromatogram needs to be checked for abnormal peaks; either manually or through computer programming which requires additional training for the healthcare professional running the instrument.

However some rare variants may show a normal chromatogram where the Hb-variant is hidden under the A0 peak which gives a falsely low HbA1c result.

An advantage of affinity chromatography is the absence of interference by haemoglobin variants or derivatives such as carbamylated haemoglobin, which has led to this method being commonly used as the method of choice for use in patients with haemoglobin variants (31, 32). Roling et al. showed, however, that there is an interference with HbF >20% due to the fact that HbF does not have  $\beta$ -chains, which results in a disproportionately low glycation of this haemoglobin molecule (33).

An advantage of current immunoassay methods is the high throughput volumes that can be achieved with these fully automated systems, which can be integrated into laboratory track systems and sample management facilities. Another advantage is that the majority of the immunoassays do not suffer interference from common haemoglobin variants such as HbAS, HbAC, HbAD and HbAE (34). Only some rare haemoglobin variants (substitution of the last 3, 4 or 5 amino acids by another amino acid) are known to cause interference. Capillary electrophoresis is relatively new to the market and can measure multiple samples at once but may have limited throughput. Enzymatic methods currently require a large main laboratory analyser so not suitable for satellite laboratories or use in diabetes clinics but can facilitate high throughput.

### **Performance of different method principles**

Performance of the different method principles has improved dramatically over recent years and the assessment of quality is aided by the international quality standards for HbA1c measurement (22). The global performance criteria were recently set by the IFCC task force for the implantation of HbA1c standardisation and based on the use of sigma metrics. The targets have been defined by the task force and use the concept of total allowable error (TAE) which combines the effects of both bias and imprecision into a single measure. The criteria state that a total of error of not more than 5mmol/mol HbA1c at a level of 50 mmol/mol is allowed. The sigma levels are applied to define how often the target can be expected to fall outside of the target range. For example at a level of  $2\sigma$  one in twenty of results would be expected to fall outside of the target range and this is deemed acceptable.

There are several ways that performance can be assessed, depending on need. The performance of individual analysers in CLSI (35-37) protocol driven laboratory evaluations can be used to assess the best performance of an individual instrument. This has a number of uses such as evaluation of new instrument in the laboratory or, when performed by recognised IFCC reference lab, to give an overview of the performance of an instrument using with IFCC targeted samples. A more robust way of assessing performance is through the use of data from External Quality Assessment (EQA) schemes, where samples within the schemes have been targeted to IFCC values. These schemes provide data for multiple manufacturers and instruments over a wider period of time using different lot numbers of reagents and calibrators and gives a realistic picture of performance over time in a 'real world' setting (38-41). In the near future data from a pan European study will be published showing the performance of HbA1c by country and by manufacturer.

### **Point-of-care instruments**

The principles used in point-of-care (POC) instruments are predominantly affinity separation, immuno-assay and lately also enzymatic. Recent evaluations of the performance of different HbA1c POC instruments have shown that the analytical performance has improved considerably in recent years. However, the diagnostic market is flooded with new HbA1c POC instruments from a wide range of manufacturers and countries and the analytical performance of these instruments is not yet known.

One key way to determine the utility of a POC device is through thorough laboratory evaluations however there are a limited number of robust studies where this has been done. Another mechanism is through performance in EQA programmes, however many POC devices are not entered into such programmes due to a lack of regulation or waiving of regulations and therefore there is limited data around the true performance of these instruments (20, 23, 24, 42-46). Whereas laboratory based methods can be adjusted by re-calibration if a drift in calibration is identified, this is not

possible with most POC instruments as the calibration factor is predetermined by the manufacturer and cannot be adjusted.

Aside from the inherent analytical performance issues, control of use of the devices outside of the laboratory setting needs to be closely monitored and as such an extensive and highly managed quality framework is required before these use of these devices is implemented (47).

## **Standardization of HbA1c**

### ***Standardization versus harmonization***

It is important to understand the fundamental difference between harmonization and standardization. With harmonization one tries to achieve comparable results among different measurement procedures for the same analyte (25). It usually implies there is no reference measurement procedure and no defined reference material or calibrator. This may be due to the heterogeneity of the molecules of interest or due to heterogeneity in measurement principles, common with hormone or antibody measurement. Harmonization aims to get close agreement of values within the constraints described above. Standardization differs in that there is always a reference measurement procedure and a clearly defined and characterised analyte that is available as reference material. In the case of HbA1c these reference materials are pure A1c and pure A0 both of which are registered with the Institute for Reference Materials and Measurements (IRMM) (48). Harmonization is commonly achieved by exchanging samples and aligning results. This can be done by adjusting the results with a factor (slope and an intercept) so that the results match between the two comparator methods. With standardization one tries to achieve near identical results by having calibration traceable to a reference measurement procedure and to a primary reference calibrator.

### **The history of standardisation of HbA1c**

At the time of the start of DCCT and UKPDS trials no reference material was available and as a consequence, the inter-laboratory CV was high (>20%) (49, 50). The



variability in results between different HbA1c methods and the multiple ways in which results were reported (e.g. total glycation, HbA1 and HbA1c) made it difficult for physicians to compare results in clinical practice or in large scale studies. Table 1 details the key time points in the history of HbA1c standardisation.

The DCCT and UKPDS studies used the same HbA1c measurement systems and close monitoring of samples in the two studies provided harmonization of the results. The DCCT initially utilized the Bio-Rex 70 ion exchange method before switching to the Bio-Rad Diamat HPLC after an evaluation to harmonize results from the two methods, as it provided greater automation and higher throughput (51). Although the study was completed using the Bio-Rad Diamat there continued to be a comparison of randomly blinded study samples between the two methods to verify the continuing relationship throughout the remainder of the study (52).

In 1996, the American Association for Clinical Chemistry (AACC) initiated the National Glycohemoglobin Standardisation Program (NGSP), a subcommittee for the standardization of glycohaemoglobin that aimed to harmonize HbA1c assays worldwide. At that time, no definitive primary reference method or calibrator was available, therefore standardization was not possible however harmonization was achievable. The method applied in the DCCT study was chosen as the 'reference method'. In addition, a network of laboratories, that would use this primary reference method, and of laboratories that would use secondary reference methods, was established to aid manufacturers of different HbA1c methods to make their methods traceable to the DCCT study. The NGSP consists of a steering committee and a network of reference laboratories including the Central Primary Reference Laboratory (CPRL, n=1), backup Primary Reference Laboratories (PRL, n=3 running the Bio-Rex 70 method used in the DCCT study) and Secondary Reference Laboratories (n=6, running 10 Secondary Reference Measurements Procedures (SRMPs), routine methods with 4 different measurement principles) (53). The SRLs work directly with manufacturers and laboratories to assist them in harmonizing their methods, and provide data for certification of traceability to the DCCT. The SRLs are also used to assign values to samples of the College of American Pathologists (CAP) external quality survey. The SRLs are monitored monthly against the CPRL and among the

SRMPs with 10 frozen samples. However, in this system there is no primary calibrator and the 'reference method' was subject to interference (54).

### **Harmonization systems in Japan and Sweden**

In addition to the NGSP system Japan and Sweden independently developed harmonization schemes based on cation exchange HPLC methods. In Japan the KO500 method is used as a PRM and in Sweden a Mono-S cation exchange system is used (55, 56). Both columns are more specific than the Bio-Rex70 method which was used in the NGSP harmonization model but still do not give the "true" HbA1c result due to some degree of interference in the method. The Mono-S HPLC does not have defined calibrators, instead the area under the HbA1c peak in relation to the area under all peaks in the elution profile, is used to calculate the percentage HbA1c.

The HbA1c methods in Japan are calibrated using material from the Japanese-Clinical-Chemistry-Use Certified Reference Material (JCCRM). The standardization of HbA1c in Japan was initiated in 1993, and the serial reference materials from JDS Lot 1 to JDS Lot 5 are well certified (57). Since 2013, in Japan and abroad JDS Lot 5 is used. This reference material has assigned certified IFCC (mmol/mol), NGSP (%) and JDS (%) values measured by JDS-JSCC Network Laboratories using the KO500 Method. The IFCC values were assigned by PRMs in the IFCC HbA1c Laboratory Network and the NGSP values were assigned by the Japanese NGSP SRMP (=ASRL#1) using the NGSP CPRL reference panel (100 specimens) as a calibrator. The Japanese NGSP SRMP is monitored monthly against the CPRL and among the 10 SRMPs in the NGSP network with 10 frozen samples.

In 2010 the JDS decided to report NGSP values as well JDS values with the purpose of international clinical harmonization. The NGSP values were expressed simply by adding 0.4% to the JDS values (58). The 0.4% is the unspecificity of the NGSP PRM compared to the KO500 method. Which serves to highlight the significant level of interference in the harmonization methods.

Since April 2014 only NGSP values have been used in the clinical practice of Japan, which offers some form of harmonization but it is unclear why SI units and indeed IFCC values were not adopted.

### **The need for standardization**

With the progress of technology and identification of interference in the Bio-Rex method, it was no longer scientifically acceptable to continue without a robust standardization process, constituting a true primary reference measurement procedure and defined primary reference materials. In addition legislation for in vitro diagnostic medical devices for manufacturers who sell instruments in European Community demands that manufacturers must guarantee the traceability of their routine tests to reference methods and materials of higher metrological order. This is further required by ISO/TC212 standards which dictate that manufacturers have documented traceability to assigned values and to a reference method (59). This is not possible with the NGSP harmonization model and thus there was a requirement for a true standardization process and the development of a PRM and pure calibrators.

### **IFCC standardization**

An IFCC Working Group for the standardisation of HbA1c was established in 1994 to develop a standard for HbA1c, consisting of almost pure HbA1c and HbA0, and a primary reference method for HbA1c. Whilst this was ongoing, the AACC and the ADA accepted harmonization via NGSP as an interim solution until true standardisation was achieved. The IFCC Working Group on HbA1c Standardisation succeeded in producing reference material and defining a reference measurement procedure, which was published in 2002 (60) (Figures 1 and 2). In addition, a laboratory network was established, which included the two reference methods, i.e. mass spectroscopy and capillary electrophoresis (61). Currently there are 21 laboratories worldwide running the IFCC PRM either using the HPLC-CE method or HPLC-MS. Each network laboratory used prepared mixtures of purified HbA1c and HbA0 as calibrators (62). The main task of the IFCC Network of Reference Laboratories for HbA1c is to assign values to secondary reference material and to collaborate with manufacturers of diagnostic

devices and External Quality Assessment Schemes (EQAS) organisers to ensure methods are traceable to the RMP. Figure 3 shows the traceability chain of the IFCC standardization of HbA1c. This process provides an unbroken chain between the patient results and the primary reference material, thus ensuring that patients get the right results, which can be universally compared against clinical targets and large scale study data.

### **Why does the IFCC RMP report values in mmol/mol and not %?**

The international consensus statement on the measurement and reporting of HbA1c values states that “HbA1c results are to be reported by clinical laboratories worldwide in SI (Système Internationale) units (mmol/mol – no decimals) and derived NGSP units (% – one decimal), using the IFCC-NGSP master equation (DCCT units).”

There are two factors that influenced the decision to change the reporting units to mmol/mol. Firstly, the difference between the Bio-Rex values and the true values determined by the IFCC RMP is approximately 2%, which represents the interference seen in the Bio-Rex method. For examples to change HbA1c values would be reduced by approximately 2% (i.e 6% would become 4%) although the relationship is not completely linear. This substantial change would cause considerable disruption and global confusion, however it would not be scientifically sound to adjust the true values either. As a change was needed, the change to completely different numbers and units was seen as less likely to be detrimental to patient and clinical understanding and care than a small reduction in numbers. This was proven to be the case in a study that showed that there was no change in glycaemic control as a result of the shift in reported units (63). Secondly, by law in Europe all test values must be reported in SI units where possible and % is not classed as an SI unit.

### **Can I relate IFCC standardized values to NGSP/DCCT harmonized values?**

The simple answer is yes. The interference seen in the Bio-Rex system is constant meaning that inter-comparison studies between the two networks allows for a master equation to be applied to convert values between the two systems. In addition there are master equations that link the JDS and IFCC systems (64, 65). This allows

clinicians and patients to convert between NGSP harmonized % values and the IFCC SI values with no loss of traceability to the DCCT trial results. Whilst more recent studies now inform clinical guidance many still wish to convert back to more familiar % values and this is achieved using the master equation. To facilitate this a number of clinical organizations have online conversion calculators (66).

### **Standardization versus calibration**

One source of confusion is the difference between standardization and calibration of instruments, with the terms often being used interchangeably. Standardization is the global process, led by the IFCC reference network and supporting committees that produces primary and secondary calibrators directly traceable to a defined measurand, in the case of HbA1c the defined measurand is Beta N-(1-deoxyfructos-1-yl) haemoglobin (67). These calibrators are provided to manufacturers who use them to assign values to their own calibrators that are provided to the end user to calibrate their instrument. Calibration is the process by which the values generated by the instrument when measuring calibration material are compared with the expected values of the calibration material and used to adjust the set point for future measurements.

### **How to I standardize HbA1c in my laboratory or my country?**

Again there is a simple answer to this, for the laboratory you don't need to implement your own standardization. Standardization is not done at a laboratory level, instead, in order to produce IFCC standardized HbA1c values it is important to use instruments where the manufacturer can demonstrate are traceable to the IFCC RMP. Manufacturers are responsible for demonstrating that they are working with the IFCC HbA1c network to be traceable to the IFCC PRMP. However, it is the responsibility of an individual laboratory is to choose an HbA1c method which is performing well in accuracy based external quality schemes. An IFCC or an NGSP certification does not always reflect the analytical performance of a method in the field because certification is done under ideal circumstances at the manufacturer's site often using a single lot of reagent and calibrators, one instrument and one application. Results in external quality schemes reveal the real analytical performance in the field with different reagent lot

numbers, different instruments, different applications (whole blood or hemolysate mode) and different users.

Individual laboratories should never use the results of an external quality scheme to calculate a factor to adjust laboratory values to 'bring them in line' with EQA data as this disrupts the traceability chain. If poor performance is identified in an EQA schemes laboratories should liaise with the manufacturer to elucidate the cause of the poor performance and work together to find a solution.

Standardization at a country level is a more complex consideration. Healthcare organizations should work closely with the IFCC and manufacturers to devise an implementation approach that best fits the needs of that country.

### **The heart of the standardization: IFCC secondary reference material**

At the heart of the global standardization of HbA1c methods is the scientific collaboration between the manufacturers and the IFCC, through the production and utilization of secondary reference material. The European Reference Laboratory for Glycohemoglobin (ERL) plays a key role in the standardization of HbA1c worldwide by producing the IFCC secondary calibrators (Figure 3). This secondary reference material, made from patient whole blood, is used by manufacturers of HbA1c methods to assign values to their own method-dependent calibrators. The primary reference calibrator can't be used in routine HbA1c methods as it is not commutable with these routine methods and therefore secondary reference calibrators made from patient blood are needed. Commutability of reference material is of utmost importance and is the responsibility of the manufacturer to verify. Each year 8 calibrator pools are produced from at least 10 donors. The values of these calibrators are assigned by all of the laboratories in the IFCC network running the IFCC PRMP (HPLC-CE or HPLC-MS, n=21) (Figure 1). Once values are assigned the calibrators are provided to manufacturers to enable them to produce their own calibrators and ensure traceability to the RMP. These calibrators are generally commutable to most methods, except some POC devices where fresh, not frozen whole blood is required. The ERL provides fresh patient samples with values assigned with at least 3 SRMP in duplicate to

manufacturers who can't work with frozen IFCC secondary reference material. Some manufacturers need fresh patient samples with assigned values every week due to the production of new lot numbers every week. A study in 2014, where 7 HbA1c point-of-care instruments were evaluated, showed that 3 of the evaluated instruments had a commutability problem with the IFCC secondary calibrator which was frozen. The instruments were calibrated with frozen material while it showed a negative bias with fresh patient samples (43, 44).

These pools are also used to check the stability of the published master equations between the different harmonization/standardization models (65). This equation is continuously monitored to insure that the relationship between the "true values" (IFCC) and other harmonization systems remains stable. Interestingly the ERL also plays a key role in value assignment for NGSP and IFCC certification samples and for external quality assessment sample. In total the ERL has 2 IFCC PRMS, 1 NGSP PRM and 7 IFCC SRMPs of which 5 are also NGSP SRMPs.

**Is there a blind spot in the standardization process? What do manufacturers do to ensure traceability?**

According to the IFCC traceability chain, manufacturers should use the IFCC secondary reference material to standardize their own method and generate their own calibration material for supply to laboratory users. Each of the major manufacturers, who collaborate with the IFCC network, receive 8 frozen pools once a year with values assigned by the whole IFCC network (n=21). Manufacturers who can't work with frozen material, for examples some manufacturers of POC devices, receive the pools as fresh material. Every manufacturer has their own procedure to use this material to assign values to their own method. Some manufacturers have a master lot and they harmonize the new lot with this master lot using it to check if they still produce the correct values rather than using the secondary calibrators to assign values to their own calibrators. A significant issue for some manufacturers is the high frequency with which they generate new lot numbers making it impossible to repeatedly use the secondary calibrators each time. This is particular the problem for some manufacturers of POC instruments.

Whilst a list of manufacturers who work with the IFCC is maintained it is known that there are a significant number of national companies around the world, who do not interact directly with the IFCC network but may collaborate with individual local IFCC Network laboratories. Despite several IFCC SRM also being NGSP SRMPs, an NGSP certificate does not confer direct traceability to the IFCC RMP.

### **Has the standardization of HbA1c been a great success story?**

Prior to 1993, there was no standardization which resulted in inter-laboratory CVs >20% and values were not linked to clinical outcome data and were therefore difficult to interpret for health care providers. The aim of standardization was to reduce the imprecision of methods and to ensure the minimum bias from the true HbA1c values. The latest results of the College of American Pathology (CAP) survey showed that the inter-laboratory CV is now around 3.5% and only 2 of the 26 methods surveyed had a method specific bias >0.3% (NGSP units) (38, 39, 41, 54). Seen from this perspective the standardization of HbA1c has been a great success story as there has been a considerable improvement in analytical performance.

However, there is still a considerable level of misunderstanding and confusion around the process of standardization and the roles of different organizations. The NGSP and IFCC Reference Systems serve different but complementary purposes, with the former being a harmonization process that applies defined acceptable limits for method performance that are based on historical clinical requirements and the latter being a standardization process that provides traceability to an accuracy base and allows for the application of scientifically based quality standards (22, 68). Certification of methods at a laboratory level is important in demonstrating good performance at that point in time and with the imminent introduction of IFCC laboratory certification, laboratories will be able to directly demonstrate that their instruments perform well in their own clinical or laboratory settings. To achieve this the laboratory will likely have to use an instrument where the manufacturer can clearly demonstrate traceability to the IFCC reference measurement procedure. However, this is still a snapshot of performance and engagement with an EQA scheme that uses samples with values



targeted to the IFCC RMP provides a more thorough demonstration of ongoing good performance.

A further issue that adds to the confusion is the use of 2 reporting units: NGSP in percentage units and IFCC (SI) in mmol/mol units. Whilst it is understandable that there may be reluctance to change, it is important to understand that the instruments are calibrated to the IFCC RMP and the values are then mathematically converted to the % values. The instruments of all the major manufacturers no longer 'measure' in DCCT units but instead apply the master equation to convert the values. For scientists and manufacturers it is still a complicated issue as both values need to be reported in articles and in package inserts of the manufacturer (69). Besides that, the scale of the difference between the numbers means that calculations of CV's, give different values using different units, CV's calculated in SI units can't be compared with CV's in NGSP units (18). Despite a global consensus statement, written by the major organizations involved in diabetes care worldwide (The American Diabetes Association (ADA), the European Association for the Study of Diabetes (EASD), the International Diabetes Federation (IDF), the IFCC, the International Society for Pediatric and Adolescent Diabetes (ISPAD), the JDS, and the NGSP) stating that HbA1c should be reported in SI units and derived (=calculated with published master equation) NGSP units (69), this is not universally applied. In addition Japan chose to follow the NGSP and report HbA1c only in NGSP units with the purpose of international harmonization of HbA1c values which is, seen from a scientific point of view, a step back in the standardization of HbA1c. So, although considerable progress has been made in the standardization of HbA1c acceptance and understanding of the process is still lagging and there is need for ongoing education around this important issue. Reporting values in SI units (mmol/mol) does not mean that the link to the DCCT and UKPDS studies is lost as that link is maintained through the master equation linking the NGSP and IFCC. With the use of HbA1c for the diagnosis of type 2 diabetes it is critical that the correct HbA1c values are provided as even a small positive or negative bias in HbA1c values can lead to the re-categorization of disease state for potentially millions of people.

**Future of HbA1c. Will we still analyze HbA1c in 2030?**

HbA1c has been widely accepted in clinical practice for over 25 years but there are a number of potential new markers available that may complement or eventually supersede its use. Continuous glucose monitoring (CGM) is one such development. Currently the CGMs are still relatively cumbersome, expensive, technically demanding and patients require extensive training to enable them to use the device which is commonly not reimbursed by many insurance companies. However, recent developments to miniaturize the device (as small as a rice grain) with a longer functional time (currently it is maximum of 14 days) have the potential to change this. In some diabetic centers where CGM is used a large database has been developed to explore the links between average mean glucose, HbA1c and clinical outcomes. Current evidence from the A1c-Derived Average Glucose study showed that, although the relationship between HbA1c and average glucose was linear, a wide variation was observed between individuals, meaning it is currently not considered robust enough for use in routine clinical practice (69, 70). However this may change with further long term data collection.

In future clinical practice will continue to move forward as new understanding diabetes is gained and targets for treatment will be patient centered and individualized to that patient. Malka et al designed an elegant model for a personalized approach using existing clinical measurements to personalize prospective estimates of average glucose (71)anaem. They combined a mechanistic mathematical model of hemoglobin glycation and red blood cell kinetics with large sets of within-patient glucose measurements (CGM) to derive patient-specific estimates of nonglycemic determinants of HbA1c, including mean red blood cell age.

## **Conclusion**

Whilst great advances have been made in the field of diabetes care, and HbA1c is rapidly becoming one of the best characterised biomarkers in laboratory science, it is likely that HbA1c will be the mainstay of disease diagnosis and monitoring for some time to come. It is essential, therefore, that the test is used correctly and more fully understood, with questions such as the influence of ethnicity, anaemia, haemoglobinopathies etc still to be fully addressed (72). In addition to this the scientific and clinical community still have a way to go to fully embrace the standardisation

process and move away from historical harmonization schemes to full global acceptance of standardisation.

## Disclosures

The authors do not have any relevant disclosures.

## References

1. Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, et al. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res Clin Pract.* 2018.
2. American Association of Clinical Endocrinologists Board of D, American College of Endocrinologists Board of T. American Association of Clinical Endocrinologists/American College of Endocrinology statement on the use of hemoglobin A1c for the diagnosis of diabetes. *Endocr Pract.* 2010;16(2):155-6.
3. Organization WH. Use of glycated haemoglobin (HbA1c) in the diagnosis of diabetes mellitus. Abbreviated report of a WHO consultation. Geneva: World Health Organization, 2011 Contract No.: WHO/NMH/CHP/CPM/11.1.
4. John WG, Diabetes UKDoHACo. Use of HbA1c in the diagnosis of diabetes mellitus in the UK. The implementation of World Health Organization guidance 2011. *Diabetic medicine : a journal of the British Diabetic Association.* 2012;29(11):1350-7.
5. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. *N Engl J Med.* 1993;329(14):977-86.
6. Nathan DM, Cleary PA, Backlund JY, Genuth SM, Lachin JM, Orchard TJ, et al. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. *N Engl J Med.* 2005;353(25):2643-53.
7. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. *Lancet.* 1998;352(9131):837-53.
8. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-year follow-up of intensive glucose control in type 2 diabetes. *N Engl J Med.* 2008;359(15):1577-89.
9. Duckworth WC, Abraira C, Moritz TE, Davis SN, Emanuele N, Goldman S, et al. The duration of diabetes affects the response to intensive glucose control in type 2 subjects: the VA Diabetes Trial. *J Diabetes Complications.* 2011;25(6):355-61.
10. Action to Control Cardiovascular Risk in Diabetes Study G, Gerstein HC, Miller ME, Byington RP, Goff DC, Jr., Bigger JT, et al. Effects of intensive glucose lowering in type 2 diabetes. *N Engl J Med.* 2008;358(24):2545-59.
11. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, et al. Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes. *N Engl J Med.* 2015;373(22):2117-28.
12. Hayward RA, Reaven PD, Wiitala WL, Bahn GD, Reda DJ, Ge L, et al. Follow-up of glycemic control and cardiovascular outcomes in type 2 diabetes. *N Engl J Med.* 2015;372(23):2197-206.
13. Duckworth W, Abraira C, Moritz T, Reda D, Emanuele N, Reaven PD, et al. Glucose control and vascular complications in veterans with type 2 diabetes. *N Engl J Med.* 2009;360(2):129-39.
14. Group AC, Patel A, MacMahon S, Chalmers J, Neal B, Billot L, et al. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. *N Engl J Med.* 2008;358(24):2560-72.
15. Marso SP, Daniels GH, Brown-Frandsen K, Kristensen P, Mann JF, Nauck MA, et al. Liraglutide and Cardiovascular Outcomes in Type 2 Diabetes. *N Engl J Med.* 2016;375(4):311-22.
16. Dormandy JA, Charbonnel B, Eckland DJ, Erdmann E, Massi-Benedetti M, Moules IK, et al. Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study

(PROspective pioglitAzone Clinical Trial In macroVascular Events): a randomised controlled trial. *Lancet*. 2005;366(9493):1279-89.

17. Inzucchi SE, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, et al. Management of hyperglycemia in type 2 diabetes, 2015: a patient-centered approach: update to a position statement of the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care*. 2015;38(1):140-9.

18. Weykamp CW, Mosca A, Gillery P, Panteghini M. The analytical goals for hemoglobin A(1c) measurement in IFCC units and National Glycohemoglobin Standardization Program Units are different. *Clin Chem*. 2011;57(8):1204-6.

19. Fraser CG, Petersen PH. Analytical performance characteristics should be judged against objective quality specifications. *Clin Chem*. 1999;45(3):321-3.

20. Gill JP, Shephard MD. The Conduct of Quality Control and Quality Assurance Testing for PoCT Outside the Laboratory. *Clin Biochem Rev*. 2010;31(3):85-8.

21. Sacks DB, Arnold M, Bakris GL, Bruns DE, Horvath AR, Kirkman MS, et al. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. *Clin Chem*. 2011;57(6):e1-e47.

22. Weykamp C, John G, Gillery P, English E, Ji L, Lenters-Westra E, et al. Investigation of 2 models to set and evaluate quality targets for hb a1c: biological variation and sigma-metrics. *Clin Chem*. 2015;61(5):752-9.

23. DG B. Quality control and quality assurance in point-of-care testing. In: CP SJA, Hicks JM, editor. *Point-of-care testing 2nd ed*. Washington DC: AACC Press; 2004. p. 137-45.

24. Martin CL. Quality control issues in point of care testing. *Clin Biochem Rev*. 2008;29 Suppl 1:S79-82.

25. Miller WG, Myers GL, Gantzer ML, Kahn SE, Schonbrunner ER, Thienpont LM, et al. Roadmap for Harmonization of Clinical Laboratory Measurement Procedures. *Clinical Chemistry*. 2011;57(8):1108-17.

26. Khera PK, Smith EP, Lindsell CJ, Rogge MC, Haggerty S, Wagner DA, et al. Use of an oral stable isotope label to confirm variation in red blood cell mean age that influences HbA1c interpretation. *Am J Hematol*. 2015;90(1):50-5.

27. Jaisson S, Leroy N, Meurice J, Guillard E, Gillery P. First evaluation of Capillarys 2 Flex Piercing(R) (Sebia) as a new analyzer for HbA1c assay by capillary electrophoresis. *Clin Chem Lab Med*. 2012;50(10):1769-75.

28. John WG, Little R, Sacks DB, Weykamp C, Lenters-Westra E, Hornsby T, et al. Multicentre evaluation of the Premier Hb9210 HbA1c analyser. *Clin Chem Lab Med*. 2015;53(2):319-27.

29. Fleming JK. Evaluation of HbA1c on the Roche COBAS Integra 800 closed tube system. *Clin Biochem*. 2007;40(11):822-7.

30. Lenters-Westra E, English E. Evaluating new HbA1c methods for adoption by the IFCC and NGSP reference networks using international quality targets. *Clin Chem Lab Med*. 2017;55(9):1426-34.

31. Little RR, Roberts WL. A review of variant hemoglobins interfering with hemoglobin A1c measurement. *J Diabetes Sci Technol*. 2009;3(3):446-51.

32. Weykamp CW, Penders TJ, Muskiet FA, van der Slik W. Influence of hemoglobin variants and derivatives on glycohemoglobin determinations, as investigated by 102 laboratories using 16 methods. *Clin Chem*. 1993;39(8):1717-23.

33. Rohlfing CL, Connolly SM, England JD, Hanson SE, Moellering CM, Bachelder JR, et al. The effect of elevated fetal hemoglobin on hemoglobin A1c results: five common hemoglobin A1c methods compared with the IFCC reference method. *Am J Clin Pathol*. 2008;129(5):811-4.

34. NGSP. HbA1c Assay Interferences 2017. Available from: <http://www.ngsp.org/interf.asp>.

35. Institute CaLS. Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline. NCCLS Document EP05-A. PA: CLSI Wayne; 1992.

36. Institute CaLS. Method Comparison and Bias Estimation using Patient Samples; Approved Guideline. CLSI Document EP09-A PA: CLSI Wayne; 1995.

37. Institute CaLS. Evaluation of the Linearity of Quantitative Measurement Procedures: A statistical Approach; Approved Guideline. CLSI Document EP06-A (2003). . PA: CLSI Wayne; 2003.

38. Kaiser P, Spannagl M, van Campenhout C, Lenga Y, Siebelder C, Weykamp C. HbA1c: EQA in Germany, Belgium and the Netherlands using fresh whole blood samples with target values assigned with the IFCC reference system. *Clin Chem Lab Med*. 2016;54(11):1769-75.

39. Mosca A, Paleari R, Carobene A, Weykamp C, Ceriotti F. Performance of glycated hemoglobin (HbA(1c)) methods evaluated with EQAS studies using fresh blood samples: Still space for improvements. *Clin Chim Acta*. 2015;451(Pt B):305-9.
40. Thelen MHM, Jansen RTP, Weykamp CW, Steigstra H, Meijer R, Cobbaert CM. Expressing analytical performance from multi-sample evaluation in laboratory EQA. *Clin Chem Lab Med*. 2017;55(10):1509-16.
41. Weykamp C, Siebelder C. Evaluation of Performance of Laboratories and Manufacturers Within the Framework of the IFCC model for Quality Targets of HbA1c. *J Diabetes Sci Technol*. 2017:1932296817741320.
42. Biochemists AAOc. PoCT 2016 [04/05/16]. Available from: <http://www.aacb.asn.au/resources/poct>.
43. Lenters-Westra E, Slingerland RJ. Six of eight hemoglobin A1c point-of-care instruments do not meet the general accepted analytical performance criteria. *Clin Chem*. 2010;56(1):44-52.
44. Lenters-Westra E, Slingerland RJ. Three of 7 hemoglobin A1c point-of-care instruments do not meet generally accepted analytical performance criteria. *Clin Chem*. 2014;60(8):1062-72.
45. Schaffert L, English, E, Heneghan, C, Price, CP, Van den Bruel, A, Plüddemann, A. Horizon Scan 0044 Point-of-Care HbA1c tests - diagnosis of diabetes Oxford2016. Available from: <http://www.oxford.dec.nihr.ac.uk/reports-and-resources/horizon-scanning-reports/point-of-care-hba1c-tests-diagnosis-of-diabetes>.
46. Hirst JA, McLellan JH, Price CP, English E, Feakins BG, Stevens RJ, et al. Performance of point-of-care HbA1c test devices: implications for use in clinical practice - a systematic review and meta-analysis. *Clin Chem Lab Med*. 2017;55(2):167-80.
47. Misra S, John, W. G., Alberti, G., Barth, J. H., English, E., Oliver, N. The use of POCT HbA1c devices in the NHS Diabetes Prevention Programme: Recommendations from an expert working group commissioned by NHS England, NHS (Commissioned report). NHS England, 2016.
48. Hub ES. IRMM Catalogue of Reference Materials 2016. Available from: [https://ec.europa.eu/jrc/sites/jrcsh/files/rm\\_catalogue\\_0.pdf](https://ec.europa.eu/jrc/sites/jrcsh/files/rm_catalogue_0.pdf).
49. John WG. Glycated haemoglobin analyses--assessment of within- and between-laboratory performance in a large UK region. *Ann Clin Biochem*. 1987;24 ( Pt 5):453-60.
50. Peterson CM, Jovanovic L, Raskin P, Goldstein DE. A comparative evaluation of glycosylated haemoglobin assays: feasibility of references and standards. *Diabetologia*. 1984;26(3):214-7.
51. Feasibility of centralized measurements of glycated hemoglobin in the Diabetes Control and Complications Trial: a multicenter study. The DCCT Research Group. *Clin Chem*. 1987;33(12):2267-71.
52. Little RR, Rohlfing CL, Wiedmeyer HM, Myers GL, Sacks DB, Goldstein DE, et al. The National Glycohemoglobin Standardization Program: A five-year progress report. *Clinical Chemistry*. 2001;47(11):1985-92.
53. NGSP Network Laboratory Members 2016.
54. College of American Pathology External Quality Scheme for HbA1c 2016 [December 2016]. Available from: <http://www.ngsp.org/CAP/CAP16c.pdf>.
55. Shima K, Endo, J., Oimomi, M., Omori, Y., Katayama, Y., Kanazawa, Y., Kawai, T., Kawamori, R., Kanno, T., Kiyose, H., Kuwajima, M., Nakashima, K., Nagamine, Y., Baba, S., Hoshino, T. Interlaboratory Difference in GHb Measurement in Japan. The Fifth Report of the GHb Standardization Committee, the Japan Diabetes Society. *J Japan Diab Soc*. 1998;41(4):317-23.
56. Jeppsson JO, Jerntorp P, Sundkvist G, Englund H, Nylund V. Measurement of hemoglobin A1c by a new liquid-chromatographic assay: methodology, clinical utility, and relation to glucose tolerance evaluated. *Clin Chem*. 1986;32(10):1867-72.
57. Tominaga M, Makino H, Yoshino G, Kuwa K, Takei I, Aono Y, et al. Japanese standard reference material for JDS Lot 2 haemoglobin A1c. I: Comparison of Japan Diabetes Society-assigned values to those obtained by the Japanese and USA domestic standardization programmes and by the International Federation of Clinical Chemistry reference laboratories. *Ann Clin Biochem*. 2005;42(Pt 1):41-6.
58. Committee of the Japan Diabetes Society on the Diagnostic Criteria of Diabetes M, Seino Y, Nanjo K, Tajima N, Kadowaki T, Kashiwagi A, et al. Report of the committee on the classification and diagnostic criteria of diabetes mellitus. *J Diabetes Investig*. 2010;1(5):212-28.
59. Standardization IOF. ISO/TC 212 Clinical laboratory testing and in vitro diagnostic test systems. Available from: <https://www.iso.org/committee/54916.html>.

60. Jeppsson JO, Kobold U, Barr J, Finke A, Hoelzel W, Hoshino T, et al. Approved IFCC reference method for the measurement of HbA(1c) in human blood. *Clinical Chemistry and Laboratory Medicine*. 2002;40(1):78-89.
61. Approved laboratories of the IFCC network laboratories for HbA1c 2016 [December 2016]. Available from: [www.ifcchba1c.net/network/approved](http://www.ifcchba1c.net/network/approved).
62. 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(2):240-8.
63. Kilpatrick ES, Rigby AS, Atkin SL, Barth JH. Glycemic control in the 12 months following a change to SI hemoglobin A1c reporting units. *Clin Chem*. 2013;59(10):1457-60.
64. Geistanger A, Arends S, Berding C, Hoshino T, Jeppsson JO, Little R, et al. 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;54(8):1379-85.
65. Hoelzel W, Weykamp C, Jeppsson JO, Miedema K, Barr JR, Goodall I, et al. 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*. 2004;50(1):166-74.
66. NGSP. Convert between NGSP, IFCC and eAG 2010. Available from: <http://www.ngsp.org/convert1.asp>.
67. International Federation Of Clinical C, Laboratory Medicine ISD, Nordin G, Dybkaer R. Recommendation for term and measurement unit for "HbA1c". *Clin Chem Lab Med*. 2007;45(8):1081-2.
68. Lenters-Westra EE, E. Understanding the Use of Sigma Metrics in Hemoglobin A1c Analysis. *Clinics in Laboratory Medicine*. 2016.
69. Hanas R, John G, International Hb ACC. 2010 consensus statement on the worldwide standardization of the hemoglobin A1c measurement. *Clin Chem*. 2010;56(8):1362-4.
70. Borg R, Kuenen JC, Carstensen B, Zheng H, Nathan DM, Heine RJ, et al. Associations between features of glucose exposure and A1C: the A1C-Derived Average Glucose (ADAG) study. *Diabetes*. 2010;59(7):1585-90.
71. Malka R, Nathan DM, Higgins JM. Mechanistic modeling of hemoglobin glycation and red blood cell kinetics enables personalized diabetes monitoring. *Sci Transl Med*. 2016;8(359):359ra130.
72. English E, Idris I, Smith G, Dhatariya K, Kilpatrick ES, John WG. The effect of anaemia and abnormalities of erythrocyte indices on HbA1c analysis: a systematic review. *Diabetologia*. 2015;58(7):1409-21.