Hemoglobin A1c

Lenters-Westra, Wilhelmina Berendina

Hemoglobin A_{1c}:

standardisation, analytical performance and interpretation

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STELLINGEN BEHORENDE BIJ HET PROEFSCHRIFT

"Hemoglobin A_{1c}:

standardisation, analytical performance and interpretation"

1. De analytische prestaties van de meerderheid van de onderzochte HbA_{1c} POC instrumenten is onvoldoende. *(dit proefschrift)*

2. Firma's van HbA_{1c} POC instrumenten dienen controle materialen te leveren met nauwere grenzen. *(dit proefschrift)*

3. Gebruikers van POC instrumenten moeten verplicht worden om deel te nemen aan interne en externe kwaliteitsprogramma's. *(dit proefschrift)*

4. Een fabrikanten NGSP certificatie geeft geen garantie voor de kwaliteit van HbA_{1c} POC instrumenten. *(dit proefschrift)*

5. Eén op de vijf laboratoria in Nederland en België die gebruik maken van verschillende HbA_{1c} methoden, gebruikt een meetmethode waarbij de uitkomst te zeer kan afwijken van de echte waarde. *(dit proefschrift)*

6. Een meerderheid van de diabetes zorgverleners verwacht betere analytische prestaties van de HbA_{1c} methode dan in werkelijkheid het geval is. *(dit proefschrift)*

7. De beslissing van de ADA om alle HbA_{1c} POC uit te sluiten voor het stellen van de diagnose diabetes en alle laboratorium gebaseerde HbA_{1c} methoden toe te laten voor de diagnose stelling van diabetes, is onjuist. *(dit proefschrift)*

8. De diagnose stelling van diabetes met verschillende HbA_{1c} methoden is onvoldoende onderzocht.

9. De waarde van een consensus statement is beperkt indien partijen die betrokken zijn, hun eigen strategie volgen na ondertekening van deze verklaring.

10. Indien dit proefschrift getoetst zou worden door de medisch ethische commissie, zou het worden afgekeurd vanwege ernstige verwaarlozing van sociale contacten door de auteur.

11. Men kan met ideeën flirten maar men moet trouwen met de feiten. *(Regardz)*

12. Normaal is het gemiddelde van alle afwijkingen. *(Miranda Swinkels)*

Erna Lenters-Westra
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Copromotor:	Dr. R.J. Slingerland
Beoordelingscommissie:	Prof. dr. I.P. Kema Prof. dr. M.Y. Berger Prof. dr. B.H.R. Wolffenbuttel

Paranimfen:	Carla Siebelder
	Sabrina Schilling

Knowledge is an unending adventure at the edge of uncertainty
(Jacob Bronowski)

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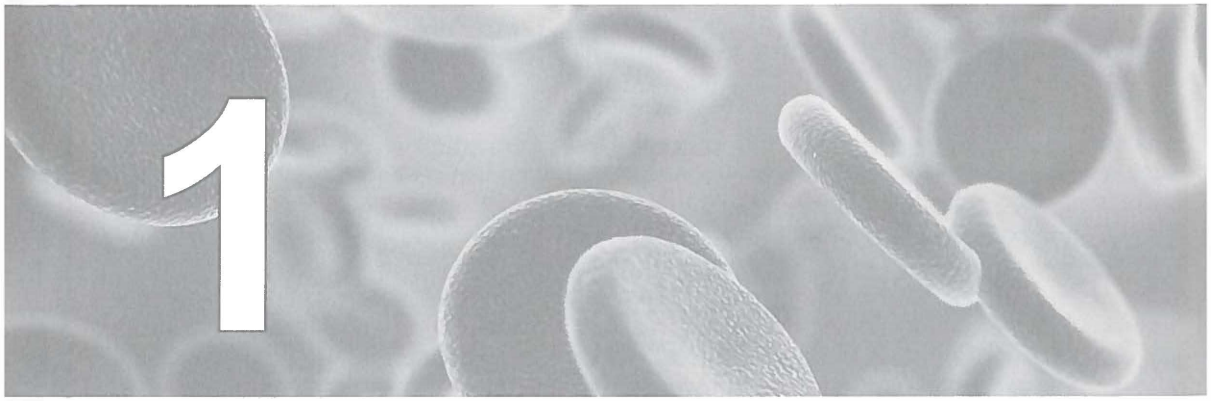

General introduction and outline of the thesis

Adapted from
Glycated Hemoglobin A_{1c} in the management and diagnosis of diabetes mellitus: historical overview and current concepts

Erna Lenters-Westra
Roger K. Schindhelm
Henk J.G. Bilo
Robbert J. Slingerland

Submitted

Abstract

Since the discovery of the relation between increased concentrations of fast hemoglobin fractions in patients with diabetes compared to such concentrations in subjects without diabetes by Samuel Rahbar and co-workers in 1969, glycated hemoglobin A1c (HbA_{1c}) has become a "gold standard" for glucose management in patients with diabetes. Recently, HbA_{1c} has been advocated as a diagnostic marker for diabetes, which further underlines the importance of HbA_{1c}. There are currently more than 30 methods available on the market with an analytical performance ranging from poor to state of the art. This review presents an historical overview of the advances made in the improvement of the analytical performance of the HbA_{1c} assay during the last four decades. Furthermore, current concepts of the HbA_{1c} assay will be discussed, including the recent introduction of HbA_{1c} point-of-care testing. Finally, recommendations for the current minimally required analytical performance characteristics regarding the HbA_{1c} assay are presented.

Introduction

Over the last decades, the prevalence of diabetes has reached epidemic proportions in Western societies, and is even higher in some developing countries. This is mainly due to population growth, ageing and a changing lifestyle, resulting in inactivity and increased prevalence of obesity[1-5]. The World Health Organization (WHO) has estimated that the global prevalence of diabetes will increase from 2.8% in 2000 to 4.4% by 2030[6]. The increased prevalence of both obesity and diabetes will have a profound impact on diabetes- and obesity-related complications and diseases[7]. Individuals with diabetes do have an increased disease burden, for example caused by the development of macrovascular and/or microvascular complications[8,9]. The development of such complications can - amongst others - be prevented or delayed by striving for optimal glycaemic control.

Therefore, striving for optimal glycaemic control is common practice in the management of diabetes. HbA_{1c} is one of the important factors taken into account when judging the degree of glucose control, and it is used to signal the need for adjusting therapy regimens and to aid in patient education. Studies such as the Diabetes Control and Complications Trial (DCCT) and the UK Prospective Diabetes Study (UKPDS) have supported the assumption that adequate glycaemic control in the general patient population may help reduce the risk of developing diabetes-related microvascular and macrovascular complications[10, 11]. Recently, the American Diabetes Association (ADA) has advocated the use of HbA_{1c} to diagnose diabetes[12] due to the global standardisation of the HbA_{1c} assay and the associated improvement of the analytical performance of the assay[13-16]. The WHO and the International Diabetes Federation (IDF), however, recommend against the use of HbA_{1c} to diagnose diabetes[17].

The basis for the current wide-spread use of HbA_{1c} in clinical practice and medical research was laid in the nineteen sixties of the previous century with the discovery of HbA_{1c} [18,19]. At first, various assays with different cut-off points and performances showed considerable differences in results, which considerably hampered a proper assessment and comparison. Since then, major improvements in analytical performance and standardisation have been made. This review presents a historical overview of the advances made in the improvement of the analytical performance of the HbA_{1c} assay during the last four decades. Furthermore, current concepts of the HbA_{1c} assay will be discussed, including the recent introduction of HbA_{1c} point-of-care testing (POCT). Finally, recommendations for the minimally required analytical performance characteristics of the HbA_{1c} assay are presented.

Biochemistry of glycated hemoglobin A_{1c}

Hemoglobin in healthy individuals consists of approximately 97% adult hemoglobin (HbA), 2.5% HbA_2 and 0.5% fetal hemoglobin (HbF). In a healthy person, approximately 94% of HbA is non-glycated, while 6% is glycated. Glycated hemoglobin consists of HbA_{1a} and HbA_{1b} (minor components: taken together ~1%) and HbA_{1c} (main component: ~5%) (Figure 1). From a chemical point of view, HbA_{1c}

is formed when glucose inextricably binds to the N-terminal (valine) of the β-chain of the hemoglobin molecule. Sixty percent of the glucose is bound to the N-terminal valine of the β-chains of the hemoglobin and the remainder is bound to the N-terminal valine of the α-chains and lysine side chains of the α- and β-chains of the hemoglobin molecule. Initially, the reaction between glucose and hemoglobin is reversible, but ultimately an Amadori rearrangement yields an irreversible and stable ketoamine (Figure 1).

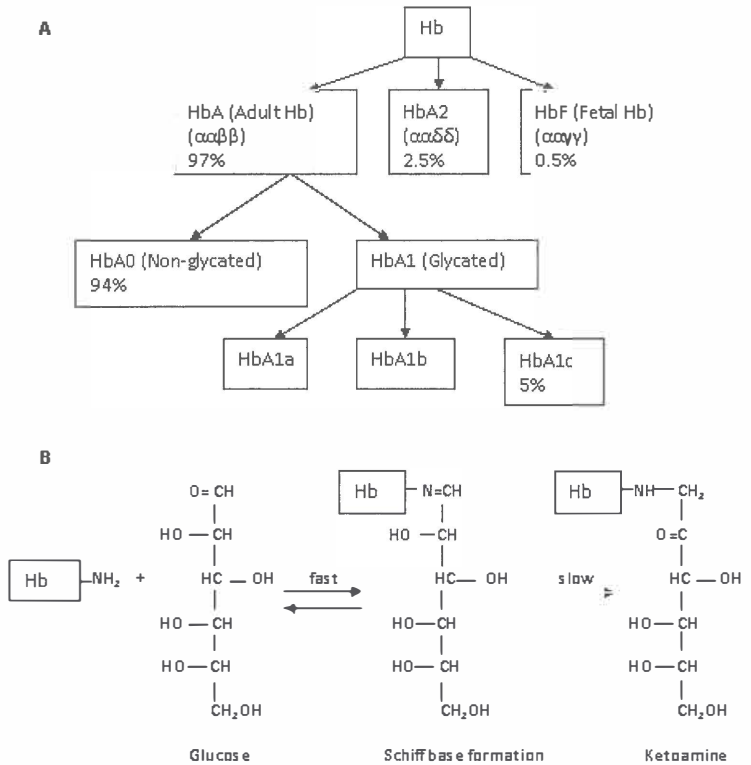

Figure 1

A: Heamoglobin types of healthy adults. Hemoglobin in healthy individuals consists of approximately 97% adult hemoglobin (HbA), 2.5% HbA_2 and 0.5% fetal hemoglobin (HbF). In a healthy person, approximately 94% of HbA is non-glycated, while 6 % is glycated. Glycated hemoglobin consists of HbA_{1a} and HbA_{1b} (minor components: taken together ~1%) and HbA_{1c} (main component: ~5%).

B: The N-terminal valine of the β chain reacts with glucose to the aldimide (Schiff base or labile HbA1c), which undergoes an Amadori rearrangement to the stable ketoamine (HbA1c).

The formation of HbA_{1c} is mainly dependent on the interaction between blood glucose concentrations and the life span of red blood cells [20,21]. According to the study of Cohen et al, red blood cell age for subjects without diabetes and with diabetes ranged from 38-59 days and 39-56 days, respectively, with a maximum life span of approximately 100-120 days[22]. The impact of differences in mean red blood cell age on measured HbA_{1c} is large and might lead to inappropriate clinical decision making. However, longitudinal intrasubject variability in red blood cell survival needs to be further investigated and confirmed[23]. With respect to erythrocyte kinetics, red blood cell survival curves demonstrated curvilinear (instead of linear) disappearance with a half-life being about 30 days[22,24]. As a consequence of the continuous

turnover of red blood cells, the ambient blood glucose concentration will be represented in a kind of sliding scale. Therefore, approximately 50% of a given HbA_{1c} value is the result of the glucose exposure during the previous 30 days, 40% is the result of the glucose exposure during the previous 31 to 90 days and 10% is formed by glucose exposure during the previous 91 to 120 days[25]. The life span of red blood cells -and thus HbA_{1c} - is affected by a number of genetic, haematological, and illness-related factors, which should be taken into account when interpreting the results[26]. Especially factors that change erythropoiesis and erythrocyte destruction may affect HbA_{1c}.

For optimal monitoring by HbA_{1c} measurement of patients with diabetes with regard to glucose control, the analytical coefficient of variation (CVa) and the within-person biological variation (CVw) are relevant. In a recent study, Braga et al systematically reviewed the published studies on the biological variability of HbA_{1c} and concluded that the published studies had methodological limitations. These limitations restricted their ability to come to clear-cut conclusions. The authors also provided a rough estimate of mean CVw in healthy persons of approximately 1.8% - 1.9%[27]. In patients with diabetes, fluctuations in HbA_{1c} levels are not random. They should be considered a true phenomenon, because they are caused by changes in the patient's glycaemic state. As a result, the CVw may be much higher in patients with diabetes than in healthy persons.

Historical overview

Discovery of glycated hemoglobin A_{1c}

In 1969, Samuel Rahbar and co-workers discovered higher concentrations of fast hemoglobin fractions in patients with diabetes compared to subjects without diabetes[18,19]. After that, it still took some time before, HbA_{1c} became a "gold standard" for glucose management in patients with diabetes. Trivelli was the first one to suggest a relationship between fast hemoglobin fractions, mean blood glucose concentrations and long-term complications in patients with diabetes[28]. The term "fast" is derived from the fact that these components eluted faster from a cation-exchange column than the other components. The fractions were described in the order in which they were eluted from the column: HbA_{1a}, HbA_{1b} and HbA_{1c}, respectively. In 1975, Bunn et al observed that the glycation process included the formation of a Schiff base (aldimine) and that the majority of aldimine is converted into a stable ketoamine (Figure 1). They also concluded that it was probably a non-enzymatic reaction because of the slow rate of the HbA_{1c} formation[29]. Eventually, in 1978 the first commercial HbA_{1c} method became available. After that, however, it took another 10 years before the ADA recommended the routine use of the HbA_{1c} assay in clinical practice.

HbA_{1c} methodology

There are currently more than 30 HbA_{1c} assays available on the market (Table 1), all of which are based on either of two principles: charge differences and structural differences. The former principle is used in ion-exchange chromatography (high-performance liquid chromatography; HPLC) and electrophoresis-based assays, whilst the latter principle is used in immunoassays and in assays based on boronate affinity chromatography.

Table1 : Overview of the most common used HbA_{1c} methods and some point-of-care methods.

Principle	Manufacturer	Method/analyzer name
Ion exchange chromatography HPLC	Bio-Rad	Variant Variant II Variant Turbo Variant Turbo 2.0
	Tosoh	D 10 A1C 2.2 G5 G7 G8
	Arkray/Menarini	HA 8140 VP and TP mode HA 8160 VP and TP mode HA 8180 VP mode
	Drew Scientific	DS360
	Trinity	CLC 330 CLC 385 PDQ Ultra[2]
Affinity Chromatography	Axis-Shield	Afinion* Nycocard*
	Infopia	Clover*
	Bio-Rad	in2it* Micromat II or GDX A1C Test*
	Abbott	Architect
	Beckman	Synchron systems (CX, LX, Unicel DxC)
	Siemens	Dimension systems (ExL, RxL, Vista, Xpand) Advia systems DCA instruments (2000, Vantage)*
Immuno-assay	Roche	Tina-quant Gen.2 Cobas c501, c111 Tina-quant Gen.2 Cobas Integra 400/800 Tina-quant Gen.2 Hitachi/Modular
	Ortho	Vitros 5.1
	Olympus	AU systems
	DiaSys	InnovaStar* OneHbA_{1c} FS on Hitachi 917
	Bayer	A1CNow*
	Thermo Fisher	Architect systems

*Point-of-Care instrument

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 β-chain. As already stated, examples of such methods are cation-exchange chromatography and electrophoresis. The latter is not often used anymore in routine clinical laboratory settings. Cation-exchange chromatography is a process that allows the separation of the mixture based on the charge properties of the molecules in the mixture. Charged hemoglobins and other hemoglobin components are eluted at varying times depending upon the net charge of the molecule in relation to a gradient of increasing ionic strength passed through a cation-exchange column. Figure 2 shows chromatograms of different cation-exchange HPLCs including the chromatogram of the Bio-Rex70 method used in the DCCT and UKPDS study (see standardisation). The different chromatograms show the improvements made in cation-exchange HPLC. The chromatogram of the Bio-Rex70 method shows poor resolution (no sharp HbA_{1c} peak), reflecting poor specificity in comparison to the chromatogram of the Tosoh G8 (sharp HbA_{1c} peak).

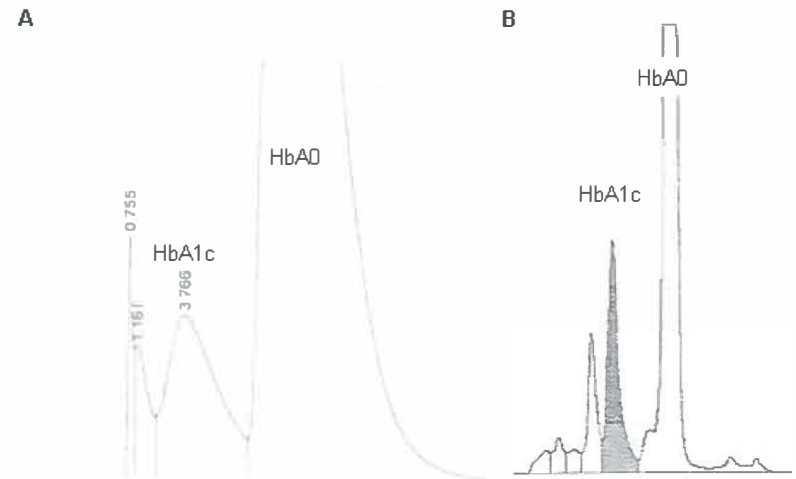

Figure 2
Chromatograms of two different cation-exchange HPLCs; Bio-Rex70 method (A) and Tosoh G8 (B). The different chromatograms show the improvements made in cation-exchange HPLC. The chromatogram of the Bio-Rex70 method shows poor resolution (no sharp HbA_{1c} peak), reflecting poor specificity in comparison to the chromatogram of the Tosoh G8 (sharp HbA_{1c} peak).

In general, the advantage of cation-exchange HPLCs is a low CVa. Most of the HPLCs are capable of having a CVa <2.0%, and newer HPLCs are even capable of having a CVa <1.0%, which makes these techniques superior for monitoring patients in comparison to other methods[30,31]. The disadvantage of cation-exchange HPLC is the interference with some hemoglobin variants. This may yield falsely lower or higher HbA_{1c} results. To avoid mistakes due to interference of hemoglobin variants, every chromatogram still needs to be checked for abnormal chromatograms, either manually or through computer programming. This demands certain skills of the technicians running the instrument.

The longer run time for the measurement of HbA_{1c} by HPLC might be a problem for commercial laboratories due to the amount of samples which need to be analysed every day. In order to solve this problem, several HPLC machines can be connected to each other, which will eventually increase the total CVa. For mid-volume laboratories, attempts are made to connect an HPLC instrument to a haematology instrument, with one technician taking care of the analyses.

Methods based on structural differences

Affinity Separation:
Affinity separation is based on the covalent binding of cis-diols of glucose in glycated hemoglobin to a boronate matrix. It measures "total" glycation (Figure 3). In HPLC, non-glycated hemoglobin will leave the column without being attached to the boronate matrix. Glycated hemoglobin will be released from the column when changing buffers. The chromatogram shows two peaks, a non-glycated peak and a glycated peak.

The advantage of affinity chromatography is the absence of interference by hemoglobin variants or derivates, which means that this method has been affirmed as the "reference" method for use in patients with hemoglobin variants for quite some time[32-34]. Rolfing et al. showed, however, that there is an interference with HbF >20% due to the fact that HbF does not have β-chains that result in disproportional low glycation of this hemoglobin molecule[35]. In the past, affinity-chromatography HPLCs were able to compete with cation-exchange HPLCs with respect to CVa, but now external quality schemes prove that this is no longer the case[36].

Immunoassays:
Immunoassays are based on specific HbA_{1c} antibodies that recognize the first 3, 4 or 5 amino acids and the glucose attached to the N-terminal of the β-chain of the hemoglobin molecule. Total hemoglobin is usually measured bichromatically. Assay designs differ substantially from each other, ranging from immunoturbidimetry (Figure 3) to latex agglutination inhibition methods (using monoclonal antibodies).

A major advantage is the high throughput ability of these instruments. This is the reason why these methods are widely used in commercial laboratories in which many samples need to be analysed every day. Another advantage is that the majority of the immunoassays does not interfere with common hemoglobin variants such as HbAS, HbAC, HbAD and HbAE. Immunoassays only interfere with rare hemoglobin variants (substitution of the last 3, 4 or 5 amino acids by another amino acid). A disadvantage, in comparison to the majority of the cation-exchange HPLCs, is the relative high CVa. External quality schemes show that only the new cation-exchange HPLCs are capable of having an interlaboratory CV of ≤ 2.0%[36].

Point-of-care instruments

Point-of-care testing is defined as: "diagnostic testing that is performed near to or at the site of the patient care with the result leading to possible change in the care of

the patient"[37]. The principles used by point-of-care (POC) instruments are based on affinity separation or immunoassay.

The advantage of POC instruments is that they provide results rapidly after blood collection, which leads to less patient inconvenience. In addition, studies have confirmed that immediate feedback of HbA_{1c} results helps to improve glycaemiccontrol in patients with type 1 diabetes and insulin-treated patients with type 2 diabetes[38-40]. The disadvantage of most HbA_{1c} point-of-care instruments is the poor analytical performance, resulting in high CVa, bias from reference methods, and lot numbers dependency[41]. Furthermore, interference with hemoglobin variants might be a problem for POC instruments with principles based on immunoassay.

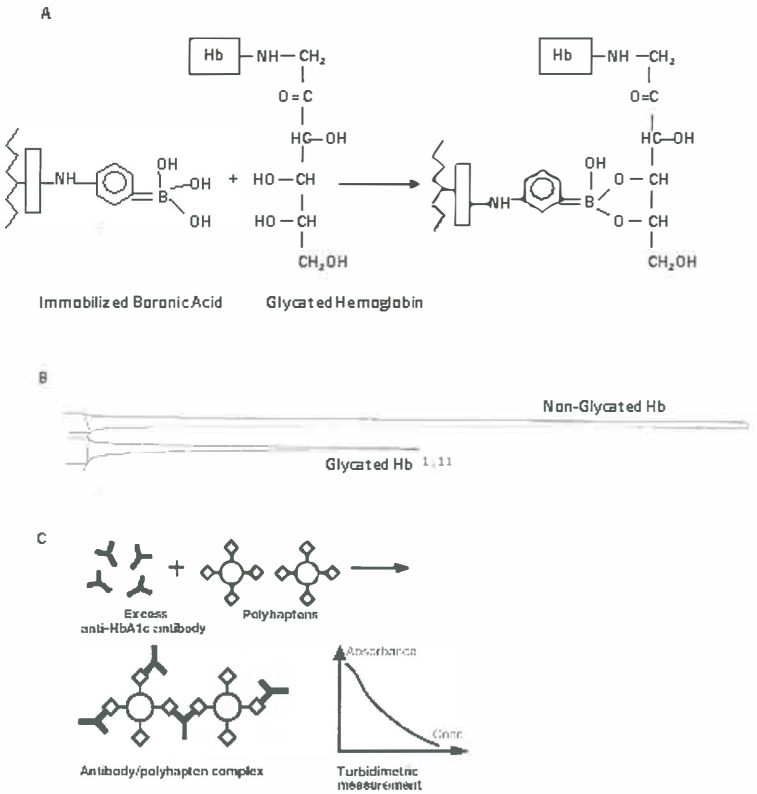

Figure 3

A: Principle of affinity separation. Affinity separation is based on the covalent binding of cis-diols of glucose in glycated hemoglobin to a boronate matrix. It measures "total" glycation. In HPLC, the non-glycated hemoglobin will leave the column without being attached to the boronate matrix. Glycated hemoglobin will be released from the column when changing buffers.

B: The chromatogram of an affinitychromatography HPLC shows two peaks, a non-glycated peak and a glycated peak.

C: Principle of an immunoassay, based on immunoturbidimetry. An excess of anti-HbA1c antibodies is added to an hemolyzed patient sample. Anti-HbA1c will bind to HbA1c in the patient sample. The excess of anti-HbA1c is agglutinated with polyhaptens and an antibody/polyhapten complex is formed. The resulting immune complexes lead to cloudiness or turbidity of the solution, which can be measured photometrically. Total hemoglobin is measured bichromatically during the preincubation phase in the same cuvet (reprinted with permission of Roche Diagnostics, Rotkreuz, Switzerland).

Standardisation of the Hemoglobin A_{1c} methods

No reference material was available until 1993, and as a consequence, the interlaboratory CV was high (>20%). The variability in results between different HbA_{1c} methods and variability in how results were reported (e.g. total glycation, HbA1 and HbA_{1c}) made it difficult for physicians to use specific HbA_{1c} targets in clinical practice. In many cases, HbA_{1c} values available in an individual clinic were not related to those reported in clinical studies from which target values were derived. Therefore, it became obvious that this important parameter for the management of patients with diabetes mellitus should be standardized.

Over the last decades, major improvements in the standardisation of the HbA_{1c} method have been made (Table 2). The DCCT study in patients with type 1 diabetes mellitus, which was published in 1993, clearly demonstrated that the risk of the development and progression of especially microvascular complications was closely related to the degree of glycaemic control[10]. In this study, HbA_{1c} was measured with a HPLC system applying the Bio-Rex 70 cation-exchange resin[42]. In 1994, the American Association for Clinical Chemistry (AACC) initiated the National Glycohemoglobin Standardization Program (NGSP), a subcommittee for the standardisation of glycohemoglobin that aimed to harmonize HbA_{1c} assays worldwide. The ultimate goal of the NGSP was to facilitate individual laboratories to relate their HbA_{1c} assay results to those of the DCCT study[43]. At that time, no definitive primary reference method was available. The method applied in the DCCT study was therefore 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 HbA_{1c} methods to make their methods traceable to the DCCT study[44]. The NGSP standardisation, however, was clinically based instead of scientifically based. Calibration of this method was arbitrarily chosen. This was one of the reasons for the International Federation of Clinical Chemistry (IFCC) to develop a scientifically based HbA_{1c} reference method instead. An IFCC Working Group for the standardisation of HbA_{1c} was established in 1994 to develop a standard for HbA_{1c}, consisting of almost pure HbA_{1c} and HbA0, and a primary reference method for HbA_{1c}. In the meantime, the AACC and the ADA accepted clinical standardisation based on DCCT numbers (via NGSP) as an interim solution until definite standardisation was established[45]. The IFCC Working Group on HbA_{1c} Standardisation succeeded in producing reference material. The development of the reference method for HbA_{1c} analysis based on enzymatic cleavage of the hemoglobin molecule was published in 2002[46]. In addition, a laboratory network was established, which included the two reference methods, i.e. mass spectroscopy and capillary electrophoresis[47]. Each network laboratory used prepared mixtures of purified HbA_{1c} and HbA0 as calibrators[48]. The main task of the IFCC Network of Reference Laboratories for HbA_{1c} was to assign values to secondary reference material and to collaborate with manufacturers of diagnostic devices and External Quality Assessment Schemes (EQAS) organisers. This secondary reference material, made from patient whole blood, is currently the basis for the standardisation of HbA_{1c} worldwide and is used by manufacturers of HbA_{1c} methods to assign values to their own method-dependent calibrators.

Table 2: Overview of the standardisation of HbA_{1c}

Year	Standardisation activity / publication of important study	Reference
1993	Publication of the DCCT study	N Engl J Med. 1993;329:977–86.
1994	AACC subcommittee GHB standardisation (NGSP)	
1995	IFCC working group for the standardisation of HbA_{1c}	www.ifcchba1c.net
1996	AACC and ADA accept clinical standardisation on DCCT numbers (via NGSP) as interim until definite standardisation	www.ngsp.org/bground.asp
1997	Japan chooses for own system based on KO500 HPLC	J Japan Diab Soc. 1994;37:233–43 J Japan Diab Soc. 1998;41:317-23
1998	Sweden accepts standardisation based on Mono-S HPLC	Ann Clin Biochem. 1994;31:355–60
	Publication of the UKPDS study	Lancet 1998;352:837-53
2002	IFCC working Group published definitive reference method	Clin Chem Lab Med. 2002;40:78–89
2003	Start implementation group with members of the ADA, EASD, IDF and IFCC which resulted in the design of the A1c Derived Average Glucose study (ADAG-study)	Diabetologia 2004;47:R53-R54.
2004	Publication of the master-equations between different standardisation systems	Clin Chem. 2004;50:166–74
2007	Consensus achieved	Diabetes Care 2007;30:2399-400
2008	Publication of the ADAG study	Diabetes Care 2008;31:1473-78
2009	Implementation of new IFCC numbers in some countries	www.ifcchba1c.net/IFCC_LatestNews.asp
2010	Revision of consensus statement	Diabetes Care 2010;33:1903-4
	Disbandance of IFCC working group after fulfilment of its tasks	
	Start integrated project	

Besides the two global standardisation programs there were also two national standardisation programs. One in Sweden, which was based on the Mono-S HPLC method, and one in Japan, which was based on the KO500 HPLC system[49-51].

The relation between the different standardisation programs (NGSP, IFCC, Sweden en Japan) has been studied since 2002. This resulted in the publication of the so-called "master equations" in 2004[52-54]. The published master equations made it possible to recalculate HbA_{1c} from IFCC numbers to DCCT, Swedish and Japanese HbA_{1c} values. As suspected, these master equations yielded lower values with the IFCC primary reference method due to higher specificity of the IFCC method when compared with the methods used in the other standardization programs. Implementation of these lower values, if expressed in the same units as the DCCT numbers (HbA_{1c} %), might confuse the patient and the health care professional. This was one of the reasons why the IFCC working Group decided to express IFCC numbers in SI units (mmol HbA_{1c}/mol Hb) resulting in about ten times higher values[55].

The major clinical diabetes organisations, including the ADA, the European Association for the Study of Diabetes (EASD) and the IDF, were asked to assist the IFCC Working Group with the implementation of the IFCC reference system and the worldwide implementation of the new HbA_{1c} values. Confusion and deterioration of glycaemic control as a result of this introduction had to be avoided[56]. The choice between the more specific lower values (in percentages) and the later proposed higher values of HbA_{1c} in SI units (mmol HbA_{1c} per mol Hb) gave rise to the idea to express HbA_{1c} in the same units as day-to-day glucose monitoring[57,58]. The A1c-Derived Average Glucose (ADAG) study group designed a study to determine if this would be possible. In addition, the ADAG study group aimed to gain a better understanding of the relationship between HbA_{1c} and average blood glucose by using frequent capillary measurements and continuous glucose monitoring[59].

The ADAG study became part of the implementation of the IFCC HbA_{1c} reference system as mentioned in the consensus statement agreed upon by the ADA, EASD, IDF and IFCC[60]. The publication of the results of the ADAG study in 2008 resulted in a worldwide discussion whether or not estimated Average Glucose (eAG) should also be reported as an interpretation of the HbA_{1c} values, in addition to reporting HbA_{1c} in IFCC/SI units and its derived NGSP/DCCT values[61,62]. In general, the majority of experts in Europe considered the study results to be unconvincing due to major limitations of the study. These experts decided not to report eAG until these limitations were resolved[63]. The consensus statement was revised in 2010, and reporting eAG was no longer part of the consensus statement[64]. However, eAG was already in use for years in the US, based on the DCCT study and will still be reported there for educational purposes besides HbA_{1c} in DCCT numbers. HbA_{1c} is not reported in IFCC numbers in the US. Notwithstanding the fact that the consensus statement was signed by representatives of the major clinical diabetes organisations ADA, EASD, IDF and IFCC, it became clear that every country has chosen or will choose its own way of reporting HbA_{1c} values.

The IFCC Scientific Division concluded in 2010 that the IFCC working Group had fulfilled its mission to develop a reference method and materials and therefore

disbanded the working group. However, the laboratory network is still operative. The educational work and clinical aspects will be intensified in a new IFCC Integrated Project.

Determination of the analytical performance of Hemoglobin A_{1c} methods

There are various ways to check the effectiveness of method standardisation and the analytical performance of an HbA_{1c} method. Two certification and/or monitoring programs are important for manufacturers.

The NGSP offers manufacturers the NGSP manufacturer certification program. The ADA and the IDF recommend laboratories to use only NGSP-certified HbA_{1c} methods. This certification process includes the exchange of 40 patient samples with a Secondary Reference Laboratory (SRL), using a certified Secondary Reference Method (SRM) and an assessment of agreement analysis[65].

The IFCC offers a monitoring program to prove traceability to a method of "higher order", the IFCC primary reference method. This method is mandatory for manufacturers in Europe according to the European Union In-Vitro Diagnostic directive of 1998[66]. This monitoring program consists of 24 interconnected, fresh-frozen, pooled patient samples. The 24 specimens are distributed to be used over a time span of one year, with a deadline every two weeks, which enables manufacturers to have an up-to-date view every two weeks. Once a cycle has been completed, an annual report can be requested which shows accuracy, precision and linearity information.

Both certification/monitoring programs provide information for the manufacturers on the analytical performance of their method. However, different approaches were used in the value assignment of both certification/monitoring programs. The value assignment of the samples used in the IFCC monitoring program was done by at least 12 approved IFCC primary reference measurement procedures, and reflects the true HbA_{1c} value. In addition, it also provides information about imprecision (12 samples in duplicate measured at different times in the year) and linearity. In contrast, in the NGSP certification program, the method of the manufacturer is compared with an NGSP-certified secondary reference method and only reflects agreement with the secondary reference method.

A more useful and informative way to check the analytical performance of an HbA_{1c} method is to do so at the user's or laboratory site. Testing can be done by following an evaluation protocol and internal and external quality controls. In general, laboratories evaluate a method when they consider replacing the current method with another method. In order to draw justified conclusions, a proper evaluation protocol and reference method is essential. The Clinical Laboratory Standard Institute (CLSI)/National Committee on Clinical Laboratory Standards (NCCLS) provides certified protocols to evaluate the test's/instrument's performance characteristics[67-69].

Internal quality controls are used by laboratories to monitor their own performance. The primary objective is attained when the system generates a proper alert. The system generates an alert if an error occurs in an analytical run. Measures can then be taken immediately to ascertain and correct the source of the error. The results of these controls can be used to calculate the overall CVa.

The main objective of external quality assessment is to establish between-laboratory comparability. External Quality Assessment (EQA) is a system whereby a set of reagents and techniques are assessed by an external source and the results of the testing laboratory are compared with those of an approved reference laboratory. External quality control schemes are meant to investigate analytical performance and results produced in the field, reflecting many different laboratories, instruments, lot numbers etc. Most EQA organisers use pooled, fresh whole blood to avoid a method-dependent matrix effect, which might be introduced by using lyophilised material. Therefore, the criteria to pass or fail are based on total error. This encompasses bias and imprecision, with no distinction between bias and imprecision.

Hemoglobin A_{1c} in the management and diagnosis of diabetes: analytical goals

The degree of glucose control can be assessed by frequent home blood glucose measurements, but the most widely acknowledged and reliable assessment is considered to be the measurement of the HbA_{1c} concentration. As such, HbA_{1c} was also the main parameter in most outcome studies. In general, a target value of HbA_{1c} of less than 53 mmol/mol (7.0% DCCT) is considered by many to be the treatment goal in order to reduce the risk of diabetes-related complications[10,11]. The ADA and EASD consensus algorithm for the initiation and adjustment of therapy states that a sustained HbA_{1c} level above 53 mmol/mol (7.0% DCCT) and a difference of 5 mmol/mol (0.5% DCCT) between two consecutive HbA_{1c} values should prompt the health care provider to consider changing therapy in order to reach the predefined target value[70]. The ADA recommends performing the HbA_{1c} test at least twice a year in patients with stable glycaemic control or four times per year in patients with changes in therapy or with HbA_{1c} levels above the target value[71]. The changes in therapeutic regimes are therefore guided by (relevant) changes in serial measurements of HbA_{1c} Therefore, most diabetes care professionals rely on the HbA_{1c} level to decide whether treatment changes are to be advised to patients or not.

From an analytical point of view, the difference between two serial HbA_{1c} measurements depends on the within person biological variation (CVw) obtainable from the literature[27,72] and the analytical variation (CVa) of the HbA_{1c} laboratory assay, established with internal quality controls. These two sources of variation can be combined in the so-called reference change value (RCV), which is defined as the critical difference between two consecutive HbA_{1c} measurements representing a significant change in health status at a probability of 95% (RCV (%) $= \sqrt{2} \times 1.96 \times \sqrt{[(CV_a)^2 + (CV_w)^2]}$)[73,74]. By taking a statistically significant difference of 5 mmol/mol (0.5% DCCT) at an HbA_{1c} concentration of 53 mmol/mol (7.0% DCCT) as the goal for HbA_{1c} measurement, one can calculate an appropriate goal in terms

of CVa. Assuming that the mean CVw is 1.8%, in line with the data of Braga et al[27], then the maximum allowable CVa is 2.9% (IFCC values) or 1.9% (DCCT values).

Recently, the American Diabetes Association (ADA) has advocated the use of HbA_{1c} for the diagnosis of diabetes[12] as a result of the global standardisation of the HbA_{1c} assay with associated improvement of the analytical performance of the assay[13-16]. Therefore, freedom from bias is critical because fixed cut-off points are then used both as targets for glycaemic control and for the diagnosis of diabetes. In order to compare a patient result with a target value of 53 mmol/mol (7.0 % DCCT), total error (TE (%) = bias (%) ± 1.96 CVa (%)) should be taken into account. If 5 mmol/mol (0.5% DCCT) is again considered as clinically significant (and thus as a total error), and if the maximum allowable CVa is 2.9% (IFFC values) or 1.9% (DCCT values, then the maximum allowable bias is 2.0 mmol/mol (0.24% DCCT). If the CVa is 1.0%, the maximum allowable bias is 4.0 mmol/mol (0.36 %DCCT). However, the CVw may vary from person to person. In order to optimally monitor each individual, more stringent criteria might be necessary. As a rule of thumb, CVa should be less than one half the CVw[75].

Analytical performance of glycated hemoglobin A_{1c} methods

Point-of-care instruments

Point-of-care (POC) instruments are widely used by health care professionals for a variation of tasks and measurements. POC instruments for the determination of HbA_{1c} are classified as CLIA-waived tests (Clinical Laboratory Improvements Amendments). Waived tests are defined as simple laboratory analyses and procedures that (1) have been cleared by the Food and Drug Administration (FDA) for home use (2) employ methodologies that are as simple and accurate as to render the likelihood of erroneous results negligible; or (3) pose no reasonable risk of harm to the patient if the test is performed incorrectly[76]. According to CLIA rules POC instruments do not have to fulfil quality requirements in the same way as laboratory based methods. For example: CLIA-waived POC instruments are not obliged to join external quality schemes, and therefore the real analytical performance is not known. A recent study showed that 6 out of 8 HbA_{1c} POC instruments do not meet the general accepted performance criteria[41]. In this study, the bias ranged from -9.6 mmol/mol (-0.9% DCCT) to +4.3 mmol/mol (0.4% DCCT), and 6 out of the 8 POC instruments had a CVa >3.0% in the clinically relevant range. Using these instruments for the diagnosis of diabetes would lead to tens of millions of people who would be wrongly diagnosed with diabetes, or millions who would not receive diabetes treatment of proven value[77]. In addition, the high CVa of POC instruments for monitoring HbA_{1c} may lead to overmanagement of the patient.

Laboratory-based HbA_{1c} methods

External quality schemes can be used to judge the overall analytical performance of laboratory-based methods. The mean bias gives a good impression of the

effectiveness of the standardisation of HbA_{1c} and the intra method CV gives a good impression of the precision of the HbA_{1c} method. The most recent survey of the College of American Pathology (CAP) reveals that approximately 26% of the HbA_{1c} methods have a mean bias of >0.2% DCCT and approximately 50% of the HbA_{1c} methods have an intra method CV>3.0% (DCCT values)[36]. As a result, patient management can not be done in an optimal way (clinically significant difference of 5 mmol/mol or 0.5% DCCT) when patient go from one hospital to another, even if the laboratories use the same HbA_{1c} method.

A recent study showed that one in five laboratories in the Netherlands, using various HbA_{1c} methods do not meet the criteria for optimal diabetes care management[78]. Of the HbA_{1c} laboratory based methods (n=220), 35% had a CVa of >1.9% (DCCT values).

In view of analytical performance of the HbA_{1c} method, we can conclude that great improvements have been made by the work of the NGSP and the IFCC working group for the standardisation of HbA_{1c} in cooperation with manufacturers. However, both from the perspective of individual patients, and based on the required accurate performance when aiming to use HbA_{1c} as diagnostic parameter, we believe that the analytical performance of some HbA_{1c} methods is insufficient.

Conclusion

HbA_{1c} has become a "gold standard" for the management of patients with diabetes and has recently been accepted by some national organisations as defining parameter for the diagnosis of diabetes (due to global standardisation of the HbA_{1c} assay with associated improvement of the analytical performance of the assay). There are currently more than 30 methods available on the market with an analytical performance ranging from poor (some POC instruments) to very reliable (newer cation-exchange HPLC methods).

In order to optimally monitor the patient with diabetes, and to check whether a target goal has been achieved, we believe the maximum allowable CVa is 2.9% (IFFC values) or 1.9% (DCCT values) and the maximum allowable bias is 2.0 mmol/mol (0.24% DCCT).

It is important that the limitations of current HbA_{1c} methods are understood by health care professionals, because these limitations may have important clinical implications. Clinical chemists can play a valuable role in choosing a method with acceptable analytical performance characteristics. They can also help clinical decision making by providing healthcare professionals with the necessary information (measurement uncertainty and/or RCV) to properly interpret HbA_{1c} results.

Outline of the thesis

Chapter 2 describes the method used to determine values to the samples used in the ADAG study. Well documented HbA_{1c} value determination of the samples in the ADAG study traceable to the IFCC reference method is very important. This HbA_{1c} value determination, using certified IFCC secondary reference methods and material, is described and the effect of additional off-line calibration was investigated in an attempt to explore the possibilities of improvement of the uncertainty expressed in 95% CI between the four IFCC secondary reference methods.

In **chapters 3 to 6** the analytical performance of 8 different HbA_{1c} point-of-care instruments was studied. This performance was studied, since according to Clinical Laboratory Improvement Amendments (CLIA) rules, users of point-of-care instruments are not obliged to join external quality schemes and as a result, there is no real notion of the performance of these instruments. Recently, the American Diabetes Association (ADA) has advocated the use of HbA_{1c} for the diagnosis of diabetes. Therefore it was of utmost importance to know the analytical performance of these instruments.

In **chapter 7** the analytical performance of a new laboratory based HbA_{1c} method (Arkray ADAMS HA-8180 HPLC) was studied.

When the ADA proposed to use HbA_{1c} as discerning marker for the diagnosis of diabetes, there was considerable apprehension regarding the consequences of the use of poorly performing HbA_{1c} methods for the diagnosis of diabetes. External Quality Assurance Schemes give information on the "analytical performance on average" of different HbA_{1c} methods but do not give insight in the analytical performance of individually laboratories using various methods. In **chapter 8** attention for this point is asked.

Chapter 9 also focuses on the potential role of point-of-care testing of HbA_{1c} and glucose in the diagnosis of pre-diabetes and diabetes. It gives an overview of the principles, pitfalls and analytical performance of glucose and HbA_{1c} point-of-care testing and summarises the studies that have applied point-of-care testing of glucose and HbA_{1c} in the diagnosis of (pre-) diabetes.

As mentioned before, External Quality Assurance Schemes give information on the "analytical performance on average" of different HbA_{1c} methods. CVa of 220 individual laboratories using various HbA_{1c} methods were obtained, and the RCV was calculated. Data are presented in **Chapter 10**.

Guidelines in the management of the patients with diabetes are well documented and are presumed to be widely used by all health care professionals dealing with the treatment of diabetes. In **chapter 11** we discuss the findings of a survey distributed among health care professionals regarding their attitudes towards cut-off points for treatment decisions in diabetes mellitus based on HbA_{1c}.

In **chapter 12** the summary and conclusions of this thesis and the recommendations and future perspectives are provided.

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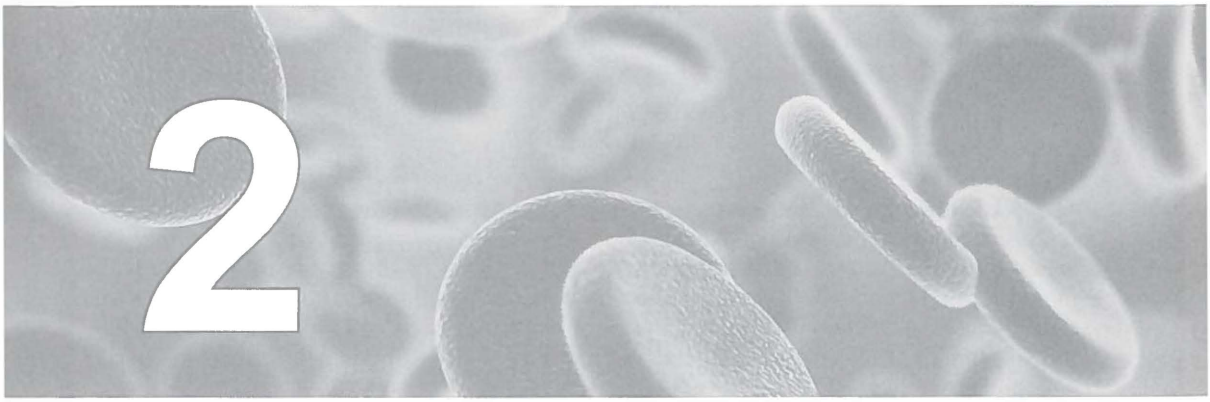

Hemoglobin A_{1c} determination in the A1c-Derived Average Glucose (ADAG)-Study

Erna Lenters-Westra
Robbert J. Slingerland

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Abstract

Background

The A1c-Derived Average Glucose (ADAG)-study was commenced to gain a better understanding of the relationship between HbA_{1c} and average blood glucose and to investigate if HbA_{1c} could be expressed in the same units as day-to-day glucose monitoring. Owing to the impact of the outcome of this study it was very important to determine HbA_{1c} values with a minimum of uncertainty and as close as possible to the International Federation of Clinical Chemistry (IFCC) primary reference method, which is the only valid anchor of HbA_{1c} standardisation.

Methods

Approximately 2300 samples were analyzed with four IFCC secondary reference methods. Additional off-line calibration with IFCC secondary reference material with assigned IFCC values was performed to improve the uncertainty in the HbA_{1c} value determination.

Results

Additional off-line calibration improved the 95% confidence interval between the four different HbA_{1c} methods at HbA_{1c} of 6.00% from ± 0.28% (5.72% - 6.28%) to ± 0.20% (5.80% - 6.20%) and at HbA_{1c} of 9.00% from ± 0.43% (8.57% - 9.43%) to ± 0.24% (8.76% - 9.24%).

Conclusion

The HbA_{1c} results used in the ADAG study were determined with currently the lowest uncertainty technically feasible by using four certified IFCC secondary reference methods and additional off-line calibration with IFCC secondary reference material.

Introduction

At the onset of the National Glycohemoglobin Standardization Program (NGSP) in 1995, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) reference system for HbA_{1c} was still under development. No gold standard was available at that time, so it was understandable that the BioRex 70 method used in the Diabetes Control and Complications Trial (DCCT)[1], and to which also the results of the United Kingdom Prospective Diabetes Study (UKPDS)[2] were calibrated, was used as a reference method in this standardisation system. Today, most if not all commercial methods are producing DCCT-aligned values[3,4].

With the publication of the definitive scientific based IFCC approved reference method for the measurement of HbA_{1c} in human blood[5], the work of the IFCC working group for HbA_{1c} standardisation was almost completed, but the last and most difficult step had to be taken: implementation of the reference system in daily life[6-11]. The problem of the lower values of the IFCC compared to the NGSP/DCCT values, as a result of more specific measurement of HbA_{1c}, had to be resolved.

The major clinical diabetes organizations (American Diabetes Association (ADA), European Association for the Study of Diabetes (EASD) and the International Diabetes Federation (IDF) were asked to assist the IFCC working group with the implementation of the IFCC reference system and the worldwide acceptance of a new numbering system, bearing in mind that confusion and deterioration of glycemic control as a result of its introduction had to be avoided[12]. The choice between the more specific lower values (in percentages) and in contrast the later proposed higher values of HbA_{1c} in SI units (mmol HbA_{1c} per mol Hb) gave rise to the concept to express HbA_{1c} in the same units as day-to-day glucose monitoring[13, 14]. The A1c-Derived Average Glucose (ADAG)-study group designed a study to determine whether this could be possible and to gain a better understanding of the relationship between HbA_{1c} and average blood glucose using frequently capillary measurements and continuous glucose monitoring[15]. This ADAG study became part of the implementation of the IFCC HbA_{1c} reference system, as mentioned in the consensus statement agreed on by the ADA, EASD, IDF and IFCC[10]. If this study fulfils its a priori specified criteria, an estimated HbA_{1c} Derived Average Glucose (eADAG) value calculated from the measured HbA_{1c} result should also be reported as an interpretation of the HbA_{1c} values, besides reporting HbA_{1c} in IFCC/SI units and its derived NGSP/DCCT values.

Well-documented HbA_{1c} value determination of the samples in the ADAG study traceable to the IFCC reference method is of utmost importance. The European Reference Laboratory for Glycohemoglobin acted as the central laboratory in this study for HbA_{1c} value determination. This HbA_{1c} value determination, using certified IFCC secondary reference methods and material, is described and the effect of additional off-line calibration was investigated to explore the improvement of the uncertainty expressed in 95% confidence interval (CI) between the four IFCC secondary reference methods.

Methods and materials

Between September 2006 and November 2007, approximately 2300 samples from 460 patients with type 1 diabetes mellitus, type 2 diabetes mellitus and non-diabetic persons were obtained from 10 clinical centers participating in the ADAG-study. During a period of 4 months, 5 EDTA whole blood samples per patient were collected and stored at -80ºC until shipment on dry-ice to the central laboratory. The five samples per patient were analyzed singular in 1 run in 1 day with four different HbA_{1c} methods. The methods used were: Roche HbA_{1c} on Modular-Analytics and Roche Tina-quant Gen.2 HbA_{1c} on Integra 800, both immuno-assays (Roche Diagnostics Ltd, Rotkreuz, Switzerland), Primus Ultra^2, affinity chromatography HPLC (Primus Diagnostics, a Trinity Biotech Company, Kansas City, MI, USA) and Tosoh G7, cation-exchange HPLC (Tosoh Bioscience N.V./S.A., Tessenderlo, Belgium). The four methods used are certified IFCC and NGSP secondary reference methods with documented results in the IFCC and NGSP monitoring program[3,4,16]. The Tosoh is not an officially certified secondary reference method for the NGSP but the performance is the same as the other certified cation-exchange HPLC methods in the NGSP laboratory network.

The protocol of the ADAG-study described optimal storage conditions of the samples and exclusion of samples from patients which were carriers of hemoglobin-variants. Despite this, chromatograms from the Tosoh G7 showed ageing peaks as a result of improper storage conditions of approximately 200 samples[17]. A total of 34 chromatograms showed presence of hemoglobin-variants which means that some patients were not screened or recognised for being carriers of hemoglobin-variants[18]. Yet, these samples were excluded from the study. The information given by the Tosoh G7 was of added value and confirms the choice of using four different methods with three different measurement principles instead of using only one method to prevent incorrect HbA_{1c} values due to interferences which are not recognizable for certain HbA_{1c} methods[19-21]. Out of the results used in the ADAG study, 92% were based on the mean of four methods. The other results were based on the mean of three methods due to analytical problems (e.g., abnormal Hb-concentration or abnormal chromatographic separation) or a result which was regarded as a clear outlier (outside mean ± 3SD).

Calibration procedure

The European Directive on In Vitro Diagnostic Devices demands that diagnostic manufacturers must guarantee the traceability of their routine test to reference methods and materials of higher metrological order which is based on ISO documents[22,23]. Once a year the leading manufacturers of HbA_{1c} assays, who support the work of the IFCC working group for the standardisation of HbA_{1c}, receive 8 EDTA whole blood pools with assigned IFCC and derived DCCT/NGSP values. The assigned IFCC values to this secondary reference material is obtained from the mean of 12 approved primary IFCC reference methods and the derived DCCT/NGSP values are obtained by using the master equation. This master equation has been established after 10 inter-comparison studies between the IFCC reference system

and the DCCT/NGSP system[6]. This secondary reference material is used by the manufacturers for value assignment to their own calibrators which are supplied to the customers using this specific method. The four methods used in this study are all off-line calibrated with this secondary reference material. By using this secondary reference material in this study, the results are one step higher in the traceability chain to the IFCC reference method for HbA_{1c} than results produced with a method which is calibrated with the calibrators supplied by the manufacturer. The step of value assignment to the calibrators at the manufacturer's site with a certain error is skipped.

The immuno-assays, Roche HbA_{1c} on the Modular-Analytics and the Tina-quant Gen.2 HbA_{1c} on the Integra 800 are normally calibrated with the calibrator supplied by the manufacturer once per month according to the recommendations of the manufacturer. The Primus Ultra^2 and the Tosoh are calibrated once per week and once per 3 months, respectively, based on long-term quality control results, with two (low and high) of the three ERL-IFCC calibrators (secondary reference material). Additional off-line calibration was applied every time value assignment took place. Three ERL-IFCC calibrators were analyzed as a patient sample at the beginning and at the end of every run. The slope and intercept were calculated (x= assigned IFCC value by the IFCC network group converted with the master equation to DCCT/NGSP value, and y= method specific measured DCCT/NGSP value, n=6). The patient samples in the same run were recalibrated with the obtained slope and intercept.

Statistics

Computations were performed using Microsoft® Excel 2002 (Microsoft Corporation, Redmond, WA, USA) software. Statistical analyses were also performed with software package Analyse-It© (Analyse-It Software Ltd., Leeds, UK) and EP Evaluator Release 8 (David G. Rhoads Associates, Inc, Kennett Square, PA, USA)[24].

Linear regression analysis was applied to compare the individual method results with the mean of the four methods. Deming regression analysis was applied to compare the different methods with each other and to calculate the 95% CI of the medical decision points[25].

Results

In Figure 1A (routinely calibrated) and Figure 2A (additional off-line calibrated), the results of all the patient samples measured with the four different methods are shown.

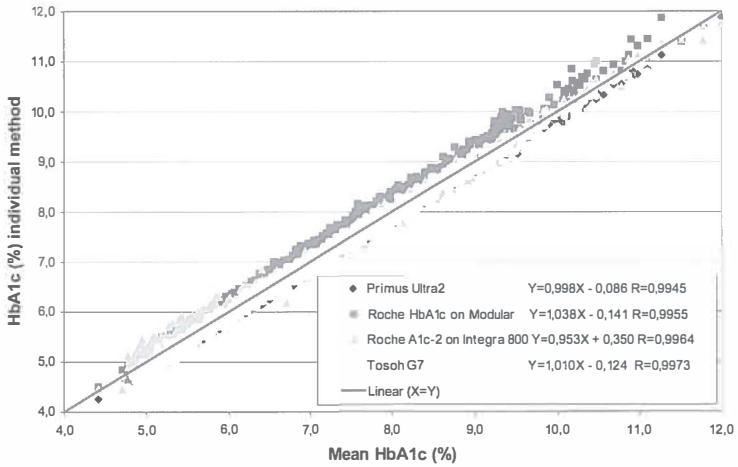

Figure 1A: Linear regression lines four HbA_{1c} methods versus x-mean of the four methods, methods normally calibrated (n~=2300).

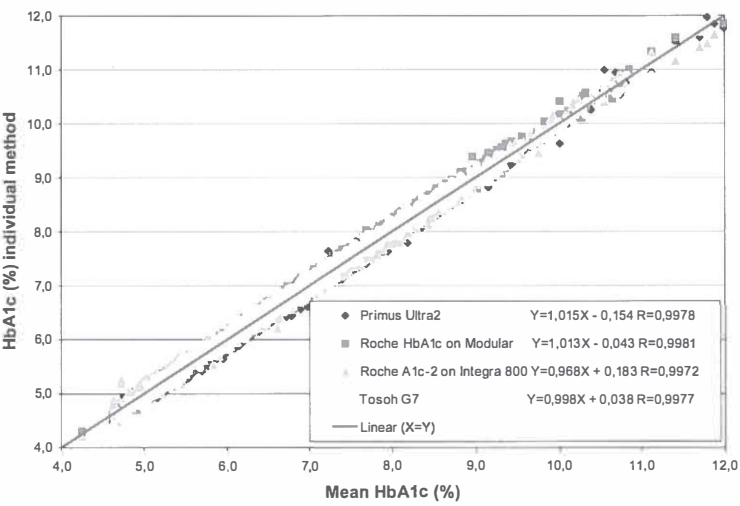

Figure 2A: Linear regression lines four HbA_{1c} methods versus x-mean of the four methods, additional off-line calibrated (n~=2300).

Figure 1B - E show the individual routinely calibrated results of the different methods.

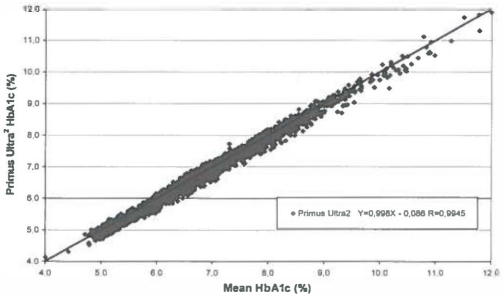

Figure 1B: *Linear regression line Primus Ultra² versus x-mean of the four methods, normally calibrated (n~=2300).*

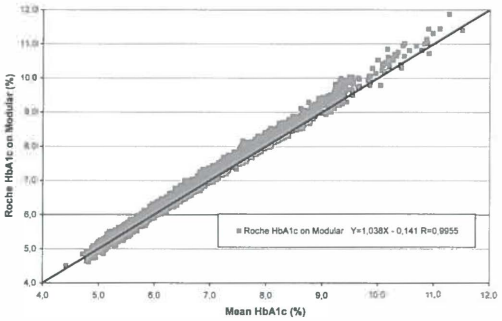

Figure: 1C: *Linear regression line between Roche HbA_{1c} on Modular versus the mean of the four methods, normally calibrated (n~=2300).*

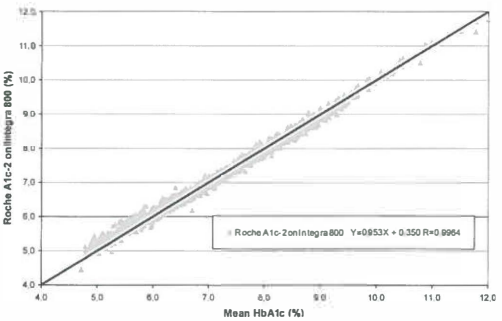

Figure 1D: *Linear regression line between Roche A1c-2 on Integra 800 versus the mean of the four methods, normally calibrated (n~=2300).*

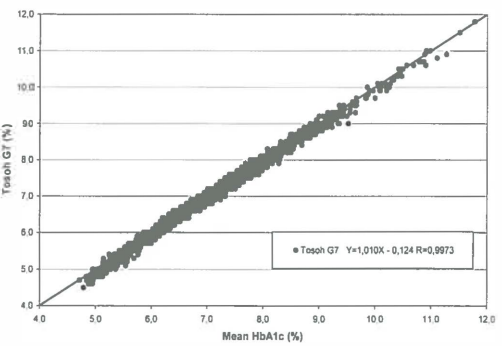

Figure 1E: *Linear regression line between Tosoh G7 versus the mean of the four methods, normally calibrated (n~=2300).*

Figure 2B - E show the individual additional off-line calibrated results of the different methods.

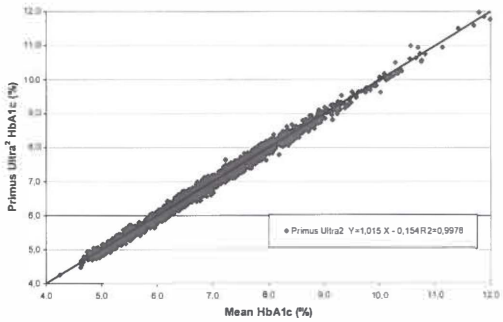

Figure 2B: *Linear regression line between Primus Ultra2 versus the mean of the four methods, additional off-line calibrated (n~=2300).*

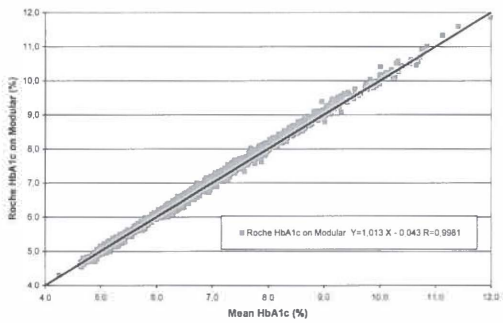

Figure 2C: *Linear regression line between Roche HbA_{1c} on Modular versus the mean of the four methods, additional off-line calibration (n~=2300).*

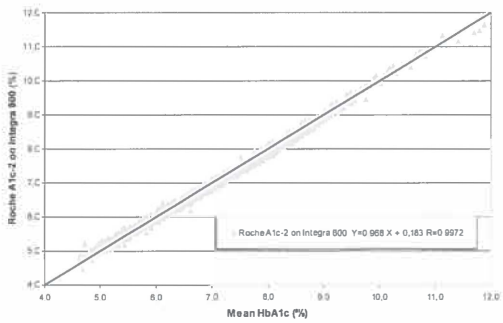

Figure 2D: *Linear regression line between Roche A1c-2 on Integra 800 versus the mean of the four methods, additional off-line calibrated (n~=2300).*

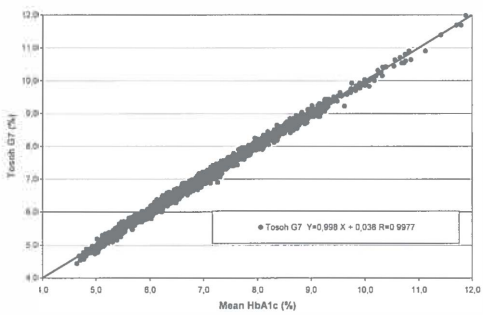

Figure 2E: *Linear regression line between Tosoh G7 versus the mean of the four methods, additional off-line calibrated (n~=2300).*

The 95% CI between the four different HbA_{1c} methods at HbA_{1c} of 6.00% improved from ± 0.28% (5.72% - 6.28%) to ± 0.20% (5.80% - 6.20%) and at HbA_{1c} of 9.00% from ± 0.43% (8.57% - 9.43%) to ± 0.24% (8.76% - 9.24%). The two controls used in this study for approval of patient results also showed improvement in coefficient of variation (CV) before and after additional off-line calibration in three of the four methods used (Table 1). The CVs of the controls of the four methods after additional off-line calibration are all <2.0%, which is desirable according to a recent review of Goodall et al [26]. A CV <2.0% allows clinicians to react on a clinical important HbA_{1c} difference of 0.5% absolute compared to a previously determined HbA_{1c}.

Table 1: Coefficient of Variation (%) of two controls of the four HbA_{1c} methods, normal and after additional off-line calibration.

Controls n=~91	Normal calibration		Additional off-line calibration	
	CV (%) of low control	CV (%) of high control	CV (%) of low control	CV (%) of high control
Primus Ultra[2]	2.23	1.09	1.85	0.93
Roche HbA_{1c} on Modular	2.30	2.31	1.67	0.95
Roche A1c-2 on Integra 800	2.73	1.54	1.85	1.19
Tosoh G7	0.75	0.82	0.72	0.42

Additional off-line calibration is especially of added value when two methods are compared with each other. Table 2 shows Deming regression analysis between the different methods before and after additional calibration. In all cases, the R and the standard error estimates (Std Err Est) improved. The 95% CI at medical decision points are very small (± 0.02%) due to a very high number of samples (n=~2300).

Table2: Deming regression analysis between the four HbA_{1c} methods, normal and after additional off-line calibration. (n=~2300).

	Normally Calibrated	95% CI of 6% HbA_{1c}	95% CI of 9% HbA_{1c}	Add. Off-line Calibrated	95% CI of 6% HbA_{1c}	95% CI of 9% HbA_{1c}
Ultra^2 (Y) vs Tosoh G7(X)	Y=0.991X + 0.018			Y=1.014X – 0.146		
Std Err Est	0.198	5.953 – 5.973	8.921 – 8.951	0.173	5.930 – 5.947	8.967 – 8.994
R	0.9899			0.9924		
Mean Bias	-0.045			-0.049		
Roche HbA_{1c} (Y) vs Tosoh (X)	Y=1.030X – 0.024			Y=1.010X – 0.040		
St Err Est	0.189	6.140 – 6.167	9.233 – 9.263	0.137	6.015 – 6.029	9.042 – 9.064
R	0.9910			0.9949		
Mean Bias	0.184			0.031		
Roche A1c-2 (Y) vs Tosoh (X)	Y=0.942 + 0.476			Y=0.969X + 0.156		
St.Err Est.	0.164	6.122 – 6.139	8.945 – 8.970	0.151	5.965 – 5.980	8.869 – 8.893
R	0.9917			0.9931		
Mean Bias	0.079			-0.054		
Ultra^2 (Y) vs Roche HbA_{1c} (X)	Y=0.960X + 0.057			Y=1.004X - 0.103		
Std Err Est	0.247	5.805 – 5.831	8.680 – 8.717	0.165	5.910 – 5.927	8.916 – 8.943
R	0.9833			0.9926		
Mean Bias	-0.224			-0.078		
A1c-2 (Y) vs Roche HbA_{1c} (X)	Y=0.916X + 0.492			Y=0.955X + 0.227		
Std Err Est	0.177	5.977 – 5.996	8.721 – 8.747	0.140	5.953 – 5.967	8.815 – 8.837
R	0.9904			0.9940		
Mean Bias	-0.103			-0.079		
Roche A1c-2 (Y) vs Ultra^2 (X)	Y=0.950X + 0.466			Y=0.951X + 0.330		
Std Err Est	0.202	6.155 – 6.175	8.998 – 9.031	0.170	6.030 – 6.046	8.878 – 8.906
R	0.9873			0.9912		
Mean Bias	0.123			0.000		

Discussion

The IFCC reference method was not developed nor investigated for routine HbA_{1c} measurement in patient samples, only for value assignment to secondary reference material. Furthermore, the method is too expensive and very time-consuming[5]. For these reasons, the value assignment in the ADAG-study was carried out with IFCC secondary reference methods. By using four IFCC certified secondary reference methods with three different measurement principles, the impact of the individual matrix effect on the ultimate result is minimized. Some samples yield a different result with a particular method. This, so-called, matrix effect is minimized by taking the mean of four methods. Also, information given by certain methods has led to exclusion of samples with ageing or interference substances which would have influenced the value determination if only one method, not free from interferences, was used for value determination.

Figure 1B and 2B show that the dispersion around the line and the 0.1% lower results of the Primus Ultra^2 compared to the other methods improved after additional off-line calibration.

Figure 1C, Figure 2C and Table 2 show the Roche HbA_{1c} on Modular-Analytics method benefits the most from additional off-line calibration. The results from this method are +0.2 absolute % higher over the whole clinical range compared to the other methods if additional off-line calibration is not applied. After additional off-line calibration the results from this Roche HbA_{1c} on the Modular-Analytics assay improved substantially in comparison with the other methods. For normal routine calibration, only calibrators from the manufacturer can be used. This is the reason why immuno-assays benefit the most from additional off-line calibration. The Tosoh G7 and the Primus Ultra^2 were already normally routinely calibrated with ERL-IFCC calibrators and this is not possible with immuno-assays.

Figure 1D shows that the Tina-quant Gen.2 HbA_{1c} method suffers from a minor calibration problem. The translation from the original HbA_{1c} calibration curve to a finally reported HbA_{1c} result is not optimal but inevitable because of the shape of the original HbA_{1c} calibration curve in the instrument. The results at low HbA_{1c} values are higher compared to other methods. The effect of additional off-line calibration to correct for this phenomenon is effective at low HbA_{1c} values and minimal at high HbA_{1c} values (Figure 2D).

Additional off-line calibration had a clear effect on immuno-assays, a moderate effect on the Primus Ultra^2 and no effect on the Tosoh G7, bearing in mind that the Primus Ultra^2 and the Tosoh G7 were already calibrated with IFCC secondary reference material on a routine basis. The dispersion of results between different HbA_{1c} methods out in the field will be much larger than the results presented here owing to the use of the calibrators supplied by the manufacturer and the additional off-line calibration applied in this study.

Conclusion

The HbA_{1c} results used in the ADAG study were determined with currently the lowest uncertainty technically feasible and as close as possible to the IFCC primary reference method by using four IFCC certified secondary reference methods and additional off-line calibration with IFCC secondary reference material.

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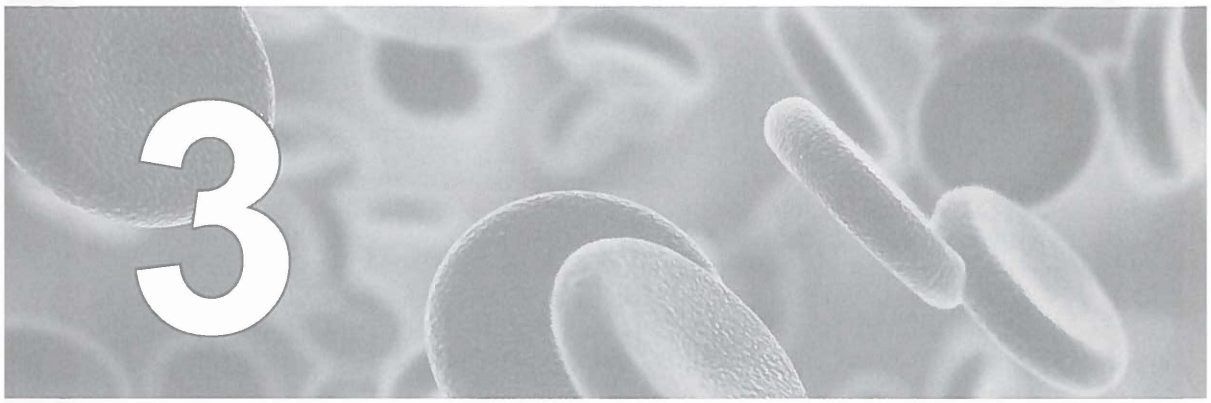

3

Hemoglobin A_{1c} point-of-care assays; a new world with a lot of consequences!

Erna Lenters-Westra
Robbert J. Slingerland

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Abstract

Background

Point-of-care instruments for the measurement of hemoglobin A_{1c} (HbA_{1c}) may improve the glycemic control of people with diabetes by providing a rapid result if the performance of the instruments used is acceptable. A 0.5% HbA_{1c} difference between successive results is considered a clinically relevant change. With this in mind, the In2it from Bio-Rad and the DCA Vantage from Siemens were evaluated according to Clinical and Laboratory Standards Institute (CLSI) protocols.

Methods

The CLSI protocols EP-5 and EP-9 were applied to investigate precision, accuracy and bias. The bias was compared with three certified secondary reference measurement procedures. Differences between capillary and venous blood was investigated by an end-user group consisting of nurse practitioners at a diabetes care center.

Results

At HbA_{1c} levels of 5.1 and 11.2%, the total coefficient of variation (CV) for the In2It was 4.9% and 3.3%, respectively, and for the DCA Vantage were 1.7 to 1.8% and 3.7 to 5.5% depending on the lot number of the cartridges. Method comparisons showed significant lot number depended results for the In2it and the DCA Vantage compared with the three reference methods. No overall difference was observed between capillary and venous blood for both methods.

Conclusion

Performance results of the In2it and the DCA Vantage showed variable and lot number dependent results. To maintain the interlaboratory CV of 5% for HbA_{1c}, the Clinical Laboratory Improvement Amendments (CLIA) rules for waived point-of-care instruments should be revised. An obligation for participating in external quality schemes and taking adequate action should be considered for POC instruments that perform poorly.

Introduction

Hemoglobin A_{1c} (HbA_{1c}), reflecting mean glycemia, is used as a risk parameter for diabetic complications and as a quality assurance indicator for the quality of diabetes care. Point-of-care (POC) instruments for HbA_{1c} are widely used in the world for the measurement of HbA_{1c}. The rapidity of obtaining a result can increase clinical effectiveness and contribute to improved outcomes for patients, but it is imperative that the result provided by the device is accurate and reliable. A faster result is only safe if it is an accurate result. POC instruments for HbA_{1c} provide relatively quick results and minimize patient inconvenience. Studies have confirmed that immediate feedback of HbA_{1c} levels improves glycemic control in type 1 and insulin-treated type 2 diabetic patients[1-3]. Information provided by the manufacturers and limited published data about the performance of POC HbA_{1c} instruments suggest that some of these instruments can compete with clinical laboratory methods[4,5].

The aim of this study was to evaluate two POC-instruments according to Clinical and Laboratory Standards Institute (CLSI) protocols under laboratory conditions and to discuss the consequences of the findings. The bias of these instruments was compared with three certified International Federation of Clinical Chemistry (IFCC) and/or National Glycohemoglobin Standardization Program (NGSP) secondary reference measurement procedures, which were calibrated with secondary reference material with assigned IFCC and derived NGSP values and with the mean of the three methods. Moreover, instruments were tested on differences obtained with capillary blood versus venous blood by the nurse practitioners at a diabetes care centre.

Methods

The evaluation consisted of an analytical part by the laboratory and an end-user evaluation by nurse practitioners at a diabetes care centre investigating user-friendliness and differences between capillary and venous blood.

The CLSI EP-10 protocol was used to get acquainted with the instruments and to get a general impression of the performances of the instruments[6]. The CLSI EP-5 protocol was used to investigate the overall precision (20 days, duplicate measurements twice a day at 2 levels)[7]. The EP-9 protocol was used to investigate the bias between the POC instruments and the three different secondary reference measurements procedures (n=40, duplicate measurements)[8]. An HbA_{1c} value determination of the samples used in the EP-10 and EP-9 protocol was done with two IFCC and NGSP certified secondary reference measurement procedures, Roche Tina-quant Gen.2 HbA_{1c} on Integra 800, immunoassay (Roche Diagnostics Ltd, Rotkreuz, Switzerland), Primus Ultra^2, affinity chromatography HPLC (Primus Diagnostics, a Trinity Biotech Company, Kansas City, MO, USA) and the certified IFCC secondary reference method Tosoh G7, cation-exchange HPLC (Tosoh Bioscience N.V./S.A., Tessenderlo, Belgium). To check overall calibration and bias,

the mean of the duplicates of the POC-instruments in the EP-9 procedure was compared to the mean of the three reference measurements procedures.

An informed consent was obtained from all patients prior to blood collection in accordance with the local ethical committee. Approximately 90% of the measurements were done by two different nurse practitioners, whereas the other 10% were done by three different nurse practitioners. The nurse practitioners were asked about user-friendliness, advantages and disadvantages of the different point-of-care analyzers.

The two POC HbA_{1c} analyzers evaluated in this study were the DCA Vantage™ (Siemens Medical Solutions Diagnostics, Tarrytown, NY, USA) which is based on inhibition of latex agglutination methodology, providing result in 6 minutes, and the In2it™ (Bio-Rad, Hercules, CA, USA) which is based on affinity separation with result available in 10 minutes.

Statistics

Computations were performed using EP Evaluator Release 8 (David G. Rhoads Associates , PA, USA)[9].

Results

Table 1 shows the precision results of the EP-5 protocol. At HbA_{1c} level of 5.1 and 11.2%, total coefficients of variation (CV) for the In2It were 4.9% and 3.3%, respectively, and for the DCA Vantage 1.7 to 1.8% and 3.7 to 5.5% depending on the lot number of the cartridges.

Table 1: EP-5 precision results from the In2it and the DCA Vantage

	In2it		DCA Vantage		DCA Vantage$	
	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2
Within-run SD	0.25	0.27	0.08	0.65	0.07	0.44
Between run SD*	#	0.10	0.04	0.10	0.04	#
Between day SD*	#	0.24	#	0.16	0.05	0.10
Total SD	0.25	0.37	0.09	0.66	0.10	0.45
Total CV	4.9	3.3	1.7	5.5	1.8	3.7

* Sample 1 and 2 are patient samples with a HbA_{1c} of 5,1% and 11,2% respectively.
\# Négligeable
$ Performed with another lotnumber

Table 2: EP-9 results of the In2it and the DCA Vantage with two different lot numbers

Deming regression lines	Lot number A	95% CI of 6% HbA_{1c}	95% CI of 9% HbA_{1c}	Lot number B	95% CI of 6% HbA_{1c}	95% CI of 9% HbA_{1c}
Primus Ultra[2] (X) vs In2it (Y)	Y=0.951X + 0.257			Y=0.965X + 0.239		
Std Err Est	0.514	5.77 - 6.15*	8.53 - 8.99*	0.255	5.95 - 6.11	8.86 - 8.99
R	0.96	(0.38)	(0.46)	0.99	(0.15)	(0.13)
Tina-quant (X) vs In2it (Y)	Y=0.928X + 0.350			Y=0.930X + 0.454		
Std Err Est	0.561	5.70 - 6.07*	8.50 - 9.02*	0.300	5.94 - 6.12	8.75 - 8.89
R	0.95	(0.37)	(0.52)	0.99	(0.18)	(0.14)
Tosoh G7 (X) vs In2it (Y)	Y=0.926X + 0.22			Y=0.980X + 0.050		
Std Err Est	0.59	5.58 - 5.94*	8.41 - 8.90*	0.308	5.83 - 6.03	8.79 - 8.94
R	0.95	(0.36)	(0.49)	0.99	(0.20)	(0.15)
Primus Ultra[2] (X) vs DCA V. (Y)	Y=0.919X + 0.576			Y=1.038X - 0.017		
Std Err Est	0.310	5.99 - 6.19	8.77 – 8.93	0.278	6.16 - 6.33	9.29 - 9.42
R	0.98	(0.20)	(0.16)	0.99	(0.17)	(0.13)
Tina-quant (X) vs DCA V. (Y)	Y=0.921 + 0.482			Y=1.003X + 0.219		
Std Err Est	0.26	5.97 - 6.05^	8.68 - 8.87^	0.249	6.16 - 6.31	9.19 - 9.31
R	0.99	(0.08)	(0.19)	0.99	(0.15)	(0.12)
Tosoh G7 (X) vs DCA V. (Y)	Y=0.975X – 0.03			Y=1.057X - 0.218		
Std Err Est	0.42	5.74 - 5.89^	8.67 - 8.81^	0.258	6.07 - 6.19^	9.16 - 9.44^
R	0.98	(0.15)	(0.14)	0.99	(0.12)	(0.28)

* Calculated by Partitioned Biases
^ Calculated by Partitioned Residuals

Table 2 gives an overview of the method comparison results achieved with the EP-9 protocol. The 95% confidence interval (CI) at medical decision points (MDP) of 6 and 9% HbA_{1c}, respectively, show that the In2it and the DCA Vantage were significantly deviant from any of the three reference methods. To check the overall calibration and bias of the POC-instruments, the mean of the duplicates of the POC-instruments were also compared with the mean of the three reference methods (Figure 1A, 1B, 2A and 2B). These figures show the predicted value (including the 95% CI) at MDP of 6 and 9% HbA_{1c} for the various POC methods.

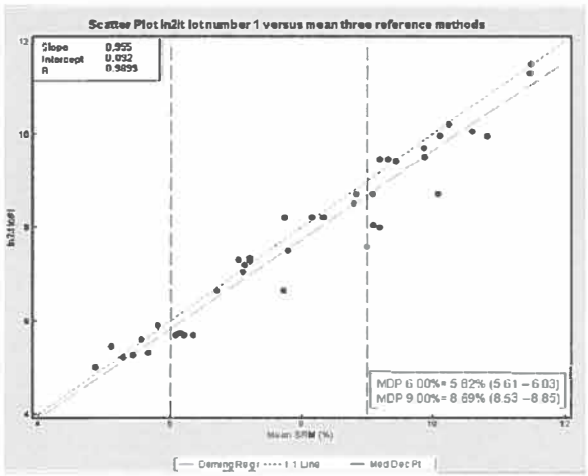

Figure 1A: *The scatter plot of the EP-9 protocol comparing the mean of the three secondary HbA_{1c} reference methods (SRM) expressed in percentage HbA_{1c} with the first lot number tested on the In2it from Bio-Rad. In this plot the upper dashed diagonal line is the line x=y, the other dashed diagonal line is the Deming regression line. The dashed vertical lines represent the medical decision points (MDP). The medical decision points were calculated with EP-evaluator.*

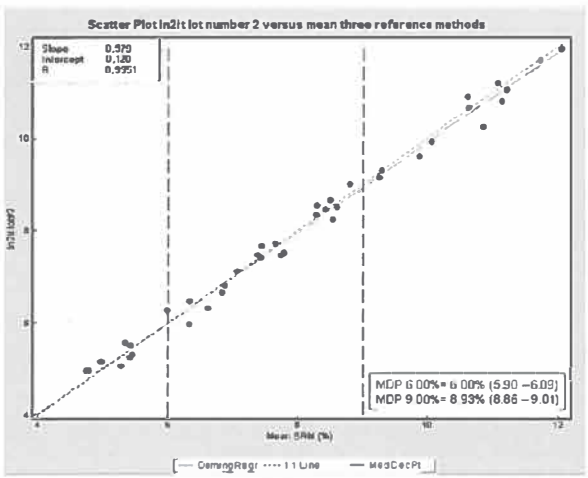

Figure 1B: *The scatter plot of the EP-9 protocol comparing the mean of the secondary HbA_{1c} reference methods (SRM) expressed in percentage HbA_{1c} with the second lot number tested on the In2it from Bio-Rad. In this plot the upper dashed diagonal line is the line x=y, the other dashed diagonal line is the Deming regression line. The dashed vertical lines represent the medical decision points (MDP). The medical decision points were calculated with EP-evaluator.*

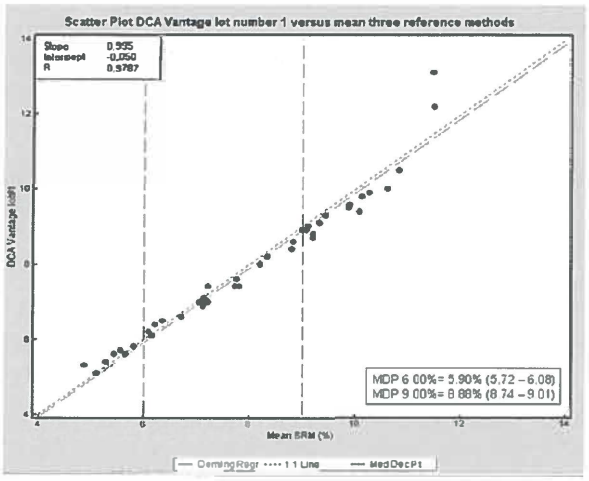

Figure 2A: *The scatter plot of the EP-9 protocol comparing the mean of the three secondary HbA_{1c} reference methods (SRM) expressed in percentage HbA_{1c} with the first lot number tested on the DCA Vantage from Siemens. In this plot the upper dashed diagonal line is the line x=y, the other dashed diagonal line is the Deming regression line. The dashed vertical lines represent the medical decision points (MDP). The medical decision points were calculated with EP-evaluator.*

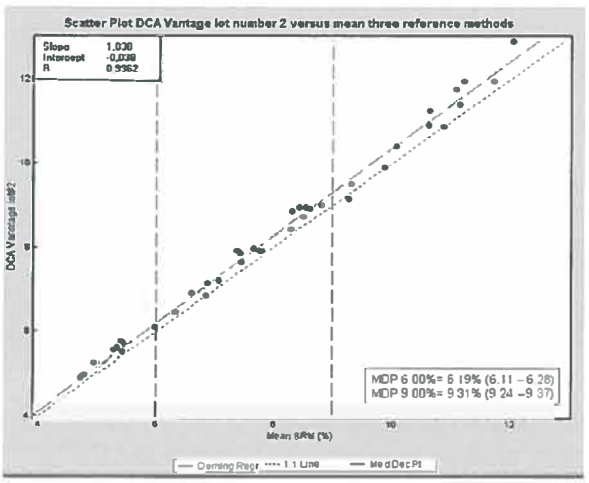

Figure 2B: *The scatter plot of the EP-9 protocol comparing the mean of the secondary HbA_{1c} reference methods (SRM) expressed in percentage HbA_{1c} with the second lot number tested on the DCA Vantage from Siemens. In this plot the lower dashed diagonal line is the line x=y, the other dashed diagonal line is the Deming regression line. The dashed vertical lines represent the medical decision points (MDP). The medical decision points were calculated with EP-evaluator.*

Results from the DCA Vantage were not within the specifications of the manufacturer. The total CV at high HbA_{1c} values was 5.5% (Table 1). Differences between duplicates seen in the EP-9 protocol with the In2it were also unusual according to the manufacturer. Seven of the 40 samples showed a difference of more than 1.1% absolute at different HbA_{1c} values (mean absolute difference between duplicates for the In2it was 0.52, DCA Vantage 0.21, Ultra[2] 0.06, Tosoh G7 0.05, Tina-quant 0.08). To rule out particular problems with the lot number used, the EP-9 protocol for both methods was repeated with another lot number. Also the EP-5 protocol was repeated for the DCA Vantage (see Table 1 and 2). Use of a second lot number diminished the mean difference in duplicates for the In2it from 0.52 to 0.27% absolute HbA_{1c} percentage and remained the same for the DCA Vantage and the reference methods.

No significant difference was found in both methods between capillary and venous blood. The MDP of 6% HbA_{1c} for the In2it was 6.10% (95% CI 5.97 to 6.23%) and for the DCA Vantage 5.93% (95% CI 5.81 to 6.06%). The MDP of 9% HbA_{1c} for the In2it was 9.11% (95% CI 8.96 to 9.27%) and for the DCA Vantage 9.07% (95% CI 8.95 to 9.19%).

Nurse practitioners considered both instruments to be user-friendly. The noise produced by the In2it the first 3 minutes and the last minute of the run time was considered as inconvenient and disturbing by one nurse practitioner.

Discussion

Point-of-care HbA_{1c} instruments are used more and more frequently. So far, the consequences of the introduction of these new types of instruments with their specific characteristics have not been discussed thoroughly in the literature. The evaluation of two types of POC instruments, the In2it and the DCA Vantage, is used here as an example to discuss several important consequences associated with the introduction of POC instruments in this field.

Results of the evaluation of the In2it and the DCA Vantage showed that there is a lot number-dependent performance of both methods. The precision of the In2it expressed in total CV and standard error of estimates in the EP-9 is still a matter of concern. The second lot number showed better results. Unfortunately, one never knows if the precision of a particular lot number is acceptable because no duplicate measurements are run in daily life with POC-instruments. The overall calibration of the second lot number for the In2it, as reflected in the overall bias, was acceptable between 6 and 9% HbA_{1c}. Results from the first lot number were influenced by bad duplicates.

The DCA 2000 was one of the first point-of-care instruments and was evaluated in several studies [10-12] Notable is that in all of these studies results from the DCA 2000 were lower compared with the methods used in the laboratory. Also, a recent evaluation of the DCA Vantage, the successor to the DCA 2000, showed a clear bias but was still considered to have acceptable imprecision and good agreement [13]. EP-9 results for the two lots of DCA reagents showed different regression lines. The results were too high (mean bias 0.27) for lot B and slightly low for lot A compared with the mean of the three reference methods and with the individual reference methods. The manufacturer may have overcompensated the calibration of the second lot number in response to results from the first lot number used in this study. From an analytical point of view, the imprecision of the first DCA Vantage lot at high HbA_{1c} levels was too high (CV was 5.5%) and was not within the specifications of the manufacturer. The second lot number gave better results (total CV was 3.7%)

Apart from point-of-care instruments, inter-laboratory variation is still a matter of concern and has stabilized at approximately 5%[14]. Holmes and colleagues concluded that the between-method variability is still a potential source of inaccuracy when HbA_{1c} results are interpreted based on fixed clinical decision thresholds [15].

This is especially the case when POC-instruments and laboratory methods are used randomly in the same facility. In order to reduce the interlaboratory (interhospital) CV, the NGSP reduced the acceptable bias for manufacturer certification to ±0.85% in 2007 and the College of American Pathologists (CAP) began using the NGSP accuracy grade as the only grading system. In addition, the acceptable total error limit of ±15% was lowered to ±12% and will be reduced further in future CAP surveys [16]. By tightening NGSP certification criteria and lowering the acceptable total error limit in the CAP survey (to ±6% by 2011), poor performing methods must improve or they will fail to be NGSP certified and some of their users will not pass CAP proficiency testing. Unfortunately, CLIA waived POC-instruments, which sustain part of the interlaboratory CV, are not obliged to join external quality schemes. The end users simply have to follow manufacturer's instructions and might therefore escape from the rules imposed on laboratory methods[17]. This is a so-called "hole in the dike". At one end, proficiency testing criteria will be tightened (laboratory methods) and at the other end there will be no rules or very limited rules for CLIA-waived point-of-care instruments.

The introduction of POC HbA_{1c} instruments in the market will diminish the number of patient samples that are analyzed on one instrument; as a consequence, the Gaussian curve describing HbA_{1c} results within a certain population is expected to get broader even if the performance of the new instruments will be the same as the HbA_{1c} methods used in the laboratory. Point-of-care instruments increase the total number of analyzers per 1000 persons with diabetes. Therefore, inter-instrument CV and the intercartridge CV are extra source of variability added to the total CV in comparison to a laboratory method. So far, the current CLSI evaluation protocols do not cover this phenomenon sufficiently.

Results achieved by the NGSP and later on by the IFCC working group for the standardisation of HbA_{1c} to decrease the interlaboratory variability from 20% in 1993 to approximately 5% in 2008 should be supported by adjusting the CLIA-waived rules for HbA_{1c} point-of-care instruments. Annual NGSP manufacturer certification should be done, and every laboratory instrument and every POC instrument should be obliged to join external quality schemes. Adequate actions (improve method or withdrawal from the market) must be administered if the performance of a laboratory and/or point-of-care instrument is not acceptable.

The manner in which quality controls are being handled may also need to be redefined. To run a quality control occasionally on POC instruments is adequate because it may tell something about the cartridge used but does not provide any guarantee for the next cartridge. However, the consequences of a bad cartridge may be less severe than a bad reagent in the laboratory (it may involve only one result on the POC instrument versus hundreds in the laboratory). Nevertheless, all POC-instruments must be equipped with an electronic check on performance. Moreover, the cartridges need to be equipped with an internal HbA_{1c} control. This might not only be true for POC HbA_{1c}, but in general also applies for other POC tests using separate cartridges. In the end, evaluations of POC instruments must be done by end-users. However, if manufacturers are capable of producing cartridges without cartridge-to-cartridge variability, the need for an internal quality control might be less

important. To achieve this goal, standards need to be tightened at the level of manufacturers.

Results presented here were obtained by the work of an experienced technician and are therefore likely the best results one can achieve analytically; EP-5 and EP-9 results obtained by less experienced end users may be less precise. Although the usage of POC HbA_{1c} instruments has some negative consequences that need to be addressed, it is also important to keep in mind that producing HbA_{1c} results at the time of the patient's visit can improve patient care as well.

Conclusion

Performance results from the In2it and the DCA Vantage showed high variability and lot dependent results. To maintain the interlaboratory CV of 5% for HbA_{1c}, the rules for CLIA-waived point-of-care instruments should be revised. An obligation for participating in external quality schemes and taking adequate action should be considered for POC instruments that perform poorly.

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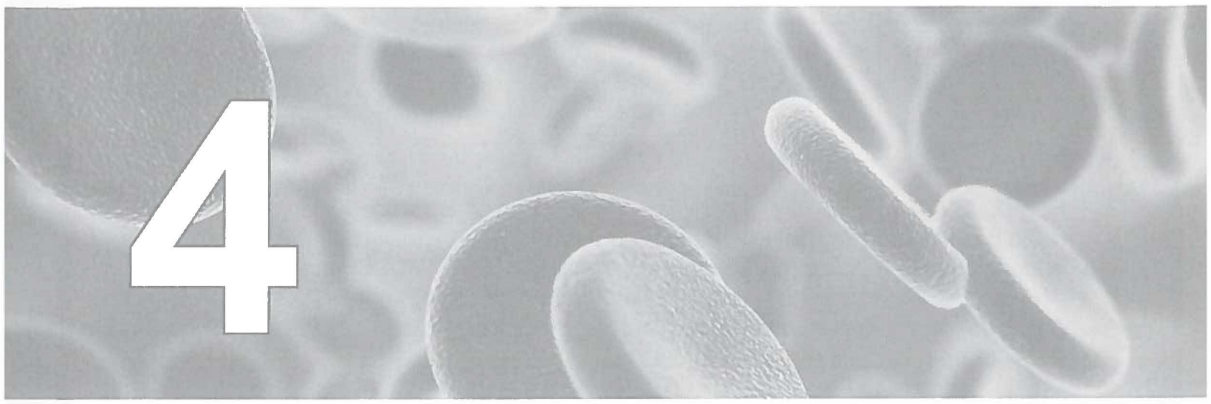

4

Six of eight Hemoglobin A_{1c} point-of-care instruments do not meet the general accepted analytical performance criteria

Erna Lenters-Westra
Robbert J. Slingerland

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Abstract

Background

Hemoglobin A_{1c} (HbA_{1c}) point-of-care (POC) instruments are widely used to provide rapid turnaround results in diabetic care centers. We investigated the conformance of various HbA_{1c} POC instruments (In2it from Bio-Rad, DCA Vantage from Siemens, Afinion and Nycocard from Axis-Shield, Clover from Infopia, InnovaStar from DiaSys, A1CNow from Bayer and Quo-Test from Quotient Diagnostics) with generally accepted performance criteria for HbA_{1c}.

Methods

The Clinical and Laboratory Standards Institute (CLSI) protocols EP-10, EP-5 and EP-9 were applied to investigate imprecision, accuracy and bias. We assessed bias using 3 certified secondary reference measurement procedures and the mean of the 3 reference methods. Assay conformance with the National Glycohemoglobin Standardization Program (NGSP) certification criteria, as calculated from analyses with 2 different reagent lot numbers for each HbA_{1c} method, was also evaluated.

Results

Because of disappointing EP-10 results, 2 of the 8 manufacturers decided not to continue the evaluation. The total CVs from EP-5 evaluations for the different instruments with a low and high HbA_{1c} value were: In2it 4.9% and 3.3%, DCA Vantage 1.8% and 3.7%, Clover 4.0% and 3.5%, InnovaStar 3.2% and 3.9%, Nycocard 4.8% and 5.2%, Afinion 2.4% and 1.8%. Only the Afinion and the DCA Vantage passed the NGSP criteria with two different reagent lot numbers.

Conclusions

Only the Afinion and the DCA Vantage met the acceptance criteria of having a total CV<3% in the clinically relevant range. The EP-9 results and the calculations of the NGSP certification showed significant differences in analytical performance between different reagent lot numbers for all HbA_{1c} POC instruments.

Introduction

Diabetes is one of the most challenging health problems of the 21st century. The International Diabetes Federation estimates that more than 250 million people around the world have diabetes[1]. Currently diagnosis and follow-up is usually done in special diabetes care centers. Many patients have their blood drawn a week before they visit the physician to ensure that laboratory results are available for appropriate clinical action. By providing results rapidly following blood collection, point-of-care (POC) instruments could minimize patient inconvenience and possibly avoid an extra visit to the clinic. Studies have confirmed that immediate feedback of hemoglobin A_{1c} (HbA_{1c}) results improves glycemic control in type 1 and insulin-treated type 2 diabetic patients[2-4].

Limited information is available regarding the analytical performance of POC instruments that measure HbA_{1c}, and whether National Glycohemoglobin Standardization Program (NGSP) certification ensures the accuracy of every instrument used in the field. The information provided by the manufacturers and the limited published data about the performance of POC HbA_{1c} instruments suggest that some of these instruments can compete with clinical laboratory methods in terms of analytical performance[5,6].

The aim of this study was to evaluate all available HbA_{1c} POC instruments according to the Clinical and Laboratory Standards Institute (CLSI) protocols and to check whether the instruments would pass the NGSP criteria with 2 different reagent lot numbers as judged by comparison with 3 certified International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and/or NGSP secondary reference measurement procedures. A manufacturer NGSP certification is performed by experienced technologists at the manufacturer's site under ideal circumstances and may not reflect the analytical performance of the instruments in the field.

Materials and Methods

The 8 POC HbA_{1c} analyzers evaluated in this study were:

- The DCA Vantage™ (Siemens Medical Solutions Diagnostics, Tarrytown, NY), which is based on latex agglutination inhibition immunoassay methodology and provides results in 6 min. This is the successor of the DCA 2000™.
- The In2it™ (Bio-Rad, Hercules, CA), which is based on affinity separation, with results available in 10 min.
- The Afinion™ (Axis-Shield, Oslo, Norway), which is based on affinity separation, with results available in 5 min.
- The Nycocard (Axis-Shield, Oslo, Norway), which is based on affinity separation, with results available in 3 min.
- The Clover (Infopia, Kyunggi, Korea), which is based on affinity separation, with results available in 5 min.

- The InnovaStar (DiaSys, Holzheim, Germany), which is based on agglutination immunoassay and provides results in 11 min. At the time of this study the InnovaStar was not yet launched on the market and the manufacturer considered the outcome of this evaluation as a starting point to further improve the method.
- The A1cNow^{+} (Bayer Health Care, Sunnyvale, CA), which is an immuno-assay, with results available in 5 min.
- Quo-Test^{TM} (Quotient Diagnostics, Surrey, UK), which is based on affinity separation and the use of fluorescence quenching, with results available in 3 min.

Apart from the InnovaStar all methods were NGSP certified as of May 2009[7].

We used the CLSI EP-10 protocol to become familiar with the instruments and to get an overall impression of performance[8]. The results were sent to the manufacturers for their approval to continue with the evaluation. After we obtained manufacturer's approval, we used the CLSI EP-5 protocol to further investigate assay imprecision (duplicate measurements twice per day on 2 samples for 20 days)[9]. In contrast to the other instruments, the Afinion and the Nycocard do not work with hemolyzed material. Therefore, for this purpose with those 2 instruments we used the 2 controls supplied by the manufacturer.

The CLSI EP-9 protocol was performed twice with 2 different reagent lot numbers, and was used to investigate the bias between the POC instruments and the 3 different secondary reference measurements procedures (n=40, 5 days, duplicate measurements)[10]. HbA_{1c} value determination of the samples was performed with 3 certified secondary reference measurement procedures:

- Roche Tina-quant Gen.2 HbA_{1c} on Integra 800, immunoassay, IFCC and NGSP certified (Roche Diagnostics).
- Primus Ultra^2, affinity chromatography HPLC, IFCC and NGSP certified (Primus Diagnostics, a Trinity Biotech Company)
- Tosoh G7, cation-exchange HPLC, IFCC certified (Tosoh Bioscience N.V./S.A.).

The secondary reference measurement procedures have documented good results in the IFCC and NGSP monitoring program and were calibrated by using the IFCC secondary reference material with assigned IFCC and derived NGSP values[11-13]. To check overall calibration and bias independently of the chosen secondary reference method, the results of the POC instruments in the EP-9 procedure were compared to the mean of the 3 reference measurements procedures. The overall differences in slope and intercept of the regression lines with respect to the 2 reagent lot numbers used were tested by Chow statistics in SPSS version 16.0 with a univariate general linear model that incorporated an interaction-term (lot number * method). A *P*-value of the interaction-term of <0.05 was considered as statistically significant[14].

The results of the EP-9 protocol were also used to calculate the NGSP certification criteria with 2 reagent lot numbers and 3 different reference measurement procedures. The 95% CI of the differences between methods (test method and reference method) should fall within ± 0.85% HbA_{1c} to pass the NGSP criteria. We used the formula: Total Error = bias ± 1.96 x SD of differences[15].

Statistics

We performed computations using Microsoft® Excel 2002 (Microsoft Corporation) software. Statistical analyses were also performed with the software package Analyse-It® (Analyse-It Software), EP Evaluator Release 8 (David G. Rhoads Associates)[16] and SPSS version 16.0 (SPSS).

Results

Two out of the 8 manufacturers (local distributor of the A1CNow instrument, and Quotient Diagnostics of the Quo-Test instrument) concluded that the EP-10 outcome data did not warrant progression to the EP-5 and EP-9 protocols and decided to discontinue the study (data not shown). At the time of this study, the Quo-Test was a prelaunch instrument and was still in development. The bias found with the EP-10 protocol of the A1CNow was probably due to EDTA interference problems. Normally HbA_{1c} POC instruments are used to measure HbA_{1c} directly in capillary blood. Both methods were NGSP certified.

The results of the EP-5 protocol are shown in Table 1. Imprecision ranged from 1.4% CV at an HbA_{1c} value of 6.3% for the Afinion to 5.3% CV at an HbA_{1c} value of 6.1% for the Nycocard.

The results of the EP-9 protocol are shown in Table 2, along with the calculations of the NGSP certification criteria and associated P-values. The different POC instruments were compared to the 3 reference measurement procedures with 2 different reagent lot numbers. None of the instruments passed the NGSP criteria with 2 lot numbers compared with 3 reference methods. Only the DCA Vantage and the Afinion passed the current NGSP criteria with 2 different lot numbers when compared with just 1 reference method that had the same measurement principle. Based on the Chow-statistics testing for differences in regression lines with respect to the lot numbers used, all regression lines except In2it vs Tina-quant were statistically significantly different (Table 2).

The graphs of the comparisons between the different POC instruments with 2 reagent lot numbers and the mean of the 3 reference measurement procedures are shown in Fig.1. In addition to the Chow-statistics, which demonstrated between-lot differences in the regression lines, the differences in mean bias between the lot numbers of all instruments seen in Fig. 1 reflected lot number instability, and were largest for the Clover (Clover 0.82, DCA Vantage 0.36, Nycocard 0.29, In2it 0.23, Afinion 0.18, InnovaStar 0.15).

Table 1. EP-5 total CV imprecision results from the different POC-instruments. (In brackets the HbA_{1c} value of the sample/control)

	In2it	DCA Vantage	Clover	InnovaStar	Nvcocard	Afinion
Patient sample 1	4.9% (5.1%)	1.8% (5.1%)	4.0% (5.0%)	3.2% (5.2%)	4.8% (4.8%)	2.4% (4.7%)
Patient sample 2	3.3% (11.2%)	3.7% (11.2%)	3.5% (11.9%)	3.9% (11.5%)		
Nycocard normal control					5.3% (6.1%)	
Nycocard abnormal control					5.2% (11.6%)	
Afinion control CI						1.4% (6.3%)
Afinion control CII						1.8% (8.2%)

Table 2: EP-9 results, calculations of NGSP certification criteria and P-values calculated with Chow-statistics to test for the overall differences in slope and intercept per method for reagent lot number 1 and 2.

Linear regression lines	Lot number 1	Bias	SD of dif	Total Error	NGSP criteria	Lot number 2	Bias	SD of diff	Total Error	NGSP criteria	P-value
In2it (Y)											
vs Ultra^2 (X)	Y=0.95X + 0.26	-0.071	0.414	-0.88	Fail	Y=0.96X + 0.24	-0.040	0.265	-0.60	Pass	<0.001
vs Tina-quant (X)	Y=0.93X + 0.36	-0.160	0.454	-1.05	Fail	Y=0.93X + 0.48	-0.112	0.338	-0.77	Pass	0.061
vs Tosoh G7 (X)	Y=0.93X + 0.22	-0.300	0.460	-1.20	Fail	Y=0.98X + 0.06	0.113	0.310	-0.72	Pass	<0.001
DCA V. (Y)											
vs Ultra^2 (X)	Y=0.92X + 0.59	-0.056	0.343	-0.73	Pass	Y=1.04X + 0.03	0.316	0.286	0.88	Fail	<0.001
vs Tina-quant (X)	Y=0.92X + 0.50	-0.141	0.298	-0.73	Pass	Y=1.00X + 0.24	0.244	0.248	0.73	Pass	<0.001
vs Tosoh G7 (X)	Y=0.97X – 0.01	-0.310	0.290	-0.88	Fail	Y=1.06X – 0.21	0.244	0.282	0.80	Pass	<0.001
Afinion (Y)											
vs Ultra^2 (X)	Y=0.88X + 0.66	-0.230	0.318	-0.85	Pass	Y=1.00X – 0.14	-0.122	0.213	-0.54	Pass	<0.001
vs Tina-quant (X)	Y=0.83X + 0.94	-0.427	0.473	-1.35	Fail	Y=0.96X + 0.11	-0.176	0.258	-0.52	Pass	<0.001
vs Tosoh G7 (X)	Y=0.87X + 0.63	-0.390	0.410	-1.19	Fail	Y=0.98X – 0.08	-0.224	0.284	-0.78	Pass	<0.001
Nycocard (Y)											
vs Ultra^2 (X)	Y=0.94X + 0.89	0.405	0.406	1.20	Fail	Y=0.94X + 0.56	0.057	0.335	0.71	Pass	<0.001
vs Tina-quant (X)	Y=0.88X + 1.18	0.212	0.505	1.20	Fail	Y=0.90X + 0.81	0.003	0.403	-0.79	Pass	<0.001
vs Tosoh G7 (X)	Y=0.93X + 0.83	0.240	0.440	1.10	Fail	Y=0.92X + 0.62	-0.050	0.380	-0.79	Pass	<0.001
Clover (Y)											
vs Ultra^2 (X)	Y=0.96X – 0.45	-0.792	0.251	-1.28	Fail	Y=0.98X + 0.12	-0.037	0.299	-0.62	Pass	<0.001
vs Tina-quant (X)	Y=0.90X – 0.18	-0.985	0.345	-1.66	Fail	Y=0.94X + 0.38	-0.090	0.371	-0.82	Pass	<0.001
vs Tosoh G7 (X)	Y=0.94X – 0.51	-0.950	0.310	-1.56	Fail	Y=0.96X + 0.20	-0.140	0.370	-0.86	Fail	<0.001
InnovaStar (Y)											
vs Ultra^2 (X)	Y=0.89X + 0.57	-0.277	0.399	-1.06	Fail	Y=0.99X – 0.09	-0.158	0.374	-0.89	Fail	<0.001
vs Tina-quant (X)	Y=0.84X + 0.82	-0.470	0.490	-1.43	Fail	Y=0.96X + 0.13	-0.231	0.356	-0.93	Fail	<0.001
vs Tosoh G7 (X)	Y=0.89X + 0.46	-0.437	0.372	-1.17	Fail	Y=0.98X – 0.06	-0.261	0.358	-0.96	Fail	<0.001

Shaded row means same measurement principle as investigated POC method

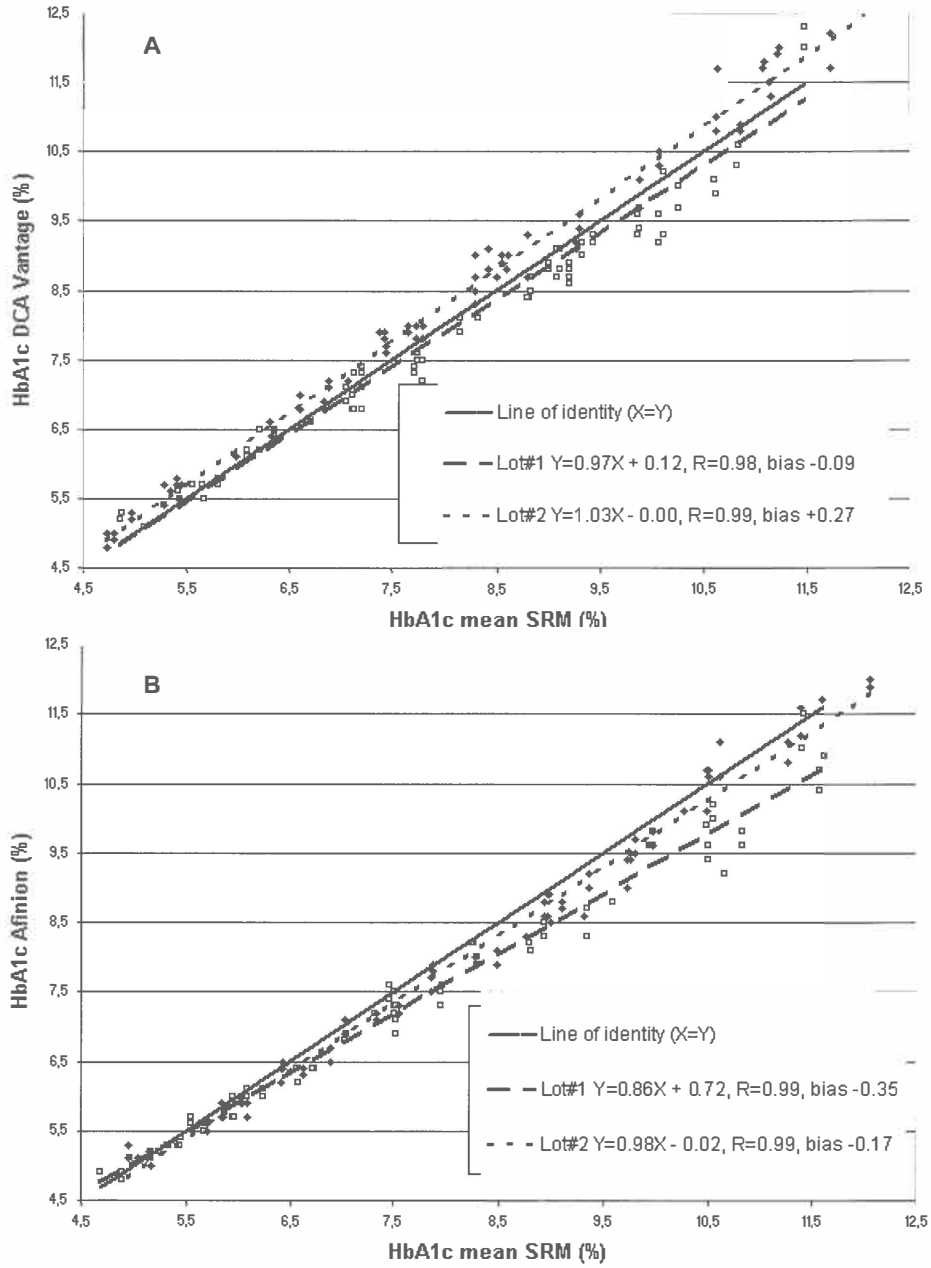
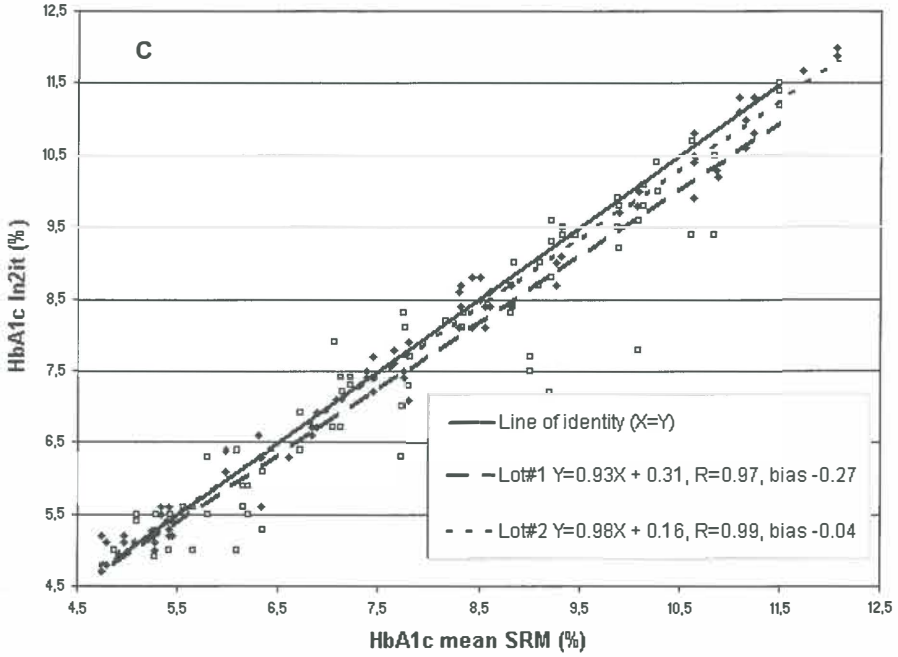


Figure 1. *HbA_{1c} results for two different lot numbers from (A) the DCA Vantage, (B) Afinion, (C) In2it, (D) Clover, (E) Nycocard, and (F) InnovaStar point-of-care instruments compared to the mean HbA_{1c} results from three secondary reference measurement procedures. The P-values of the regression lines between the two lot numbers of all POC instruments were <0.001, which confirmed the statistically significant differences between the regression lines.*



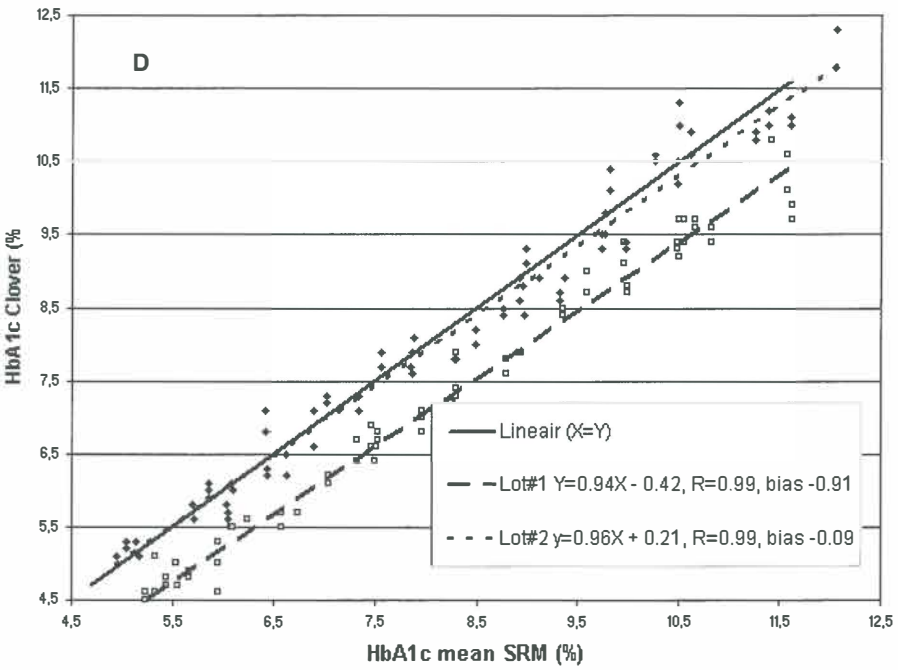
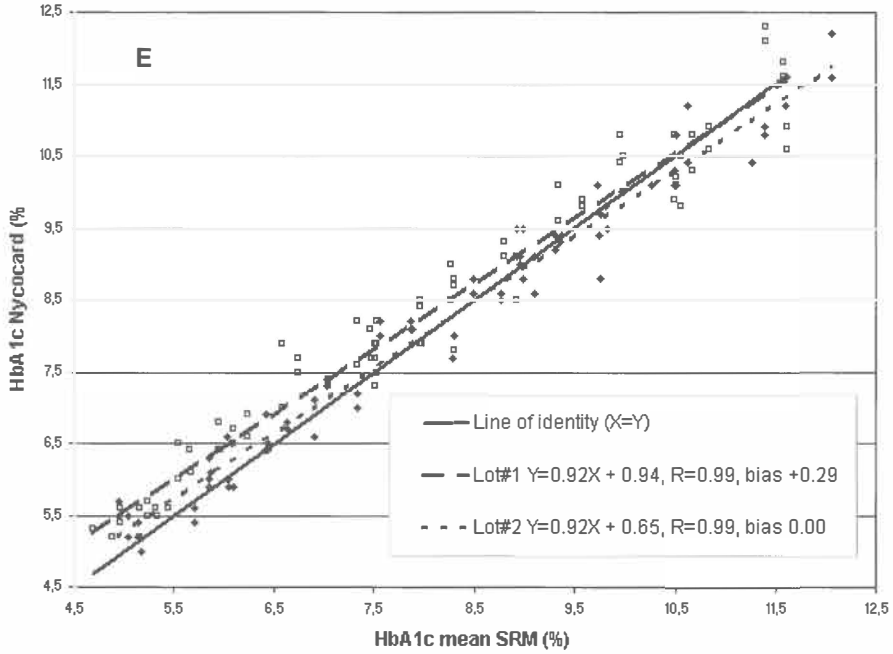


Figure 1. Continued



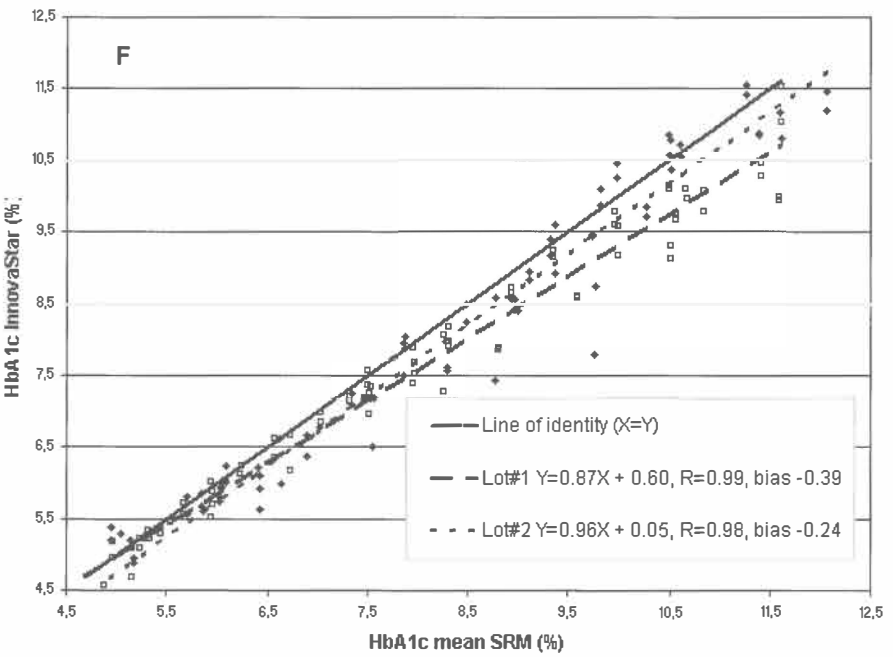


Figure 1. Continued

Discussion

There is demonstrated benefit in using POC instruments for the measurement of HbA_{1c} in certain clinical situations[2-4], but recently concerns have been raised about the performance of NGSP-certified POC instruments compared with laboratory-based methods[17]. The overall imprecision as determined by means of an EP-5 protocol is very important for interpretation of HbA_{1c} results (variability in the patient vs analytical variability). The Diabetes Complication Control Trial (DCCT) found that 10% reduction in HbA_{1c} levels resulted in a 43-45% lowering of risk of retinopathy[18]. For optimal clinical monitoring and for effective differentiation of an HbA_{1c} of 7.0% from that of 7.6% an imprecision of less than 2% CV is required, assuming an intraindividual biological variation of 2%[19,20]. This criterion is very strict, however, and difficult to meet, even for certain laboratory-based methods (immunoassays). It would therefore seem inappropriate to impose this goal on POCT devices measuring HbA_{1c}. Currently, an imprecision of <3% CV is a more realistic, though not optimal goal[21]. Only the Afinion and the DCA Vantage were able to meet this criterion in the clinically relevant range (Table 1). The acceptable CVs of these 2 methods make them potentially equivalent to laboratory-based methods, if the problem of lot number instability is resolved and assured.

All of the instruments showed statistically significantly different regression lines for the different lot numbers compared to the mean of the 3 reference methods (Fig. 1). The calibration of the In2it is adequate but the variability of the instrument reflected by a high total CV in the EP-5 protocol, and a high standard error of estimates with the first lot number is still a matter of concern. The second lot number gave better results. Unfortunately, it is impossible to predict whether the precision of a particular reagent lot number is acceptable because no duplicate measurements are run routinely with POC instruments.

The first reagent lot number of the DCA Vantage showed slightly lower results in the clinically relevant range with a low variability (1.8%CV) and higher results in the high range with higher variability (3.7% CV). A recent evaluation of the DCA Vantage also showed lower results compared with the laboratory method; therefore adjustment of the calibration by the manufacturer was justified[22]. However, Fig. 1A shows that the manufacturer of the DCA Vantage may have overcompensated in adjusting the calibration of the second lot number.

The Afinion also demonstrated a calibration problem. The results were consistently lower than the results from the reference methods, independent of the lot number used.

The Nycocard system showed the worst imprecision of all the systems (Table 1) raising questions regarding its suitability for clinical use. The manual nature of this test may possibly explain the poor precision. The CVs presented here were obtained by the work of an experienced technologist and would likely be worse if the method were used by many different inexperienced personnel.

However, the Nycocard passed the NGSP criteria with the second lot number compared with 3 reference methods. The bias of the second lot number was very small, which allowed a higher SD of differences.

The lot number dependency of the Clover was unacceptable (Fig. 1D) and the total imprecision was also too high for optimal clinical use. Because of the poor results seen with the first lot number, the software version of the instrument was successfully updated. All results of the controls were within the limits provided by the manufacturer, whereas the patient results of the first lot number proved to be too low. As a possible way to address such problems, manufacturers should be encouraged to narrow the range of acceptable values for provided QC materials sufficiently to enable users to meet the requirements for good clinical test results.

The InnovaStar method was still under development at the time of this study. The manufacturer regarded the outcome of this study as a starting point to further improve the method. In general lower results were obtained compared with the reference methods.

The measurement principle used with 5 of the 8 methods was affinity separation. This measurement principle is well accepted as being free of interference from hemoglobin variants, a very important attribute for use in areas of the world with a high prevalence of hemoglobinopathies. Healthcare professionals must be aware of potential interferences of rare hemoglobin variants, especially when they use immunoassay-based POC instruments[23, 24].

The NGSP uses 1 comparative secondary reference method for certification, which is usually the same method type. The NGSP also states that manufacturer certification is performed only once per year with 1 lot of reagent and it is up to the manufacturer to ensure consistency among different lots[7,15]. Passing or failing outcomes for NGSP certification of the tested POC methods are clearly dependent on lot number and reference method (Table 2). The NGSP criterion (which specifies that the 95% CI of the differences between methods should fall within ± 0.85% HbA_{1c}) will be tightened to ± 0.75% HbA_{1c} by January 2010[25]. When this criterion is taking into account only 9 of the 36 comparisons would pass the NGSP criteria and only the DCA Vantage would pass it with 2 different lot numbers compared with just 1 reference method.

The reproducibility of the production of the different reagent lots of the POC instruments investigated appears inadequate at this moment for optimal clinical use of the test results. A manufacturer NGSP certification does not guarantee accuracy of a result produced in the field. We often observed significant differences between lots of reagents in this study. The Nycocard instrument data demonstrated that it is possible to pass the NGSP criteria while the total CV is >5%. Adjustments or additions to the criteria might be considered by the NGSP. For example, we believe the SD of differences should not exceed 0.30% HbA_{1c}. However, a manufacturer NGSP certification is still necessary and is an important tool to prove the optimal analytical performance of a method. In addition users of POC instruments should be required to run daily controls with tight ranges and, as with any HbA_{1c} method, users should participate in external proficiency-testing schemes.

It is important that the limitations of current POC instruments and laboratory methods be understood by healthcare professionals, because these limitations may have important clinical implications. Clinical chemists can play a valuable role by providing healthcare professionals with the information they need (measurement uncertainty) to properly interpret laboratory and POC HbA_{1c} results.

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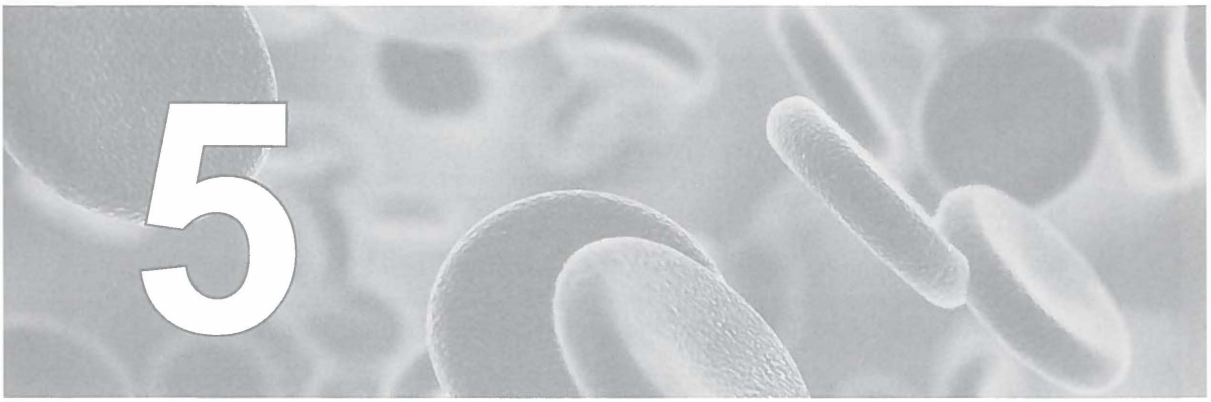

Evaluation of the Quo-Test Hemoglobin A_{1c} point-of-care instrument: second chance

Erna Lenters-Westra
Robbert J. Slingerland

Clinical Chemistry 2010;56:1191-1193

We previously reported the evaluation of 8 different Hemoglobin A_{1c} (HbA_{1c}) point-of-care instruments[1]. Two of 8 manufacturers withdrew from that study after initial unpromising results. One of the 2 instruments withdrawn was the Quo-Test A1c (Quotient Diagnostics), which was withdrawn because of a technical problem. The manufacturer claimed to have resolved the problem and asked us to re-evaluate the instrument.

The Quo-Test method is based on affinity separation and the use of fluorescence quenching and gives results in 3 min. The instrument was certified by the National Gycohemoglobin Standardization Program (NGSP) as of September 2009[2].

We used the same approach for evaluation as in the initial study, following the CLSI EP-5 protocol for imprecision and the CLSI EP-9 protocol for method comparison. Because the American Diabetes Association (ADA) has recommended HbA_{1c} as the preferred test for the diagnosis of diabetes[3], we added an additional sample of approximately 6.5% HbA_{1c} in the EP-5 protocol. The EP-9 protocol was performed twice with 2 different lot numbers and compared with 3 IFCC and NGSP secondary reference measurement procedures (SRM):

- Roche Tina-quant Gen.2 HbA_{1c} on an Integra 800, immunoassay, IFCC and NGSP SRM (Roche Diagnostics)
- Primus Ultra^2, affinity chromatography HPLC, IFCC and NGSP SRM (Primus Diagnostics, a Trinity Biotech Company)
- Tosoh G8, cation-exchange HPLC, IFCC SRM (Tosoh Bioscience N.V./S.A.)

To check overall calibration and bias, we compared the EP-9 protocol results to the mean of the 3 SRM results and also used the EP-9 protocol results to calculate the NGSP certification criterion with 2 reagent lot numbers.

In monitoring therapy, the reproducibility of HbA_{1c} assays is critical. The total CV should be at least <3% (realistic goal) and for optimal clinical use <2% (desirable goal)[1]. The total CVs in the EP-5 protocol for the Quo-Test at HbA_{1c} values of 5.0%, 6.2% and 10.2% were 5.9%, 4.5%, and 2.9%, respectively.

Comparisons between the Quo-Test with 2 reagent lot numbers and the mean of the 3 SRM are shown in Fig.1 with the individual EP-9 results and the NGSP certification calculations. The 95% CI of the differences between the SRM and test method should fall within ±0.75% HbA_{1c} (total error) to pass the current NGSP criteria[4]. The Quo-Test NGSP certification was granted in September 2009[2] before the tightening of the NGSP criteria from ±0.85% HbA_{1c} to 0.75% HbA_{1c}. To evaluate this method in the same way as the other methods in our previous study[1], we used the old criteria. The calibration of the first lot number appeared adequate, but with the EP-5 protocol we observed high variability reflected by a high total CV, and a high SE of estimates was still a matter of concern. The discrepancy with the second lot number may have been attributable to problems associated with up scaling of the production of cartridges.

The Quo-Test just passed the NGSP criteria compared with 1 SRM procedure (Tosoh G8) with 1 lot number but failed the NGSP criteria for all the other comparisons (Fig. 1). Tests performed by using Chow-statistics for the overall differences in slope and intercept per method for lot number 1 and 2 showed significant differences in analytical performance between the 2 lot numbers (P<0.001).

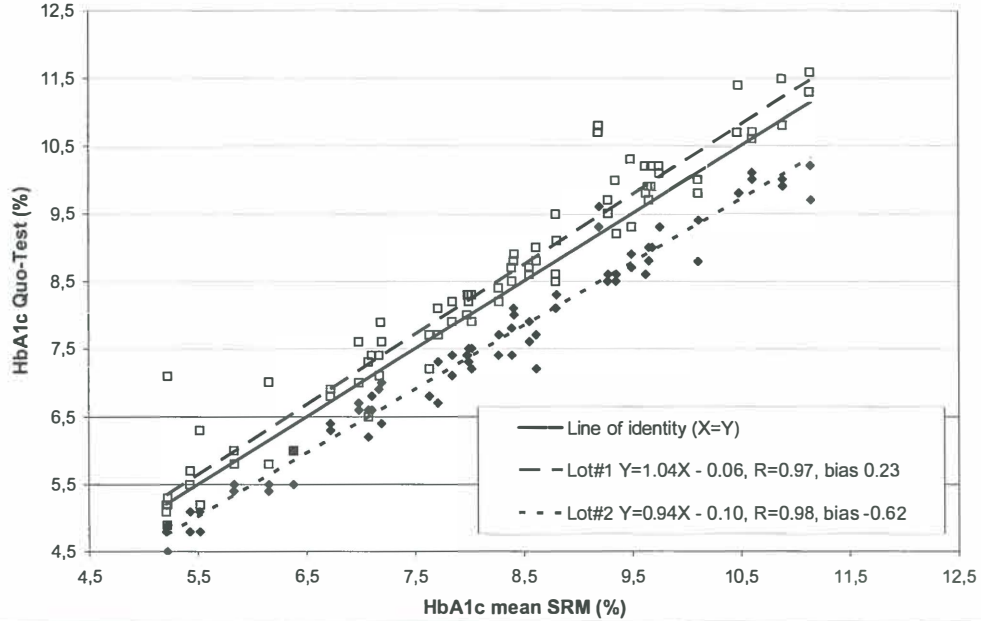

Figure 1: *HbA_{1c} results for 2 different lot numbers from the Quo-Test point-of-care instrument compared to the mean HbA_{1c} results from 3 secondary reference measurement (SRM) procedures (individual EP-9 regression lines and NGSP certification criteria shown below the graph). The P-value of the regression lines between the two lot numbers was <0.001, which confirmed the statistically significant difference between the two regression lines.*

Shaded row has the same measurement principle

Linear regression lines		Lot number 1	Bias	SD of diff	Total Error	NGSP criteria	Lot number 2	Bias	SD of diff	Total Error	NGSP criteria	P-value
Quo-Test (Y)	vs Ultra[2] (X)	Y=1.02X + 0.02	0.192	0.443	1.06	Fail	Y=0.92X - 0.04	-0.660	0.319	-1.28	Fail	<0.001
	vs Tina-quant (X)	Y=1.08X - 0.31	0.330	0.437	1.19	Fail	Y=0.96X - 0.15	-0.506	0.329	-1.15	Fail	<0.001
	vs Tosoh G8 (X)	Y=1.04X - 0.22	0.104	0.375	0.84	Pass	Y=0.91X - 0.02	-0.725	0.323	-1.36	Fail	<0.001

The manufacturer provided 2 controls with wide ranges: low control 4.2% to 7.5% and high control: 10.5% to 15.3%. The manufacturer should narrow these ranges as was described recently[1].

Results of analysis of the analytical performance of the Quo-Test showed a high total CV, large bias with 1 lot number, failed NGSP criteria, and significant differences between lot numbers. The Quo-Test is officially NGSP certified and passed the NGSP criteria with only 1 lot number as tested at the manufacturer's site[2]. The results we report here demonstrate the large lot-to-lot variability in quality of the Quo-Test HbA_{1c} point-of-care test.

Health care professionals should be aware of the clinical implications for an HbA_{1c} value that is determined by using a point-of-care instrument[5]. Moreover, to properly interpret the result, health care professionals must know the analytical performance of the HbA_{1c} method used. This study and the previous study[1] prove that an NGSP certification does not guarantee the quality of results produced in the field and confirms the recommendation of the American Diabetes Association not to use HbA_{1c} point-of-care assays for diagnostic purposes at this time[3]. Validation of a new method is always necessary and cannot be expected to be carried out by health care professionals. For this reason we think that point-of-care devices should be guided by and fall under the responsibility of a central laboratory.

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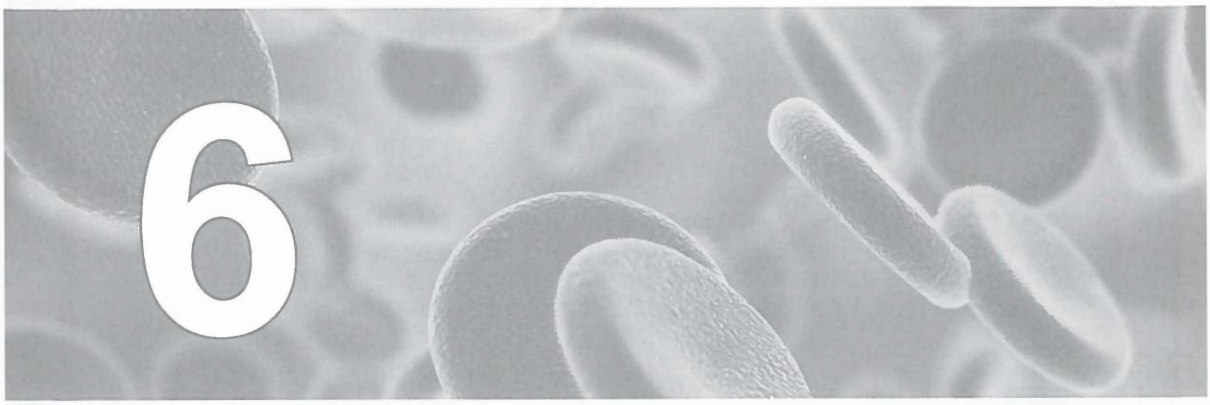

6

Point-of-care assays for Hemoglobin A_{1c}: convenient, but is performance adequate?

Randie R. Little
Erna Lenters-Westra
Curt L. Rohlfing
Robbert J. Slingerland

Accepted as letter to the editor in clinical chemistry

Abstract

Background

Hemoglobin A_{1c} (HbA_{1c}) is an essential component of routine diabetes care. There is some evidence that having the HbA_{1c} result at the time of the patient visit is beneficial and several point-of-care (POC) HbA_{1c} methods are now available. Lenters-Westra, et al previously reported less than desirable results for some POC HbA_{1c} methods. The present study re-examines three of the previously studied methods.

Methods

Two different lots of A1cNow, Afinion, and In2it reagents were evaluated in either one or two different laboratories. For each method and lot, imprecision was evaluated following CLSI EP-5. Each lot was also compared to a National Glycohemoglobin Standardization Program (NGSP) network laboratory following the NGSP certification protocol. Differences in results among reagent lots were evaluated using an overall test of coincidence of least squares regression lines and a likelihood ratio test.

Results

The total CVs for the Afinion and In2it were ≤3%. The A1cNow CVs were between 3.4 and 5.1%. The 95% CI of the differences compared to NGSP were outside acceptance limits for 2 of 4 lots of A1cNow reagents and one lot of Afinion reagents. Both In2it lots passed certification. There were differences among reagent lots for all of the methods evaluated.

Conclusions

The Afinion and In2it met the precision goal of ≤3%; the A1cNow did not. There were difference among reagents lots of A1cNow, Afinion and In2it and not all lots passed NGSP certification. Performance of some POC methods may not be sufficient to meet clinical needs.

Introduction

The routine determination of hemoglobin A_{1c} (HbA_{1c}) has become an essential component of the standard of care for patients with diabetes and is recommended by major clinical diabetes organization including the American Diabetes Association[1]. There is a small amount of evidence showing that having the HbA_{1c} result at the time of the doctor's visit is beneficial[2-4]. HbA_{1c} results are now available at the time of the visit with several point-of-care (POC) analyzers for HbA_{1c}. Recently there has been much discussion about whether or not the quality of POC testing for HbA_{1c} is sufficient to meet clinical needs.

Lenters-Westra and Slingerland recently evaluated eight POC methods; the Siemens DCA Vantage, Bayer A1cNow, Axis-Shield Afinion and NycoCard, Infopia Clover, DiaSys InnovaStar, Bio-Rad In2it and Quotient Diagnostics Quo-Test[5,6]. All but one of the methods tested were NGSP certified at the time of the study. Imprecision and bias were evaluated for all methods according to CLSI EP-10. Six of the eight POC methods were further evaluated using CLSI EP-5 and EP-9. Total CVs ranged from 1.4% to 5.3% for the six different methods at an HbA_{1c} level of approximately 6%. Only two methods (Afinion and DCA Vantage) had total CVs <3%. Two different lot numbers for each of the six methods were compared with NGSP Secondary Reference Methods; only two of the six methods (Afinion and DCA Vantage) passed NGSP certification with both reagent lots. In addition, there were statistically significant differences between the two lots for all methods.

NGSP certification evaluates methods at the manufacturer level using only one lot of reagents at any point in time[6]. Although CAP proficiency testing provides an excellent snapshot of the performance of each method in the clinical laboratory, POC methods are CLIA waived and thus users are not required to participate in proficiency testing. There are a few POC methods that appear on the CAP survey but only one appears with a large number of users. Therefore, inadequate performance of some of these methods in the hands of experienced users[5] raises concerns about the ability of these methods to perform well enough for diabetes monitoring, especially in the hands of less experienced users. One of the methods that was previously evaluated (Clover) showed differences of almost 1% HbA_{1c} between two lots at 7% HbA_{1c}. The Quo-Test had technical problems in the first study and was reevaluated after the manufacturer had claimed to resolve the problems. In the second study, EP5 and EP9 evaluations demonstrated high CVs and large lot-to-lot variability[7]. The manufacturer of the A1cNow did not agree with the conclusions in the first study noting that EDTA blood was used which is not in accordance with manufacturer recommendations. Manufacturers of Afinion and In2it have claimed that improvements were made to these methods since the original evaluation. The present study therefore re-examines the Afinion, A1cNow (using heparinized blood), and In2it in either one or two different NGSP laboratories.

Methods

Two different lots of A1cNow and Afinion reagents were shipped to each laboratory (total of 4 different reagents lots tested for each method) and two lots of the In2it reagents were shipped to one laboratory. For each method, each lot was evaluated for imprecision following CLSI EP-5 guidelines and using fresh or frozen whole blood and/or manufacturer quality control material in one or both of the laboratories. Precision of the A1cNow was evaluated in both laboratories; precision of the Afinion and In2it were each evaluated in one laboratory. Precision evaluation was performed using both whole blood (WB) and lyophilized manufacturer control material for the Afinion since the WB could not be frozen for this method and the fresh non-diabetic WB sample was only stable for 11 days at 4°C (EP5 recommends 20 days). All evaluations for the A1cNow were performed using heparinized WB since EDTA interferes with the A1cNow method[8]; EDTA WB was used for both the Afinion and In2it evaluations. Each lot in each laboratory was compared to an NGSP SRL method as would be done for NGSP method certification[6] using Bland Altman assessment of agreement with current NGSP manufacturer certification limits[6,9]. For the A1cNow and Afinion methods, differences in results among reagent lots between and within laboratories were evaluated for statistical significance using a likelihood ratio test. For the In2it method two reagent lots were evaluated in a single laboratory, therefore an overall test of coincidence of least squared regression lines was used to test for a statistical difference between the lots. For all tests $P<0.05$ was considered to indicate statistical significance.

Results

The imprecision data are shown in table 1. Total CVs were between 3.4 and 5.1% for the A1cNow while CVs were lower for the Afinion (between 1.2 and 2.7%), and for the In2it (between 2.4 and 3.0%). For the Afinion, imprecision for the QC material was slightly better than for the WB; the WB estimate may better reflect variability of patient WB results for the Afinion.

The 95% CI of the differences between the methods and the NGSP SRLs are also shown in Table 1. The A1cNow was compared to another immunoassay in Lab B (ESRL9) and to an ion-exchange HPLC method in Lab A (SRL7); both of these SRLs are routinely used for manufacturer certification of immunoassay methods. In Lab A both lots passed the NGSP certification criteria, while in Lab B both lots failed. For the Afinion, each lot in each laboratory was compared to the same boronate affinity HPLC method (SRL3 and ESRL8). In lab A one lot passed and one lot failed; in lab B, both lots passed. The In2it was compared to a boronate affinity HPLC method in Lab B (ESRL8); both lots passed NGSP certification.

Comparing the two lots of A1cNow reagent in each lab, there was no significant difference between pairs of lots. However, the two lots in Lab A were statistically significantly different from the two lots in Lab B. For the Afinion there were statistically significant differences in lots both within and between the two laboratories. For the In2it, there was a very small but statistically significant difference between the two lots of reagent in a single laboratory.

Table 1: Accuracy and Precision of 3 POC methods

		Total Imprecision %CV)		Bland-Altman 95% CI of differences (uper, lower)*	
A1CNow	**Sample**	**Lot#1**	**Lot#2**	**Lot#1**	**Lot#2**
Lab A (SRL7)	WB1 WB2	3.72 3.43	3.80 3.92	-0.67, 0.63	-0.63, 0.35
		Lot#3	**Lot#4**	**Lot#3**	**Lot#4**
Lab B (ESRL8)	WB1 WB2		4.1 5.1	**-0.97**, 0.23	**-1.07**, 0.36
Afinion		**Lot#1**	**Lot#2**	**Lot#1**	**Lot#2**
Lab A (SRL7)	WB1 WB2 QC1 QC2	2.70^ 1.94^ 1.44 1.15	2.06^ 2.37^ 1.39 1.34	-0.04, **0.87**	-0.08, 0.65
Lab B (ESRL8)				-0.24, 0.68	-0.30, 051
In2it		**Lot#1**	**Lot#2**	**Lot#1**	**Lot#2**
Lab B (ESRL9)	WB1 WB2		2.40 3.00	-0.59, 0.37	-0.63, 0.16

* Data are %HbA_{1c}; bold type indicates a failed result
^ Data collected over <20 days

Discussion

An analytical goal for precision of HbA_{1c} methods is <2%[10]. Many available laboratory methods are capable of within-laboratory CVs <2%, but not all of them. CVs of 3% or less, although not ideal, are certainly reasonable[5]. In the present study, the Afinion imprecision was similar to previous results with CVs under 3%. The A1cNow CVs were considerably higher than 3% and were therefore considered unacceptable. Total CVs for the In2it were improved since the original evaluation and were acceptable in the current evaluation. Lot-to-lot variability for the A1cNow and the Afinion was of some concern based on the current data. Three of four A1cNow lots and one Afinion lot did not pass NGSP certification.

It is important to consider clinical needs when selecting HbA_{1c} assay methods, including POC methods. For laboratory HbA_{1c} methods, and some POC methods it is important to examine proficiency testing data to learn about performance of each method in the field with many lots of reagents. For some POC methods, this type of data is limited. Clinicians must recognize that while POC HbA_{1c} offers convenience in some clinical settings, the performance of some POC methods may not be sufficient to meet clinical needs.

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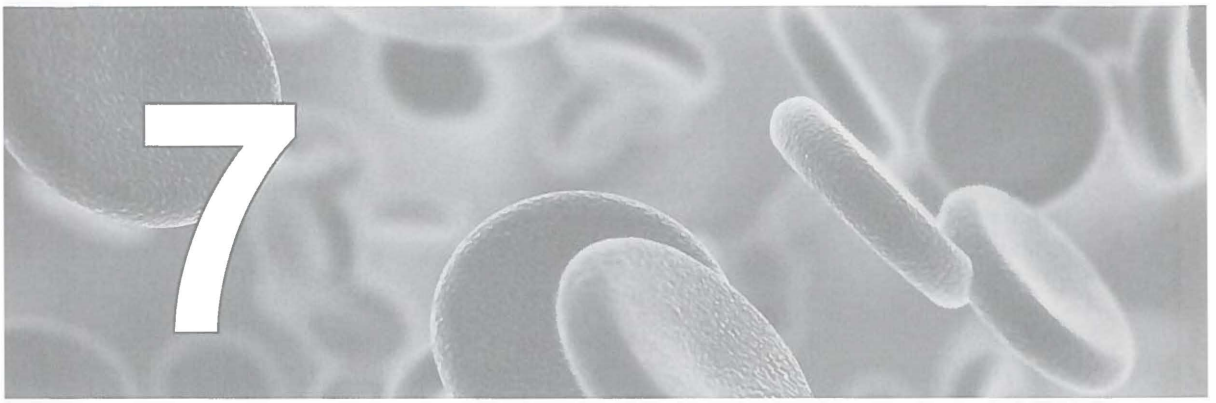

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Evaluation of the Menarini / ARKRAY ADAMS A_{1c} HA-8180V analyser for HbA_{1c}

Cas Weykamp
Erna Lenters-Westra
Hans van der Vuurst
Robbert J. Slingerland
Carla Siebelder
Willeke Visser-Dekkers

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Abstract

Background

We report an evaluation of the Menarini/ARKRAYADAMS A1c HA-8180V analyser (HA-8180V), the fifth generation Menarini/ARKRAY ion-exchange HPLC for the measurement of HbA_{1c}.

Methods

We evaluated the analytical performance, the measurement of hemoglobin variants and the performance in comparison to major analytical methods.

Results

Within-run, between-run and total CV were 0.2%, 0.4% and 0.7% at low HbA_{1c} concentrations and 0.2%, 0.2% and 0.4% at high HbA_{1c} concentrations, respectively. Trueness revealed a maximum deviation of 0.8 mmol/mol (IFCCunits) or 0.1% NGSP units) over the relevant analytical range. Linearity, carry-over and linear drift were excellent. Labile HbA_{1c}, carbamylated hemoglobin, icteric samples and variation in hematocrit did not affect HbA_{1c} outcome. Hemoglobin variants AS, AC and F do not affect HbA_{1c} outcome and are explicitly identified and correctly quantified. HbA_{1c} can not be measured in samples with AE and AD, but these variants are identified correctly. In comparison to other methods used at present, the HA-8180V shows excellent performance.

Conclusions

The HA-8180V performs at a high level and is fit for any clinical application.

Introduction

HbA_{1c} is the cornerstone for monitoring long-term time averaged glycaemic control in type 1 and 2 diabetics[1,2]. Criteria for treatment[3] as well as for accuracy and precision have become more stringent[4]. The recently implemented worldwide standardisation[5] has contributed substantially to the quality of the HbA_{1c} assay, as did technical improvements by the manufacturers of diagnostic devices. HbA_{1c} is now on the threshold of becoming applicable for screening and diagnosis of diabetes[6]. HPLC ion-exchange chromatography has a long history in the measurement of HbA_{1c}, starting with the discovery of HbA_{1c}[7]. One of the leading manufacturers in the field is Menarini/ARKRAY. In this study, we evaluated the fifth generation instrument of this company, the HA-8180V analyser. We aimed to do a standard evaluation of the new instrument, and we gave special attention to interference and detection of hemoglobin variants. We also compared the performance of the new instrument with other methods frequently used.

Materials and methods Characteristics of the HA-8180V analyser

The HA-8180V is an automated bench top analyser [dimensions: 530 (W) x 530 (D) x 530 (H) mm]. The instrument is designed to measure HbA_{1c} (range 9–195 mmol/mol IFCC units; 3%–20% NGSP units), as well as hemoglobins S, C and F. The instrument has a capacity of 100 samples per run. Specimens are either primary tubes with cap piercing (patient samples), or tubes for haemolysates (calibrators, controls, patients with small sample volume) placed in specific ARKRAY racks (9 types). There is an option to insert urgent (STAT) samples. The instrument can spin tubes in order to prevent blood sedimentation, automated reagent information codes, self-diagnostic functions, precision controls function and a large colour LCD.

Column and reagents

In total, 3.4 mL of automatically diluted (standard 1:100; anemic samples 1:50) whole blood is injected. The stainless steel ARKRAY column, maintained at 40°C in an oven, consists of a prefilter and an analytical column packed with an ion exchange resin (a hydrophilic polymer of methacrylate ester copolymer). Sealing screws are made of PEEK (polyether ether ketone). Elution is achieved in a five-step phosphate buffered gradient with increasing ionic strength. There are three buffers (80A, 80B and 80CV) in aluminium foil packs placed on top of the instrument. Hemoglobin fractions are detected with a dual wavelength (420–500 nm) LED-photodiode. At this wavelength, the absorption of oxy-and deoxyhemoglobin is equivalent and thus, ensures a stable signal irrespective of the oxy-/deoxyhemoglobin ratio in the sample.

Operation

The instrument is calibrated with two calibrators (low and high concentration). Calibration can be performed after power up, but is not required when operation is started from stand by. The reported result is derived from the ratio HbA_{1c} /HbA total, adjusted for calibration and expressed in both IFCC units (no decimal) and NGSP units (1 decimal). The instrument has two operation modes, the fast mode (runtime 48 s; no variants detected) and the variant mode (runtime 90 s; variants detected). Both modes are fast in comparison to the 170 s used by the previous generation (Model HA-8160). We evaluated the variant mode. Variants S, C and F are identified, HbA_{1c} as well as the percentage variant are reported. Variants D and E are identified, but HbA_{1c} and percentage variant are not reported.

Protocols

We used protocols CLSI EP 5, 9 and 10[8] along with Rhoads EP-evaluator release 9 software (Data Innovations Inc.) to evaluate reproducibility, trueness and linearity/carry over/linear drift, respectively. Samples with increased concentrations of labile- HbA_{1c}, increased concentrations of bilirubin and carbamylated hemoglobin were used to investigate their interference on the measurement of HbA_{1c}. Specimens with a broad range of hemoglobin concentrations were created to investigate the effect of haematocrit. Five specimens of each of the hemoglobin variants AS, AC, AE, AD and F (hemoglobin type established with capillary electrophoresis) were assayed to investigate both the effect of these variants on HbA_{1c} determination, and the ability of the instrument to detect, interprete and quantify these variants. A 24-specimen panel from the federative EQA programme of The Netherlands, Belgium, Greece and Finland[9] was assayed to allow comparison of the performance of the HA-8180 with the other major methods on the market.

Calibrators and controls

The evaluation was carried out over a period of 6 weeks. The instrument was only calibrated once, at the beginning of the study, with the calibrators supplied by the manufacturer. Throughout the study we used controls from the manufacturer.
During the evaluation we used one batch of controls, calibrators and reagents.

Reference methods

1. IFCC Reference Measurement Procedure[10];
2. Affinity Chromatography Secondary Reference Method. Primus PDQ (Primus Corporation, Kansas City, MO, USA) calibrated with calibrators lot 2009.102 supplied by the IFCC Network;
3. Capillary Electrophoresis Secondary Reference Method. Beckman Coulter P/ACE MDQ, Beckman Coulter Inc., Fullerton, CA, USA) calibrated with calibrators lot 2009.102 supplied by the IFCC Network.

Specimens and traceability

All specimens used in the study had IFCC target values assigned either by the IFCC Reference Measurement Procedure (EQA samples) or with the IFCC Secondary Reference Measurement Procedures (patient samples). NGSP target values were derived from the established IFCC targets using the Master Equation as stated in the Consensus Statement on HbA_{1c} [5].

Results

Reproducibility

The reproducibility was investigated using CLSI EP-5 protocol. With this protocol, on 20 working days, a low and a high sample are assayed in duplicate twice a day in an analytical run, with at least 10 samples. EP-5 defines four parameters for the precision, all listed in Table 1.

Table 1. Reproducibility ARKRAY HA-8180V Analyser*

Parameter	Low HbA_{1c} Level IFCC 39 mmol/mol NGSP 5.7 %	High HbA_{1c} Level IFCC 99 mmol/mol NGSP 11.2 %
Within Run CV	0.2%	0.2%
Between Run CV	0.4%	0.2%
Between Day CV	0.6%	0.2%
Total CV	0.7%	0.4%

* According to protocol NCCLS EP-5 using Menarini Controls

Trueness

Trueness was investigated according to CLSI EP-9 protocol. With this protocol, 40 samples are assayed with the method to be investigated and a comparative method. We used a set of 40 samples to which IFCC targets, as well as derived NGSP targets, have been assigned by two IFCC network labs. This allows evaluation of the trueness in absolute terms with respect to the IFCC and NGSP Reference Systems. Slopes and intercepts (calculated according to Deming and standard regression, along with their 95% confidence intervals, as well as HbA_{1c} concentrations of the HA-8180V at low, medium and high HbA_{1c} concentrations, are shown in Table 2. From the confidence intervals, it can be seen that from statistical point of view, there is a borderline significant difference between the HA-8180V and the reference systems. However, when the difference is expressed in HbA_{1c} units, it can be seen that differences do not exceed 0.8 mmol/mol (IFCC units) and 0.1% (NGSP units).

Table 2: Trueness ARKRAY HA-8180V Analyser*

	IFCC		NGSP	
	Parameters derived from EP-9			
	Deming	Regular	Deming	Regular
Slope*	1.022 (1.003 to 1.042)	1.019 (1.000 to 1.038)	1.030 (1.010 to 1.049)	1.026 (1.006 to 1.046)
Intercept*	-1.15 (-2.27 to -0.03)	-0.95 (-2.07 to 0.17)	-0.209 (-0.356to-0.062)	-0.181 (-0.328to-0.034)
	Difference between measured and (assigned) values			
HbA_{1c} level	Measured	Assigned	Measured	Assigned
Low	29.5 mmol/mol	30.0 mmol/mol	4.8%	4.9%
Medium	60.2 mmol/mol	60.0 mmol/mol	7.6%	7.6%
High	90.8 mmol/mol	90.0 mmol/mol	10.5%	10.4%

* According to protocol NCCLS EP-9 on basis of 40 samples

Linearity, carry-over and linear drift

These parameters were investigated according to CLSI EP-10 protocol. For this protocol, a low sample (L) is mixed 1on 1 with a high sample (H) to create a medium sample (M). On five consecutive working days these three samples are assayed in the following order: M-H-L-M-M-L-L-H-H-M. The data were processed according to the EP-software and the results summarised in Table 3. For results in IFCC numbers, the t-value of 5.0 for non-linearity exceeds the critical value of 4.6, indicating statistically significant non-linearity. Expressed in HbA_{1c} units, this is 0.6 mmol/mol (IFCC) or 0.06% (NGSP). For all other parameters, the t-value was below the critical value.

*Table 3: ARKRAY HA-8180V Analyser: Linearity, Carry-over, Linear Drift**

Parameter	IFCC		NGSP	
	Outcome	t-value	Outcome	t-value
Intercept	0.2200	3.6	0.0100	2.2
Slope	1.0000	0.0	1.0000	0.0
Non-linearity	-0.0007	-5.0	-0.0052	-3.1
% Carry-over	-0.0100	0.0	0.0000	0.0
Drift	0.0000	0.2	0.0000	0.0
Critical t-value		4.6		4.6

* According to protocol NCCLS EP-10

Interference

We investigated four potential interferences. From a normal EDTA-sample, specimens with interferents were created by adding 100 mmol/L glucose (high labile-HbA_{1c}), by adding plasma with a high bilirubin (icteric sample), and by removing

or adding plasma (broad hematocrit range). These samples, as well as the normal sample, were measured with the HA-8180, and the differences in results are shown in Table 4. A sample from a patient with chronic uremia (increased carbamylated hemoglobin) was analysed on the HA-8180V, and an IFCC/NGSP calibrated Primus Affinity Chromatography Instrument. Differences in results are also shown in Table 4.

Table 4: ARKRAY HA-8180V analyser. Interference of Labile HbA_{1c}, Carbamylated Hemoglobin, Hematocrit and Icteric samples

Potential Interferent	Effect IFCC units mmol/mol	Effect NGSP units %
Labile HbA_{1c} (13.8%)	<1 mmol/mol	<0.1%
Carbamylated Hemoglobin (3%)	<1 mmol/mol	<0.1%
Hematocrit (3 – 14 mmol/L)	<1 mmol/mol	<0.1%
Icteric plasma (bilirubin 268 µmol/L)	<1 mmol/mol	<0.1%

Hemoglobin variants

Five hemoglobin variants were investigated: AS, AC, AE, AD and F. For each variant, we selected five specimens with a range in HbA_{1c} to be assayed these on the HA-8180V. HbA_{1c} targets for these specimens were assigned with an IFCC calibrated capillary electrophoresis instrument. Table 5 shows the mean measured HbA_{1c} percentages using the HA-8180V compared to the targets. Table 5 also shows the measurement of the variants itself: in terms of whether an abnormal chromatogram is seen, if the HA-8180V correctly interprets the identity of the variant, and if the percentage of the variant is quantitated. Chromatograms of the respective variants are shown in Figure 1 compared to a normal chromatogram of hemoglobin type A.

Table 5: ARKRAY HA-8180V Analyser and Hemoglobin Variants: Effect on HbA_{1c} outcome and Measurement of Variants

Variant	Effect on HbA_{1c}				Measurement of Variant		
	IFCC Units mmol/mol		NGSP Units %				
	HA-8180	Target	HA-8180	Target	Abnormal Chromatogram	Correct Interpretation	Variant Quantitated
AS	40*	42	5.8	6.0	Yes	Yes	Yes
AC	49*	51	6.6	6.8	Yes	Yes	Yes
AE	Not meas.	52	Not meas.	6.9	Yes	Yes	No
AD	Not meas.	44	Not meas.	6.2	Yes	Yes	No
F	51*	49	6.8	6.6	Yes	Yes	Yes

* within the uncertainty range of the assigned values

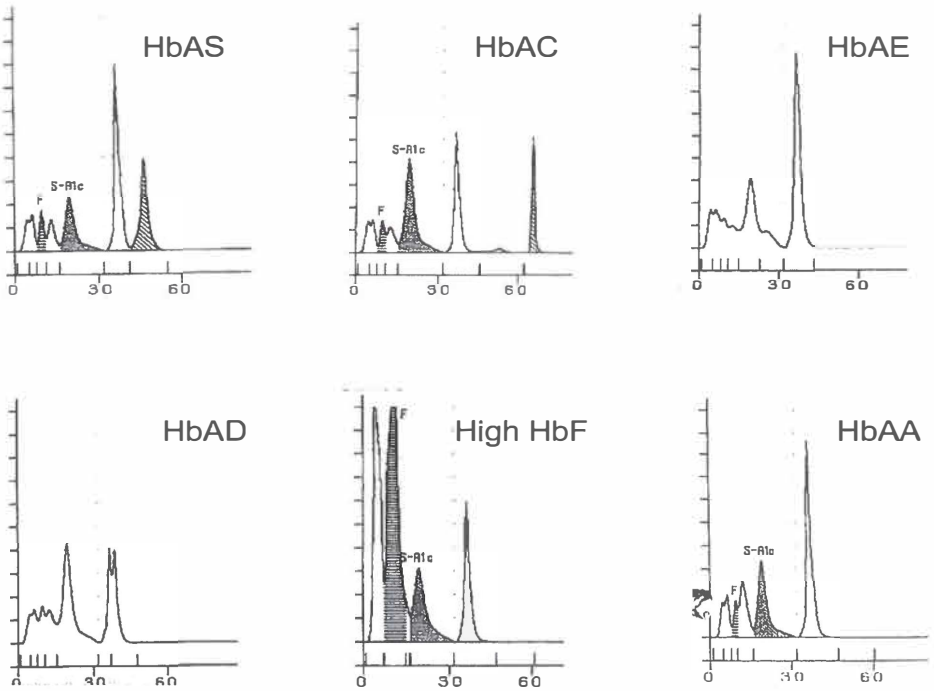

Figure 1.
HA-8180V chromatograms of hemoglobin types S, C, E, D, and F in comparison to hemoglobin A.

Reproducibility and trueness in a regular EQA programme

The samples of the 2009 federate programme (national EQA organisers share samples and software but keep their own identity) of the Netherlands (SKML), Belgium (WIV), Greece (ESEAP), and Finland (Lab quality) were analysed with the HA-8180V. The EQA programme consists of 24 specimens (12 blinded duplicates). IFCC targets and derived NGSP targets for the programme are set using the IFCC Reference Measurement Procedure. The precision of labs is calculated from the 12 blind duplicates and expressed as CV. Trueness is defined as the difference between measured HbA_{1c} and the target. Table 6 shows the CV and the bias from the target (at an HbA_{1c} concentration of 54 mmol/mol/ 7.0%) for the HA-8180V in comparison to the mean CVs as determined for the major methods in the EQA programme.

*Table 6: ARKRAY HA-8180V: Reproducibility and Trueness compared with other methods**

Method	n	CV	Deviation Target	
			IFCC mmol/mol	NGSP %
Major Methods 2009				
ARKRAY HA-8180	1	0.8%	0	0.0
ARKRAY HA 8160	157	1.3%	+1	+0.1
ARKRAY HA 8140	33	2.1%	+2	+0.2
TOSOH G8	35	0.9%	+1	+0.1
TOSOH G7	48	1.3%	+1	+0.1
TOSOH G5	8	2.0%	+1	+0.1
Bio-Rad D10	17	2.4%	+1	+0.1
Bio-Rad Variant	41	1.8%	+1	+0.1
Roche Tina Quant	73	2.8%	+1	+0.1
Historical Performance				
All methods 1993	122	5.2%	---	---
All methods 1999	143	4.9%	+3	+0.3
All methods 2005	376	2.9%	-2	-0.2
All methods 2009	438	1.8%	+1	+0.1

* on basis of the 2009 EQA programme of ERL (European Reference Laboratory)

Discussion

Therapeutic strategies rely more than ever on reproducible and unbiased measurement of HbA_{1c}. Not only for control and follow-up of diabetic patients, but recently also for diagnosis and screening[6]. The efforts of the IFCC working group[11] on standardisation of HbA_{1c} have contributed much to global standardisation.

The efforts of manufacturers contributed to improvements of analytical systems and are in fact ongoing to create the fastest, most convenient and most reliable test. In this study, we evaluated the HA-8180V, the new, fifth generation analyser from ARKRAY/Menarini. With between-run CVs of 0.2% - 0.4%, within-run CVs of 0.2%, and total CVs of 0.4% - 0.7% the evaluation revealed excellent reproducibility, far below the most stringent requirements of 2%[12]. Trueness verification of the manufacturer-calibrated instrument demonstrated traceability to the IFCC and NGSP reference measurement procedures. Evaluation of linearity, carry-over and linearity showed good results. There was no trace of interference by the common interferences (labile- HbA_{1c}, carbamylated hemoglobin, icteric samples, hematocrit). However, some statistical tests showed significant differences. It seems a paradox, but these are derived from the excellent reproducibility of the instrument:

HbA_{1c} results are reported with no decimals (IFCC units) and to one decimal (NGSP units). From a statistical point of view, one additional decimal is required, but this is clinically irrelevant.

Hemoglobin variants are an important issue. They can interfere with HbA_{1c} measurements, but when detected, provide essential information on the presence of a variant: important for the interpretation of the HbA_{1c} result and for genetic counselling. We evaluated the HA-8180V for both applications. Hemoglobin variants AS, AC and F did not affect the correct measurement of HbA_{1c}. However, in patients with AE and AD, HbA_{1c} cannot be measured. All five variants are easily recognised in the chromatogram, and correctly interpreted by the instrument (the correct name of the variant is reported).

The evolution of the quality of methods is illustrated by the performance of the various instruments in the federative EQA programme of The Netherlands, Belgium, Greece and Finland: the third generation instrument of ARKRAY (HA-8140) has a mean intra-lab CV of 2.1%. The fourth generation (HA-8160) has a CV of 1.3% and the fifth generation (HA-8180 evaluated in this study) has a CV as low as 0.8%. The continuous improvement in quality is also demonstrated by the overall interlab CV over the past 15 years: from 5.2% in 1993 to 1.8% in 2009.
Excellent reproducibility is a prerequisite for the application of HbA_{1c} in diagnosis and screening of diabetes. The final conclusion from this evaluation is that the new ARKRAY/Menarini instrument performs at a high level, and is fit for any clinical application of HbA_{1c}.

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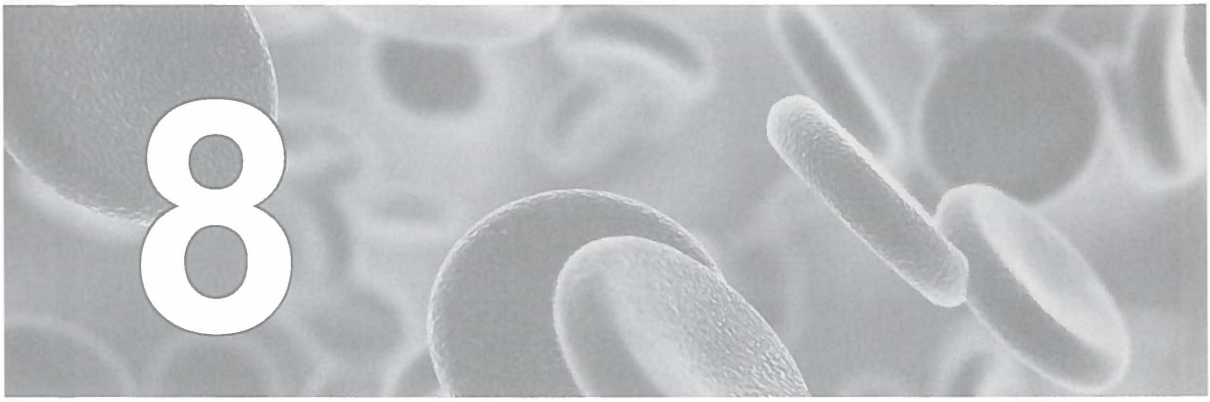

8

Glycated hemoglobin A_{1c} (HbA_{1c}) in the diagnosis of diabetes mellitus: don't forget the performance of the HbA_{1c} assay

Roger K. Schindhelm
Erna Lenters-Westra
Robbert J. Slingerland

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Glycaemic control, as reflected by HbA_{1c}, is common practice in the management of diabetes mellitus to adjust therapy regimens and to aid in patient education. The American Diabetes Association has advocated the use of HbA_{1c} in the diagnosis of diabetes mellitus[1], whereas the World Health Organization and the International Diabetes Federation recommend against its use in the diagnosis of diabetes mellitus in their 2006 consensus report[2]. The applicability of HbA_{1c} and the optimal cut-off value in the diagnosis of diabetes mellitus seem to depend on ethnicity, age, sex and diabetes prevalence[3], and other HbA_{1c} cut-off values have been proposed.

In addition, healthcare professionals should be aware of the inherent analytical variability of the HbA_{1c} laboratory assays. Although the analytical performance has improved over the last 5 years, all methods still do not concur with the primary reference methods, which may contribute to bias in epidemiological studies and lead to misclassification of patients in the diagnosis of diabetes mellitus. Indeed, the results of the College of American Pathologists 2009 GH2-B Survey Data on HbA_{1c} (4) demonstrated that only two of 25 methods have an inter-laboratory coefficient of variation of < 2% with the sample with a reference value of 6.6%. Overall in that survey, interlaboratory coefficients of variation ranged from 1.7 to 7.6% and the analytical bias ranged from –0.19 to 0.27% HbA_{1c}. For example, the assay with the worst analytical performance would yield HbA_{1c} values for the reference value of 6.6% between 5.5% and 7.7% (95% confidence interval).

Furthermore, not all studies assessing the diagnostic value of HbA_{1c} report the analytical performance (intra- and inter-assay coefficients of variation and analytical bias) of the HbA_{1c} assay used. Another point of concern is the use of point-of-care testing devices for HbA_{1c} in the diagnosis of diabetes mellitus. The American Diabetes Association recommends against the use of HbA_{1c} point-of-care testing devices in the diagnosis of diabetes[1]. In keeping with this recommendation, a recent study demonstrated that the majority of HbA_{1c} point-of-care testing devices do not comply with the generally accepted performance criteria[5], which may have serious diagnostic and therapeutic consequences. If a healthcare professional, however, should insist on applying HbA_{1c} point-of-care testing devices for the diagnosis of HbA_{1c}, we strongly recommend using those devices that fully comply with optimal analytical performance criteria[5].

In summary, the performance of the HbA_{1c} assay in the diagnosis of diabetes mellitus should be taken into account and ideally be reported in studies assessing the diagnostic value of HbA_{1c} in the diagnosis of diabetes mellitus. Furthermore, healthcare professionals should be provided with the same information to properly interpret laboratory and point-of-care HbA_{1c} results. The clinical biochemist can play a valuable role in this matter and should be encouraged to use HbA_{1c} methods with optimal analytical performance (no bias and a total coefficient of variation of <2%). Given the insufficient analytical performance of most HbA_{1c} point-of-care testing devices, caution should be exercised when applying these devices in the diagnosis of diabetes mellitus.

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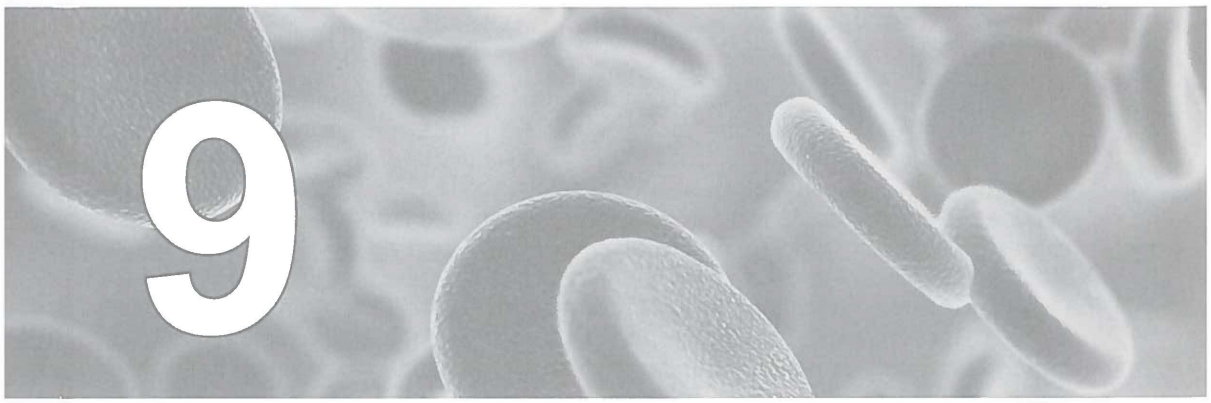

Glucose and glycated Hemoglobin A_{1c} point-of-care testing and early diagnosis of diabetes and pre-diabetes

Roger K. Schindhelm
Erna Lenters-Westra
Marion J. Fokkert
Robbert J. Slingerland

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Abstract

The number of individuals with impaired glucose metabolism ('pre-diabetes') and type 2 diabetes is reaching epidemic proportions. This increase is associated with higher cardiovascular morbidity and mortality. Early screening for diabetes and pre-diabetes (i.e. elevated glucose and/or glycated hemoglobin (HbA_{1c}) may aid in the reduction of diabetes-related complications. Point-of-care testing, defined as testing at or near the site of the patient, is able to bring diagnostic tests and its associated therapeutic actions immediately to the patient and may aid in the detection of diabetes and the reduction of complications. However, the majority of available point-of-care testing devices for glucose and HbA_{1c} do not meet generally accepted analytical performance criteria and may underestimate the true risk of diabetes. Until these analytical performance issues have been addressed properly, caution should be exercised in the use of point-of-care testing of glucose and HbA_{1c} in the diagnosis of and screening for pre-diabetes and diabetes.

Introduction

Over the last few decades the prevalence of diabetes has reached epidemic proportions in western societies and is even higher in developing countries[1-4], mainly due to population growth, ageing and obesity[1,5]. The World Health Organization (WHO) has estimated that the global prevalence of diabetes will increase from 2.8% in 2000 to 4.4% by 2030[6]. The increases in both obesity and diabetes will have a profound impact on diabetes- and obesity-related complications[7], the use of healthcare resourses[8] and the quality of life of affected patients[9].

Diabetes comprises a group of metabolic diseases characterised by elevated blood glucose levels, and the diagnosis is based on increased fasting and/or post-load (after an oral glucose tolerance test (OGTT)) plasma glucose values (see *Table 1*)[10-12]. Type 2 diabetes, which accounts for 90–95% of all diabetes cases, is characterised by inappropriate insulin secretion due to a decline in β-cell function and the presence of (obesity-related) insulin resistance[13,14], resulting, among other metabolic disturbances, in fasting and post-prandial hyperglycaemia. Glycaemic control, as reflected by glycated hemoglobin (HbA_{1c}), is common practice in the management of diabetes to adjust therapy regimens and to aid in patient education.

Recently, the American Diabetes Association (ADA) has advocated the use of HbA_{1c} in the diagnosis of diabetes[15,16] as a result of the global standardisation of the HbA_{1c} assay with associated improvement of the analytical performance of the assay[17-20]. However, the WHO and the International Diabetes Federation (IDF) recommend against the use of HbA_{1c} in the diagnosis of diabetes in their 2006 consensus report[12].

Individuals with type 2 diabetes have an increased risk of developing macrovascular and/or microvascular complications and mortality[21,22], and the risk of developing these complications may increase if good glycaemic control is not adequately maintained. Studies such as the Diabetes Control and Complications Trial (DCCT) and the UK Prospective Diabetes Study (UKPDS) have supported the notion that adequate glycaemic control in the general patient population may aid in the prevention or reduction of the risk of developing diabetes related vascular complications[23-26]. Early detection of high-risk patients with elevated glucose levels may aid in the prevention or reduction of diabetes-related complications. This could be achieved by screening individuals who are at high risk of developing diabetes by for example capillary glucose testing by point-of-care testing.

This article focuses on the potential role of point-of-care testing of glucose and HbA_{1c} in the diagnosis of pre-diabetes and diabetes. It gives an overview of the principles, pitfalls and analytical performance of glucose and HbA_{1c} point-of-care testing and summarises the studies that have applied point-of-care testing of glucose and HbA_{1c} in the diagnosis of (pre-) diabetes. Finally, the article concludes with the authors' recommendations on the applicability of point-of-care testing of glucose and HbA_{1c} in the diagnosis of diabetes.

Point-of-care Testing of Glucose and Glycated Hemoglobin A_{1c} – Principles, Practice and Pitfalls

Principles of Point-of-care Testing

Point-of-care or near-patient testing can be defined as diagnostic testing at or near the site of the patient and is able to bring the diagnostic test and its associated therapeutic actions immediately to the patient[27]. This could lead to improvement of patient care, given that appropriate quality assurance systems in point-of-care testing have been implemented[27,28]. The application of point-of-care testing of glucose and HbA_{1c} in the management of diabetes has been introduced and is regarded as standard care. Indeed, evidence is suggesting that point-of-care testing of glucose may improve glycaemic control[29], whereas point-of-care testing of HbA_{1c} was shown to be effective in the improvement of glycaemic control in some but not all studies depending on the HbA_{1c} targets[30-32]. The applicability of point-of-care testing of glucose and HbA_{1c} in the diagnosis of diabetes and pre-diabetes is less evident and still under debate, mainly due to analytical performance issues of the devices and the definition of optimal cut-off values[33-36].

Developments in Glucose Point-of-care Testing Devices

Over the last four to five decades the principles of point-of-care testing of glucose have changed considerably[37,38]. The first generation quantitative point-of-care testing devices for glucose included a modified dipstick originally designed for detecting glucose in urine that is based on an enzymatic reaction with a change of colour of the pad of the dipstick. A blood sample was applied to the strip and whipped off. Subsequently, the change in colour intensity was measured and compared with an internal calibration and translated to a quantitative result. The second generation point-of-care glucose testing devices provided automatic timing and no need for wiping off the strip, which considerably improved the performance of the device. The latest point-of-care glucose testing devices are based on enzymatic methods (glucose dehydrogenase and glucose oxidase) and electrochemical sensors instead of colorimetric assays[39-41]. These technical advances have led to an improved performance with respect to operation and sample handling and to improvement of the analytical performance of these devices. However, even state-of-the-art devices can still be improved.

Developments in Glycated Hemoglobin A_{1c} Point-of-care Testing Devices

Laboratory analysers for HbA_{1c} utilise technologies that are based on either charge differences (high-pressure liquid chromatography) or structure (boronate affinity or immunoassay combined with general chemistry). In the last five to 10 years these technologies have been incorporated into point-of-care testing devices, allowing for immediate availability of HbA_{1c} measurements[42-44]. The first HbA_{1c} point-of-care devices needed several manual handlings, while the newly developed devices are easy to use and are provided with tools to make it possible to be connected with other information systems.

Practice and Pitfalls of Point-of-care Testing of Glucose and Glycated Hemoglobin A_{1c}

Point-of-care testing for glucose has been used in a variety of settings, including hospitalised patients with diabetes, self-management of patients with diabetes, outpatient diabetes clinics, emergency departments, general practitioners' offices and pharmacies[45-47], whereas the use of point-of-care testing of HbA_{1c} is less common in clinical practice. In The Netherlands, HbA_{1c} point-of-care devices are mainly used in paediatric diabetes centres in children with type 1 diabetes. The major advantages of point-of-care testing include portability, small sample volume (whole blood) and immediate result with appropriate therapeutic action[27]. However, in general point-of-care testing of glucose is still more expensive than the laboratory reference method and higher analytical variability has been reported. In addition, quality assurance issues should be addressed properly[27,28].

Although the performance of glucose point-of-care testing devices has improved, some pitfalls in point-of-care testing should still be acknowledged. The operators should be properly trained and certified to obtain an optimal whole-blood sample and to apply the correct amount of blood volume on the point-of-care testing device[48,49]. Furthermore, patient characteristics that may adversely influence the result should be noted, including haematocrit levels[50,51], interfering drugs[52] and metabolic disorders (e.g. uraemia, hyperlipidaemia)[53,54], low (<0.35) and high (>0.55) haematocrit levels may significantly influence the result of glucose measurement by point-of-care devices as illustrated in Figure 1, and should be evaluated in patients in whom the point-of-care devices are to be applied. Finally, factors that might adversely affect the operation of the devices and the performance of the strips such as temperature, humidity and high altitude should be taken into account[55-57]. Although the use of point-of-care testing of HbA_{1c} is less common than that of glucose, similar limitations and pitfalls of point-of-care testing of HbA_{1c} may apply. In addition, immunoassay-based HbA_{1c} point-of-care devices may interfere with hemoglobin variants, which is not the case with affinity-based point-of-care testing devices[58,59].

Although the technical specifications of the point-of-care devices have improved considerably, pre-analytical, analytical and post-analytical issues should still be acknowledged and quality assurance systems should be implemented.

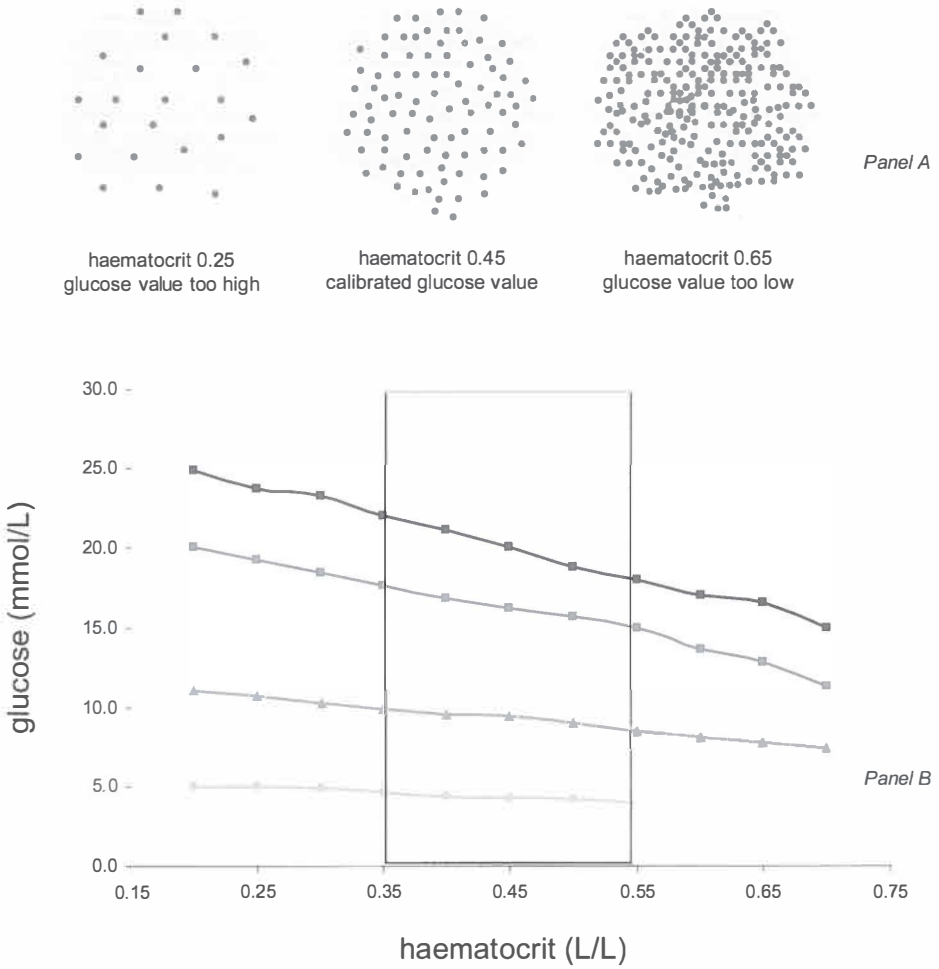

Figure 1. *Effect of haematocrit on point-of-care testing for glucose. Point-of-care testing devices for glucose are calibrated at normal haematocrit levels (e.g. 0.35-0.45 L/L) (Panel A). A whole blood sample with a low haematocrit (i.e. 0.25 L/L) has a relative excess of plasma and the measurement will yield a value that will be incorrectly too high. The opposite will be the case if the haematocrit is high. The influence of haematocrit levels at various glucose levels is depicted in Panel B.*

Analytical Performance of Point-of-care Testing of Glucose and Glycated Hemoglobin A_{1c} (Regulations and Guidelines)

Although glucose point-of-care testing devices can provide immediate results, these results may not be equivalent to the results produced by laboratory analysers[60,61]. Over the past few years various regulatory affairs bodies have issued guidelines for the analytical performance of glucose point-of-care testing devices. The US Food and Drug Administration (FDA) has cleared over 200 point-of-care testing devices for

medical use based on the review of clinical and laboratory evidence provided by the manufacturer[62,63]. A systematic review in 2007 concluded that none of the included reports on the evaluation of glucose point-of-care devices followed generally accepted recommendations of performing these evaluation studies and the authors concluded that these limitations may have affected the conclusions of these evaluation reports[64]. The Clinical and Laboratory Standards Institute (CLSI)/National Committee on Clinical Laboratory Standards (NCCLS) guideline states that >95% of the results should be within ±20% or 0.8mmol/l (whichever is greater) of the laboratory value [65]. The International Organisation for Standardisation (ISO) recommends an agreement of ±20% for levels above 4.2mmol/l or within ±0.83mmol/l for glucose levels less than 4.2mmol/l (ISO 15197)[66].

The Dutch guideline issued by the Netherlands Organisation for Applied Scientific Research (TNO) Centre for Medical Technology recommends a maximum of ±15% average deviation from a hexokinase laboratory value >6.5mmol/l and within 1mmol/l for values <6.5mmol/l [67]. Finally, the ADA has proposed the most stringent guidelines and recommends an agreement within ±10% of a laboratory method, with an eventual goal of <5% deviation [68]. Based on the TNO guidelines, an overview of the current minimal criteria for assessment of the performance of point-of-care glucose devices is presented in Table 2.

Table 2: Minimal performance criteria of point-of-care testing devices for glucose

Criterion	Comment
Accuracy	max. 15% deviation from hexokinase-method
Reproducibility	max. coefficient of variation of 10%
Haematocrit dependency (range: 0.35-0.55)	max. coefficient of variation of 10% for glucose values >6.5 mol/L or 1 mmol/L for glucose values <6.5 mmol/L
Underfilling protection	max. 10% deviation from result at minimal volume or error mark

As yet, there is no consensus on what should be considered the maximum deviation of the point-of-care devices. Currently, the ISO 15197 guideline is under revision and a maximum deviation of 15% in point-of-care testing devices for home use versus a reference method is proposed. In our view, for point-of-care testing devices for clinical uses a lower deviation should be implemented (<10%).

Performance of Glucose and Glycated Hemoglobin A_{1c} Point-of-care Testing Devices

Slingerland and co-workers found that only 60% of 30 available point-of-care testing devices available in The Netherlands complied with the TNO guidelines (15% deviation). If all criteria as set out in the TNO guidelines were tested, including reproducibility (maximum coefficient of variation of 10%), haematrocrit dependency and under-filling protection, only 20% of the devices would comply with the TNO Guideline[69]. HbA_{1c} point-of-care testing devices have been made available over the last few years and only a number of validation studies have been published. One of the most extensive validation studies was performed by Lenters-Westra and

Slingerland. They reported that the majority of HbA_{1c} point-of-care testing devices do not comply with the generally accepted performance criteria[70], and these results imply that these devices should be used with caution in the screening of diabetes and pre-diabetes[71]. Unfortunately, the most stringent ADA criteria may not be met by most glucose point-of-care testing devices. In addition, the majority of HbA_{1c} point-of-care devices do not meet generally accepted analytical performance criteria.

Point-of-care Testing of Glucose and Glycated Hemoglobin A_{1c} in the Diagnosis of Diabetes and Pre-diabetes

Diagnosis of Pre-diabetes with Point-of-care Testing of Glucose:
The diagnosis of diabetes and pre-diabetes (impaired fasting glucose and/or impaired glucose tolerance) is based on elevated fasting plasma glucose and/or post-load (after a 75g OGTT) glucose levels and/or hyperglycaemia related symptoms (see Table 1). The OGTT is regarded as the gold standard in the diagnosis of diabetes, and is preferably performed on two separate occasions. The reproducibility of the OGTT is relatively low (95% of the random test and re-test differences were less than 15% with fasting glucose and 46% with post-load glucose), mainly due to intra-individual biological variability and, to a lesser extent, to analytical variability if glucose is measured in venous plasma with a laboratory reference method with low analytical co-efficients of variation (<2%)[72-74]. Indeed, an analysis of the DCCT data demonstrated that the biological variation was higher than the variation of the glucose measurements[75].

Table 1: Diagnostic criteria of diabetes mellitus

	American Diabetes Association (15)	World Health Organisation/IDF (12)
	Diabetes	
Fasting plasma glucose§	≥7.0 mmol/L (or)	≥7.0 mmol/L (or)
Post-load plasma glucose*	≥11.1 mmol/L	≥11.1 mmol/L
HbA_{1c}**	≥6.5%	not recommended
	Impaired Fasting Glucose (IFG)	
Fasting plasma glucose	5.6 - 6.9 mmol/L	6.1 - 6.9 mmol/L (and)
Post-load plasma glucose	<7.8 mmol/L (and)	<7.8 mmol/L
HbA_{1c}	5.7 – 6.4%	not recommended
	Impaired glucose tolerance (IGT)	
Fasting plasma glucose	<7.0 mmol/L (and)	<7.0 mmol/L (and)
Post-load plasma glucose	≥7.8 and <11.1 mmol/L	≥7.8 and <11.1 mmol/L
HbA_{1c}	5.7 – 6.4%	not recommended

§: Diagnostic testing should be repeated, unless patient presents with symptoms of hyperglycaemia, or hyperglycaemic crisis, or random plasma glucose of ≥11.1 mmol/L

*: Venous plasma glucose 2 hours after an oral glucose tolerance test (75-g) and after no caloric intake for at least 8 hours

**: Based on a National Glycohemoglobin Standardization Program (NGSP) certified method and standardised to the DCCT-assay

The co-efficients of variation of glucose measured by point-of-care testing devices may be considerably higher and therefore may contribute to further lowering the reproducibility of the OGTT.

Indeed, a number of studies that compared venous plasma glucose assessed by a laboratory reference method with glucose measured in capillary whole blood by point-of-care devices showed an acceptable correlation between both values, but significantly higher co-efficients of variation in the point-of-care measured glucose values.

To date, only a limited number of studies have addressed the applicability of point-of-care glucose testing in the diagnosis of diabetes and pre-diabetes (Table 3). Rush and co-workers studied the performance of glucose point-of-care testing in an outpatient setting for the diagnosis of diabetes and pre-diabetes[33]. An OGGT was performed in more than 3,000 individuals with a laboratory-based glucose reference method and with point-of-care glucose testing to assess the comparability of the two methods. The glucose levels as measured by the point-of-care device were significantly lower compared with the laboratory reference method, and the authors recommended against the use of point-of-care glucose testing for the diagnosis of diabetes and pre-diabetes[33]. By contrast, based on their study of 200 participants in an area of Western Australia, Marley and co-workers concluded that point-of-care glucose testing could be used in the diagnosis and exclusion of diabetes if based on locally established reference values[34]. A recently published study conducted by Zhou and co-workers compared HbA_{1c} (laboratory reference method) with point-of-care glucose testing with plasma glucose values after an OGTT to diagnose diabetes and pre-diabetes. The authors concluded that point-of-care glucose testing performed significantly better than HbA_{1c} for the diagnosis of diabetes and/or pre-diabetes. Unfortunately, the authors did not present data on the comparability of point-of-care-derived glucose values with the plasma glucose values of the reference method at diagnostic values (i.e ≥7.0mmol/l). Overall, the reported areas under the receiver operating characteristics curve were less than 0.81 and 0.68 for detecting diabetes and pre-diabetes, respectively[35]. Kruiskoop and co-workers studied the applicability of glucose point-of-care testing in epidemiological studies in a subset (350 subjects) of the CoDAM study, a population-based cohort study. The concordance between capillary and venous glucose measurements was 78%[36]. The authors concluded that use of point-of-care glucose measurement is reliable and cost-effective in epidemiological settings.

Based on these studies it can be concluded that the performance of point-of-care glucose testing may suffice for epidemiological studies in the screening of diabetes and pre-diabetes, and that local cut-off values of point-of-care glucose testing may, to some extent, enhance the performance of point-of-care glucose testing. However, in screening and diagnosis of individual patients the performance of point-of-care testing of glucose may lead to a significant misclassification of patients.

Table 3. Overview of studies assessing the value of POCT of glucose in the diagnosis of diabetes

Author (year)	Cohort / Population	N	Diagnostic Criteria	Reference	(Pre)Diabetes n (%)	Sensitivity /Specificity	AUC	Authors' Conclusion
Rush et al (2008) (33)	Te Wai o Rona	3225	WHO 2006	Single OGTT	NDM 161 (5.0%) IFG 115 (3.6) IGT 299 (9.3%)	NDM: 57% / 98%	NDM: 0.88	POCT cannot be recommended as a means of screening for (pre)diabetes
Zhou et al (2009) (35)	Qingdao	2332	WHO 1999	Single OGTT	NDM 278 (11.9%) IFG/IGT 689 (29.55)	Not reported	Not reported	POCT of glucose, as a screening tool for diabetes and pre-diabetes, performed better than HbA_{1c}.
Kruiskoop (2004) (36)	CoDAM	350	WHO 1999	Single OGTT	NDM: 97 (27.7%) IGT: 77 (22.0%)	NDM: 84% / 98%	Not reported	Cost-effective inclusion schemes for epidemiological studies
Marley (2007) (34)	Australian cohort	200	Glucose ≥11.1	Fasting or non-fasting venous glucose	Not reported	NDM: 83.3% / 99.3%	Not reported	Can be used in the process of diagnosis or excluding diabetes using locally established reference values.

Diagnosis of (Pre-) Diabetes with Point-of-care Testing of Glycated Hemoglobin A_{1c}

An elegant alternative to fasting and post-glucose testing in the diagnosis of diabetes and pre-diabetes is the use of HbA_{1c}. The patient does not need to fast or to undergo an OGTT, which can be associated with some discomfort. Instead, a non-fasting blood sample can be drawn to measure HbA_{1c}, which has an overall lower analytical variability than glucose measurements (either capillary or venous plasma). Recently, the ADA has proposed the use of HbA_{1c} in the diagnosis of diabetes[15]. However, the applicability of HbA_{1c} and the optimal cut-off value (≥6.5% as proposed by the ADA) in diagnosing diabetes seem to depend on ethnicity, age, sex and diabetes prevalence[76], and therefore other HbA_{1c} cut-off values have been proposed. Furthermore, the life span of the erythrocyte should be taken into account, which may differ between individuals and may influence HbA_{1c}[77]. In addition, recent studies that applied the cut-off value of ≥6.5% reported low sensitivity and specificity of HbA_{1c} in the diagnosis of diabetes[78-81].

To the best of our knowledge, no studies that used point-of-care HbA_{1c} testing in the diagnosis of diabetes and pre-diabetes have been published to date. Given the recent observation that the majority of point-of-care HbA_{1c} devices performed poorly with respect to generally accepted analytical performance criteria, the applicability of these point-of-care testing devices in the diagnosis of diabetes and pre-diabetes may be very limited or may even discouraged until these performance issues have been properly addressed.

Based on these observations with respect to HbA_{1c} testing in the diagnosis of diabetes and pre-diabetes, we can conclude that HbA_{1c} assessed by reference laboratory methods may underestimate or overestimate the prevalence of undiagnosed diabetes and pre-diabetes. Given the low analytical performance of point-of-care HbA_{1c} devices, their use in diagnosing diabetes and pre-diabetes is not recommended.

Conclusions

The number of patients with type 2 diabetes is reaching epidemic proportions and this increase is associated with an increase in cardiovascular morbidity and mortality. Early screening of patients with undetected diabetes and pre-diabetes (i.e. elevated glucose and/or HbA_{1c}) may eventually lead to a reduction in diabetes-related complications. Point-of-care testing of glucose and HbA_{1c} have been introduced and could lead to improvement of patient care. However, currently the majority of available point-of-care testing devices for glucose and HbA_{1c} do not meet generally accepted analytical performance criteria, and may therefore underestimate or overestimate the risk of diabetes. Until these analytical performance issues have been addressed properly, we recommend against the use of point-of-care testing of glucose and HbA_{1c} in the diagnosis and screening of pre-diabetes and diabetes.

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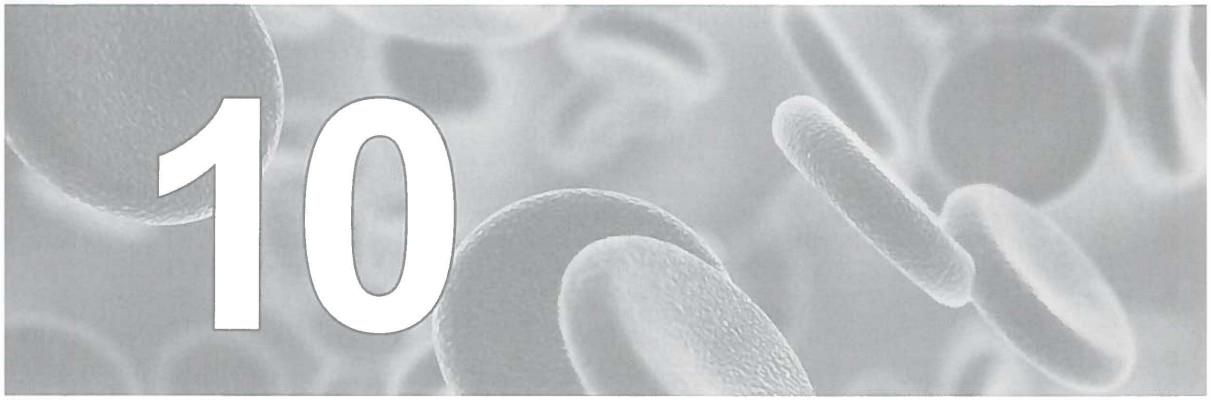

One in five laboratories using various Hemoglobin A_{1c} methods do not meet the criteria for optimal diabetes care management

Erna Lenters-Westra
Roger K. Schindhelm
Henk J.G. Bilo
Robbert J. Slingerland

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Abstract

Background

We assessed the reference change value (RCV) of currently available hemoglobin A_{1C} (HbA_{1c}) laboratory assays, which is defined as the critical difference between two consecutive HbA_{1c} measurements representing a significant change in health status.

Methods

We examined the individual laboratory coefficients of variation (CVs) in the Dutch/Belgian quality scheme based on 24 lyophilized samples and calculated the RCV per laboratory (n = 220) and per assay method. In addition, two pooled whole blood samples were sent to the participating laboratories. The individual laboratory results were compared to the assigned value ± an allowable total error (TE_a) of 6%.

Results

At HbA_{1c} values of 41.0 mmol/mol (5.9%-Diabetes Control and Complications Trial (DCCT)) and 61.8 mmol/mol (7.8%-DCCT), 99% and 98%, respectively, of the laboratories reported a value within a TE_a limit of 6%. The analytical CV of the HbA_{1c} method used in 78% of the laboratories is <2.4% based on DCCT numbers. The mean RCV at an HbA_{1c} value of 53 mmol/mol (7.0%-DCCT) for methods of Bio-Rad is 6.4 mmol/mol (0.59%-DCCT); for Arkray/Menarini, 4.7 mmol/mol (0.43%-DCCT); for Roche, 7.1 mmol/mol (0.65%-DCCT); for Tosoh, 3.6 mmol/mol (0.33%-DCCT); and for other methods, 6.9 mmol/mol (0.63%-DCCT).

Conclusions

The analytical performance of the majority of laboratory HbA_{1c} methods is within the clinical requirements. However, based on the calculated RCV, 21.8% of the laboratories using different HbA_{1c} methods are not able to distinguish an HbA_{1c} result of 59 mmol/mol (7.5%-DCCT) from a previous HbA_{1c} result of 53 mmol/mol (7.0%-DCCT). It can be presumed that differences in HbA_{1c} results of 5 mmol/mol (0.5%-DCCT) do influence treatment decisions.

Introduction

Monitoring glycemic control by using glycated hemoglobin A_{1c} (HbA_{1c}) measurements is one of the hallmarks in the management of diabetes mellitus to adjust therapy regimens and to aid in patient education[1]. In general, a target value of HbA_{1c} of less than 53 mmol/mol (7.0% Diabetes Control and Complications Trial (DCCT)) is considered by many to be the treatment goal in order to reduce the risk of diabetes-related complications[2,3]. The American Diabetes Association (ADA) and European Association for the Study of Diabetes (EASD) consensus algorithm for the initiation and adjustment of therapy states that a sustained HbA_{1c} level above 53 mmol/mol (7.0%-DCCT) should prompt the healthcare provider to consider changing therapy in order to reach the predefined target value[4]. Indeed, the ADA recommends performing HbA_{1c} testing at least twice a year in patients with stable glycemic control or four times per year in patients with changes in therapy or with HbA_{1c} levels above the target value[1]. The changes in therapeutic regimes are therefore guided by (relevant) changes in serial measurements of HbA_{1c} testing.

From an analytical point of view, the difference between two serial HbA_{1c} measurements depends on the coefficient of variation (CV): the intra-individual biological variation (CV_w) and the analytical variation (CV_a) of the HbA_{1c} laboratory assay. These two sources of variation can be combined in the so-called reference change value (RCV), which is the critical difference in the change in patient's serial test results that can be considered significantly different at a probability of 95%[5,6]. In other words, this means that if an RCV of 7 mmol/mol (0.7%-DCCT) HbA_{1c} units is found, an HbA_{1c} value of 58 mmol/mol (7.5%-DCCT) would not be significantly different from a previous HbA_{1c} value of 53 mmol/mol (7.0%-DCCT).

Currently there are more than 30 HbA_{1c} laboratory methods available on the market, and information on analytical performance of each assay method may not be readily available. In addition, the same HbA_{1c} assay may have different performance characteristics within and between various laboratories. External HbA_{1c} quality schemes reveal average analytical performance of the different HbA_{1c} methods used in the field and do not make available the results of individual laboratories to others besides the laboratory concerned. Indeed, the aggregated results, in general, indicate sufficient analytical performance of the majority of the methods but provide no insight in the performance of all laboratories individually[7].

The aim of the current study was to present the analytical performance of a large portion of available HbA_{1c} laboratory assays currently available on the market, based on the individual results of the HbA_{1c} values of the participating laboratories in the Dutch and Belgian external quality scheme. Moreover, the results of a separate ring survey with fresh whole blood are presented. Based on this information we calculated the RCV, which may aid the healthcare professional to interpret differences in serial HbA_{1c} measurement results.

Research Design and Methods

The results of two External Quality Assurance Services (EQAS) - the Stichting Kwaliteitsbewaking Medische Laboratoria (SKML) in The Netherlands and the Wetenschappelijk Instituut voor de Volksgezondheid (WIV) in Belgium - and the results of a ring survey with two pooled fresh whole blood samples were used to assess the individual laboratory performance of various HbA_{1c} laboratory methods[8]. Not all laboratories (n = 550) participated in the ring survey with two pooled fresh whole blood samples, and therefore only the results of the laboratories that submitted results in both surveys (SKML/WIV and fresh whole blood samples) were used (n = 220).

The design of the Dutch SKML and Belgian WIV scheme is based on 24 lyophilized interconnected samples. The samples are sent annually to all participating laboratories and stored at –20°C or below. Each sample is requested to be analyzed every fortnight, and the results are to be submitted to the website of SKML/WIV. The 24 samples were in fact 12 samples in duplicate. The duplicates were blinded to prevent any influence on results. From the duplicates the CV_a was calculated, using the following formula:

$$CV_a = \frac{\sqrt{\frac{\sum(\Delta)^2}{n}}}{\bar{x}\sqrt{2}} \times 100\% \quad (1)$$

where CV_a is the analytical CV, Δ is the difference between duplicates, n is the number of duplicates, and $\bar{x}$ is the mean of the duplicates.

The CV_a was used to calculate the RCV, which is the critical difference in the change in patient's serial test results that can be considered significantly different at a probability of 95%. The RCV is calculated with the following formula[5,6]:

$$RCV\ (\%) = \sqrt{2} \times 1.96 \times \sqrt{[(CV_a)^2 + (CV_w)^2]} \quad (2)$$

where CV_a is the analytical CV and CV_w is the intra-individual or within-person biological CV.

For a healthcare provider to be able to conclude that a significant difference of 5 mmol/mol (0.5%-DCCT) at a medical decision point of 53 mmol/mol (7.0% DCCT) was caused by significant changes in glycemic control of a patient instead of analytical imprecision of the HbA_{1c} method, the percentage RCV should be <7.1% ([0.5/7.0] × 100 = 7.1%). This RCV results in a CV_a of 2.4% when applying a CV_w of 1%. We used a CV_w of 1%, in line with the data presented by Rohlfing et al,[9] who stated that in patients with diabetes, fluctuations in HbA_{1c} levels are not random, but should be considered pathologic, i.e., caused by changes in glycemic state. Furthermore, we calculated the RCV with a CV_w of 3.4%, in line with the data presented by Ricos et al. available on the website from Westgard[10]. Based on the

percentage RCV and the HbA_{1c} medical decision point of 53 mmol/mol (7.0%-DCCT), the absolute RCVs of the various methods were derived (in mmol/mol and %-DCCT).

To avoid discussions about commutability of lyophilized samples for certain methods with respect to systematic error (bias), we used two pooled fresh whole blood samples that were sent halfway the SKLM/WIV scheme to the laboratories, similar to the College of American Pathologists' (CAP) survey[7]. The values were assigned with five International Federation of Clinical Chemistry (IFCC) Secondary Reference Measurement procedures on two days in duplicate[11]. The acceptance limit of an allowable total error (TE_a) of 6% was used[12]. TE_a is calculated as follows:

$$TE_a\ (\%) = \text{bias}\ (\%) \pm 1.96\ CV_a\ (\%) \quad (3)$$

Statistics: Computations were performed using Microsoft® Excel 2002 (Microsoft, Redmond, WA) software.

Results

Figures 1 and 2 show the results of the ring survey of two pooled fresh whole blood samples at respective HbA_{1c} values of 41.0 mmol/mol (5.9%-DCCT) and 61.8 mmol/mol (7.8%-DCCT). Of the laboratories, 99% and 98%, respectively, met the criterion of a TE_a of <6%.

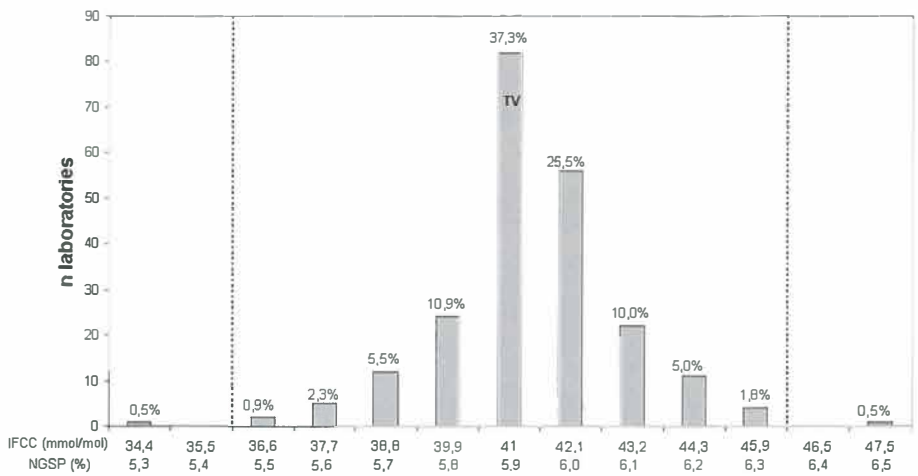

Figure 1:
Measured hemoglobin A1c results compared with the target value (TV) of 41.0 mmol/mol (5.9%-Diabetes Control and Complications Trial [DCCT]) ± allowable total error of 6%. IFCC, International Federation of Clinical Chemistry.

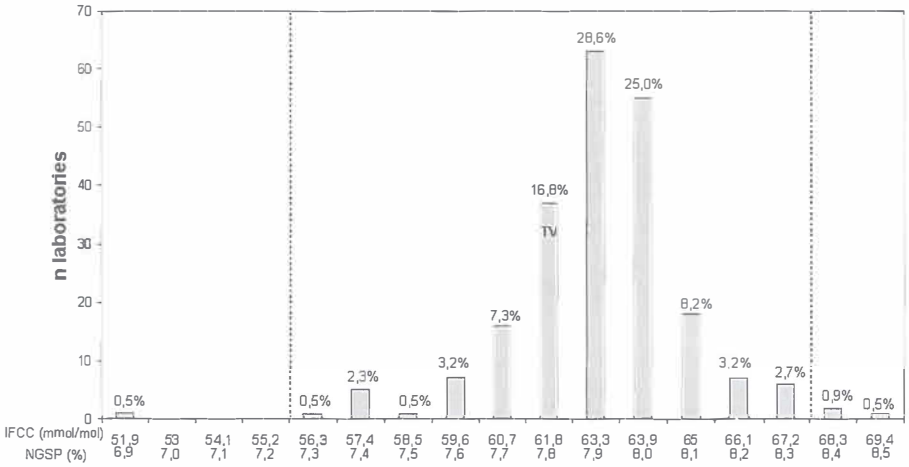

Figure 2
Measured hemoglobin A1c results compared with the target value (TV) of 61.8 mmol/mol (7.8%-Diabetes Control and Complications Trial [DCCT])± allowable total error of 6%. IFCC, International Federation of Clinical Chemistry.

The calculated CV_a in the SKML survey gives an impression of the CV used in the laboratory over a longer time, as both duplicate samples are assayed over a period of several months. Of the HbA_{1c} laboratory methods, 69%, 78%, and 86% have a CV_a of ≤2.0%, ≤2.4%, and ≤3.0%, respectively (Table 1). One of the remarkable findings is that 41.9% of the laboratories using immunoassays have a CV_a >3.0% compared with only 10.4% of the laboratories using a high performance liquid chromatography (HPLC) based method.

Table 1: Analytical Coefficient of Variation from Different Hemoglobin A1c Methods based on DCCT numbers.

	n	Range CV_a	Mean CV_a	CV_a ≤3.0%	CV_a≤2.4%	CV_a≤2.0%
Bio-Rad (HPLC)						
D-10 A1c Program	14	0.9 - 6.4	3.1	10 (71%)	8 (57%)	3 (21%)
Variant II HbA_{1c}	6	2.0 - 7.0	3.6	4 (67%)	3 (50%)	2 (33%)
Variant II Dual A1C Program	4	1.7 - 3.6	2.8	2 (50%)	1 (25%)	1 (25%)
Variant II Turbo A1C Program	8	1.6 - 2.7	2.0	8 (100%)	7 (88%)	5 (63%)
Arkray / Menarini (HPLC)						
HA-8140 DM	11	0.8 - 5.8	2.0	10 (91%)	9 (82%)	6 (55%)
HA-8160 TP	6	1.1 - 4.6	2.3	4 (67%)	4 (67%)	3 (50%)
HA-8160 VP	98	0.5 - 7.7	1.5	93 (95%)	89 (91%)	82 (84%)
Roche (Immunoassay)						
Cobas (501 -311-111)*	10	1.7 - 4.5	2.9	5 (50%)	3 (30%)	1 (10%)
Integra 400/800	14	0.7 - 14.8	3.3	11 (79%)	7 (50%)	6 (43%)
Tosoh (HPLC)						
G7	20	0.6 - 5.4	1.5	19 (95%)	18 (90%)	17 (85%)
G8	17	0.5 - 1.5	1.0	17 (100%)	17 (100%)	17 (100%)
Others#	12	1.1 - 6.1	3.0	7 (58%)	6 (50%)	6 (50%)

Bio-Rad, Hercules, CA; Arkray/Menarini, Kyoto, Japan and Florence, Italy, respectively; Roche, Basel, Switzerland; Tosoh, Tokyo, Japan.

* Whole blood and hemolysate mode.

\# Beckman Coulter (Brea, CA) Synchron LX, Beckman Unicel DxC, Siemens (Munich, Germany) Dimension RxL, Vitros 5,1 FS (Johnson & Johnson, Raritan, NJ), Trinity Biotech (Bray, County Wicklow, Ireland) Ultra2 HPLC and PDQPlus, Roche Hitachi (902-911-912-Modular) Tina-quant.

CVa, analytical coefficient of variation; HPLC, high-performance liquid chromatography.

Table 2 shows the results of the mean and the range of the absolute RCVs calculated at an HbA_{1c} value of 53 mmol/mol (7.0%-DCCT) with different intra-individual biological variations (1% and 3.4%). Fifty-nine percent of the laboratories using a method from Bio-Rad, 93% of the laboratories using a method from Arkray/Menarini, 42% of the laboratories using a method from Roche, and 95% of the laboratories using a method from Tosoh were able to meet the criterion of having a RCV of <7.1% at an HbA_{1c} value of 53 mmol/mol (7.0%-DCCT) calculated with a CV_w of 1%. Overall, almost 22% of the methods used in laboratories were not able to distinguish an HbA_{1c} result of 59 mmol/mol (7.5%-DCCT) from a previous HbA_{1c} result of 53 mmol/mol (7.0%-DCCT). This means that one in five laboratories using various HbA_{1c} methods do not meet the criteria for optimal diabetes care management.

Table 2: Absolute Reference Change Values (%-Diabetes Control and Complications Trial) at a Hemoglobin A1c Value of 53.0 mmol/mol (7.0%-Diabetes Control and Complications Trial.) Calculated with Different Intra-Individual Biological Variations

	n	CV_w 1%		CV_w 3.4%	
		Range RCV	Mean RCV	Range CV	Mean RCV
Bio-Rad (HPLC)					
D-10 A1c Program	14	0.26 - 1.25	0.63	0.68 - 1.40	0.91
Variant II HbA_{1c}	6	0.33 - 1.37	0.73	0.71 - 1.51	1.00
Variant II Dual A1C Program	4	0.38 - 0.72	0.57	0.74 - 0.86	0.86
Variant II Turbo A1C Program	8	0.37 - 0.56	0.43	0.73 - 0.84	0.77
Arkray / Menarini (HPLC)					
HA-8140 DM	11	0.21 - 1.14	0.45	0.66 - 1.30	0.79
HA-8160 TP	6	0.29 - 0.91	0.49	0.69 - 1.11	0.82
HA-8160 VP	98	0.22 - 1.50	0.36	0.67 - 1.63	0.74
Roche (Immuno-assay)					
Cobas (501 -311-111)*	10	0.38 - 0.89	0.62	0.74 - 1.09	0.89
Integra 400/800	14	0.24 - 2.87	0.68	0.67 - 2.94	0.97
Tosoh (HPLC)					
G7	20	0.23 - 1.06	0.37	0.67 - 1.24	0.74
G8	17	0.21 - 0.37	0.28	0.66 - 0.73	0.69
Others#	12	0.29 - 1.20	0.63	0.69 - 1.35	0.91

For clarity of presentation, only Diabetes Control and Complications Trial (DCCT) values are shown.
Bio-Rad, Hercules, CA; Arkray/Menarini, Kyoto, Japan and Florence, Italy, respectively; Roche, Basel, Switzerland; Tosoh, Tokyo, Japan.

* Whole blood and hemolysate mode.

\# Beckman Coulter (Brea, CA) Synchron LX, Beckman Unicel DxC, Siemens (Munich, Germany) Dimension RxL, Vitros 5,1 FS (Johnson & Johnson, Raritan, NJ), Trinity Biotech (Bray, County Wicklow, Ireland) Ultra2 HPLC and PDQPlus, Roche Hitachi (902-911-912-Modular) Tina-quant.

CV, coefficient of variation; CVw, intra-individual biological CV; HPLC, high-performance

Discussion

In this study we derived the CV_a from the individual participating laboratories in the Dutch SKML/Belgian WIV external quality scheme. Almost every laboratory was able to report HbA_{1c} results within a TE_a limit of 6%. However, based on the calculated RCVs, almost 22% of HbA_{1c} methods are not able to distinguish an HbA_{1c} result of 59 mmol/mol (7.5%-DCCT) from a previous HbA_{1c} result of 53 mmol/mol (7.0%-DCCT). This may have a profound impact on the management of patients with diabetes if changes in medication are made due to changes in serial HbA_{1c} measurements. Indeed, the International Diabetes Federation recommends starting insulin therapy above an HbA_{1c} value 58 mmol/mol (7.5%-DCCT),[13] and the ADA/EASD consensus statement states that therapy changes should be initiated if HbA_{1c} levels are above 53 mmol/mol (7.0%-DCCT)[4]. However, if the analytical variability of the laboratory assay is >2.4%, corresponding to an RCV of >5 mmol/mol (>0.5%-DCCT), a clear distinction based on patient health status between 53 mmol/mol (7.0%-DCCT) and 58 mmol/mol (7.5%-DCCT) is not possible. It is important that the limitations of current HbA_{1c} laboratory methods are understood by healthcare professionals as these may have important clinical implications.

The design of the Dutch/Belgian SKML/WIV scheme differs in approach compared to the CAP survey. The CAP survey sends three fresh pooled samples to all participating laboratories twice a year. Results are presented per method, number of laboratories applying that method, mean bias, and inter-method/laboratory CV. The current CAP acceptance limit is 8% but will be tightened to 6% in the future[12]. The design of the Dutch SKML/Belgian WIV scheme is based on 24 lyophilized interconnected samples. The advantage of lyophilized samples is the long-term stability; therefore, analytical variation can be determined over a longer period of time. However, the best way to assess the RCV is with controls, based on patient material, analyzed daily on the HbA_{1c} instrument. The results of the two pooled fresh whole blood samples showed sufficient analytical performance of almost every method used in a laboratory based on a TE_a of 6%. Although a CV_a of 3% is a realistic goal, a value of less than 2% is definitely desirable[14-16]. Indeed, our results suggest that a value of less than 2.4% should be implemented in order to be able to detect changes in HbA_{1c} levels of 5 mmol/mol (0.5%-DCCT).

We chose to use a CV_w of 1% to calculate the RCV. Ricos et al.[10] presented a CV_w of 3.4%. Applying a CV_w of 3.4% results in an absolute RCV of 5 mmol/mol (0.66%-DCCT) at a medical decision point of 53 mmol/mol (7.0%-DCCT) without taking into account analytical variation. Rohlfing et al.[9] suggested a CV_w of <1%, which seems more appropriate and was also supported by our own data (E.L.-W., unpublished data). Hence, a CV_w of 3.4% implies that the CV_a could not have a significant impact on the RCV, and therefore changes in serial HbA_{1c} would mostly rely on biological variation, which seems unlikely.

Conclusions

Thus, the analytical performance of some HbA_{1c} methods is not accurate enough to sufficiently support treatment decisions in the management of patients with diabetes when differences in serial HbA_{1c} measurements amount to 5 mmol/mol (0.5%-DCCT) or less. ADA guidelines for treatment of patients with diabetes may assume higher quality laboratory testing than might be available in the real world. Laboratories using methods with a CV_a >2.4% should consider changing to a method with better precision. In our opinion, the laboratory specific RCV should be provided to the healthcare professional in order to make this professional aware of the fact that changes in serial HbA_{1c} results might not be caused by true changes in the degree of glucose control, but also may be due to the variability of the method used to measure HbA_{1c} in a specific laboratory.

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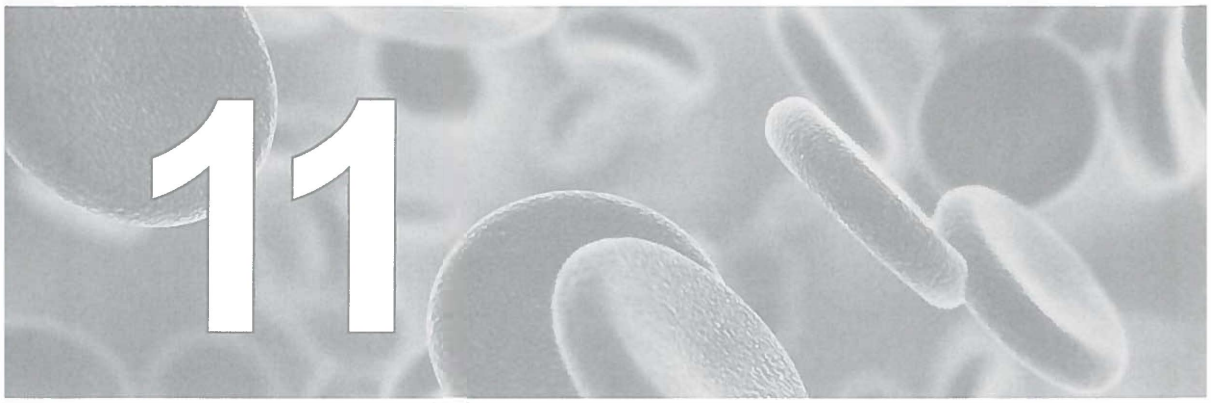


11

Interpretation of Hemoglobin A_{1c} values among different health care professionals

Erna Lenters-Westra
Roger K. Schindhelm
Robert J. Slingerland
Henk J.G. Bilo

In preparation

Abstract

Background

We assessed the daily routine regarding the use of HbA_{1c} measurement techniques, expected precision of HbA_{1c} value, and the magnitude of HbA_{1c} changes possibly eliciting treatment change advices. Therefore, we surveyed a large group of diabetes care professionals regarding these aspects.

Methods

A questionnaire with 10 questions was developed and was sent to participants through a website serving health care professionals. The survey focused on internists, pediatricians, and general practitioners ('doctors'); and diabetes specialist nurses, and primary health care practice nurses specialized in diabetes.

Results

In total 104 doctors, 177 diabetes specialist nurses and 248 primary care practice nurses responded to the survey. The majority of diabetes specialist nurses and the primary care practice nurses consider an HbA_{1c} value as an absolute value and are not aware of the fact that every HbA_{1c} result has an inherent uncertainty based on the analytical performance of the used method. Both nurses groups intended to change therapy of the patient based on very small changes in HbA_{1c} concentrations. A decrease of at least 5 mmol/mol (0.5% DCCT) or 11 mmol/mol (1.0% DCCT) at an HbA_{1c} value of 75 mmol/mol (9.0%-DCCT) after adjustment of therapy, is considered as sufficient by all health care professionals to allow the conclusion that glucose regulation has improved. In contrast, even a very small or no increase in HbA_{1c} is considered by the majority of the diabetes specialist nurses and primary care practice nurses as significant enough to allow the conclusion that glucose regulation has worsened. Most of the doctors adhere to change of 5 mmol/mol (0.5%-DCCT) as a clinically meaningful cut-off point. Approximately 35% of the health care professionals use HbA_{1c} in combination with fasting glucose for the diagnosis of diabetes.

Conclusions

There is a significant difference in interpretation of changes in HbA_{1c} results between doctors and diabetes specialist nurses/primary care practice nurses. In general, nurses consider therapy changes based on very small changes in HbA_{1c}, whereas doctors preferably agree to the clinically relevant change of 5 mmol/mol (0.5%-DCCT). Changing therapy based on relatively small changes in HbA_{1c} might lead to undue adjustments in the treatment of patient with diabetes, also in the light of the fact that the analytical performance of some of the HbA_{1c} methods is not precise and reliable enough to warrant changes in therapy based on differences less than 5 mmol/mol (0.5%-DCCT).

Introduction

Both in subjects with type 1 diabetes mellitus (T1DM) and with type 2 diabetes mellitus (T2DM), glucose control is considered of major importance. On short term, adequate glucose control is associated with disappearance for symptoms of hyperglycemia, preferably without an increase in the occurrence and symptoms of hypoglycemia. On longer term, adequate metabolic control is associated with a lower incidence and prevalence of microvascular and macrovascular complications, both in T1DM and in T2DM[1,2]. Furthermore, good metabolic control in the early years after diagnosis is associated with long term beneficial effects.

The degree of glucose control can be measured by frequent home blood glucose measurements, but the most widely acknowledged and reliable assessment is considered to be the measurement of the concentration of Hemoglobin A1c (HbA_{1c}). As such, HbA_{1c} was and is also one of the main parameters in regard to glucose control in most outcome studies[1-3]. Therefore, most diabetes care professionals rely (at least in part) on HbA_{1c} level to decide, whether treatment changes are to be advised to patients or not. As such, they presume HbA_{1c} measurements to be reliable and precise enough to allow such decisions.

In an earlier study, we showed that most point-of-care methods to measure HbA_{1c} are not precise enough to allow an estimate within 5 mmol/mol (5.0%-DCCT) of the actual value, based on an actual values of 53 mmol/mol (7.0%-DCCT)[4]. A recent study showed, that, even in The Netherlands, HbA_{1c} in 20% of the laboratories also cannot be seen as precise enough to allow an outcome within 5 mmol/mol (0.5%-DCCT) of the actual value[5]. Such facts have practical consequences and should be known to diabetes care professionals and patients. For example, in the Netherlands, an HbA_{1c} > 53 mmol/mol (7.0%-DCCT) in a person with T2DM on maximal oral therapy would be a reason to consider starting insulin therapy. Such treatment changes often have profound consequences for both patients and diabetes care workers. This also means that HbA_{1c} measurement should indeed be very precise and trustworthy.

After an informal and limited initial survey we found, that most diabetes care professionals would accept a degree of precision within 2.0 mmol/mol (0.2%-DCCT) of the actual value, and that treatment changes would be considered with HbA_{1c} changes of 5 mmol/mol (0.5%-DCCT) or more compared to earlier levels (results not shown).

The aim of this study was to assess the daily routine regarding use of HbA_{1c} measurement techniques, expected precision of HbA_{1c}, and the magnitude of HbA_{1c} changes possibly eliciting treatment change advices. Therefore, we surveyed a large group of diabetes care professionals regarding these aspects.

Materials and methods

First, a questionnaire was developed, which was tested in 10 diabetes care professionals. Second, the list was sent to participants in a health care professional website (http://www.diabetes2.nl).
The survey was meant for internists, pediatricians, general practitioners (referred together as doctors) diabetes specialist nurses (DSN), and primary health care practice workers specialized in diabetes (PCPN). The exact amounts of respondents are shown in table 1.

Table 1: List of participants

	Total	Completed questionnaires
Internists	48	93.8%
General practitioners	28	96.4%
Pediatricians	28	42.9% *
Total doctors	104	
Diabetes specialist nurses (dsn)	177	100%
Primary care practice nurses (pcpn)	248	99.6%
Total	529	

*Due to the questions specifically directed towards T2DM, part of the questions were inappropriate for pediatricians

Statistical analysis

The answers on the questionnaire were reported as bar charts showing the percentages for each group of health care professionals. Differences between the groups were tested using Fisher's Exact test. P-values < 0.05 were considered significant. SPSS version 19 was used for the analysis.

Results

Question 1

Which method do you use to measure HbA_{1c} in your practice? Answers on this question are presented in Table 2.

Table 2: HbA_{1c} measurement methods in various groups

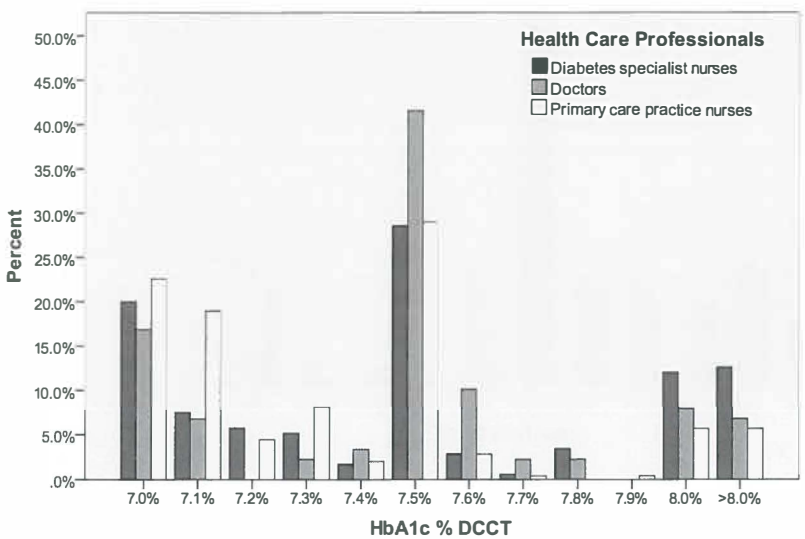
	Point-of-care	Laboratory	Both
Doctors	2.9%	95.1%	2.0%
Diabetes specialist nurses	3.3%	96.7%	
Primary care practice nurses	1.2%	98.8%	

Table 2 show that in, the Netherlands, POC instruments are not widely used for the measurement of HbA_{1c}.

Figure 1 Answers on question 2:

When someone with T2DM and < 70 years is on maximal oral therapy and you consider starting insulin, at which HbA1c level you decide to start insulin?

(For clarity reasons only DCCT values are shown, p<0.001 Fisher' Exact test)



Answers on question 2 do indeed show that, with a cut off point of 53 mmol/mol (7.0%-DCCT) as a signal for treatment changes, a level of 58 mmol/mol (7.5%-DCCT) is indeed seen as a sufficiently powerful signal to actually consider starting insulin which is in line with the IDF guidelines [6].

Figure 2 Answers on question 3:

Consider someone with T1DM (<70yrs) without signs or complaints of hypo- or hyperglycemia. HbA1c was 6.9% at the previous visit. After three month you get a new result. At which HbA1c value you would consider and propose a treatment adjustment?

(For clarity reasons only DCCT values are shown, p<0.001 Fisher's Exact test)

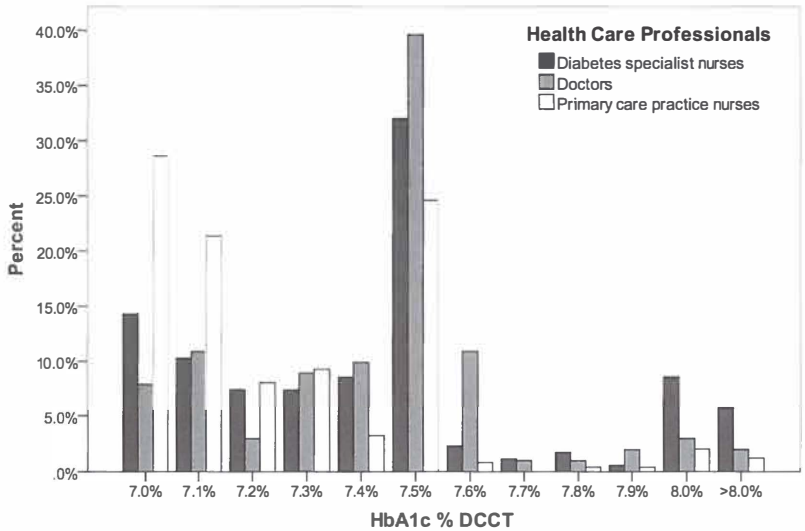
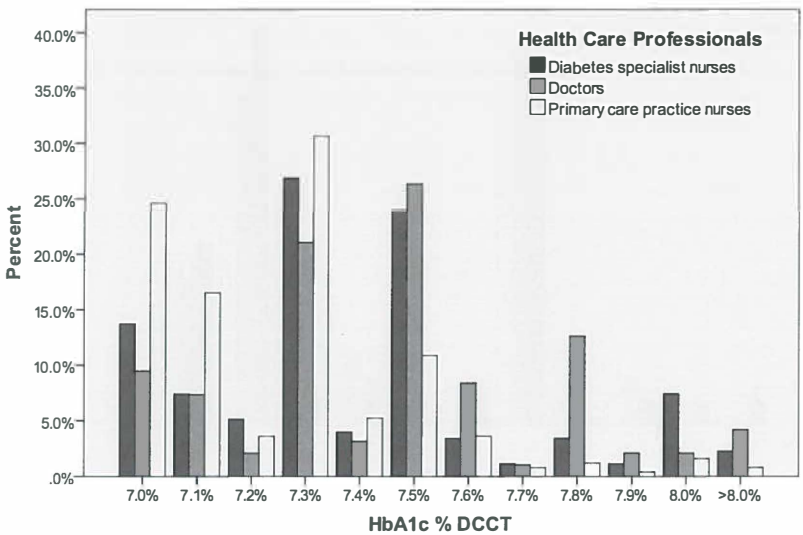


Figure 2 shows that primary care practice nurses (PCPN) are trained to strictly follow guidelines. The guidelines of the American Diabetes Association (ADA) and European Association for the Study of Diabetes (EASD) consensus algorithm for the initiation and adjustment of therapy states that a sustained HbA_{1c} level above 53 mmol/mol (7.0%-DCCT) should prompt the health care provider to consider changing therapy in order to reach the predefined target value[7]. This might explain why 28% of the PCPN react on a difference of 0.1% compared to the previous HbA_{1c} result. However, they are probably not aware of the fact that a result of 52 mmol/mol (6.9%-DCCT) is statistically the same as a result of 53 mmol/mol (7.0%-DCCT)[5]. A difference of 5 mmol/mol (0.5%-DCCT) between two consecutive results is considered by many as clinically significant. In figure 2 we see that most of the health care professionals react with a difference of 6 mmol/mol (0.6%-DCCT). However, there seems to be a kind of tendency to consider cut-off points rounded to whole or half percentage points.

Figure 3 Answers on question 4:

Consider someone with T2DM (<70yrs) without signs or complaints of hypo- or hyperglycemia and treated with a combination of insulin and metformin. Three months previously, HbA1c was 7.3%. Insulin dose was increased. At which HbA1c level you would consider further treatment changes?

(For clarity reasons only DCCT values are shown, p<0.001 Fisher's Exact test)

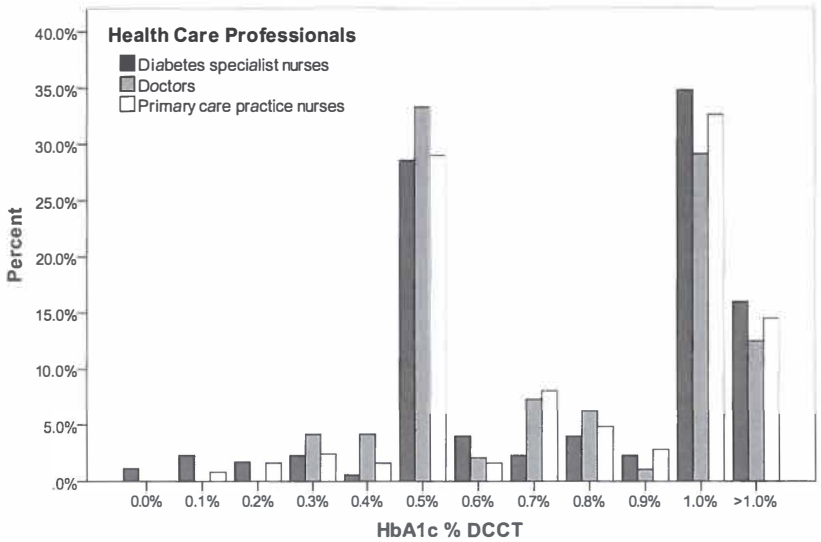


Question 4 provides a somewhat mixed answer, with care givers tending to either start treatment changes with an HbA_{1c} which stays at a consistently higher level of 56 mmol/mol (7.3%-DCCT), or – again – at the cut-off point of 58 mmol/mol (7.5%-DCCT) and 53 mmol/mol (7.0%-DCCT). Again PCPN are more focused on trying to reach lower HbA_{1c} values than doctors, specifically to reach the treatment goal of 53 mmol/mol (7.0%-DCCT).

Figure 4 Answers on question 5:

Consider someone with T2DM and an HbA1c value of 9.0%. Treatment adjustments are made. How much decrease in HbA1c value you would consider sufficient to allow the conclusion that glucose regulation has improved?

(For clarity reasons only DCCT values are shown, p=0.28 Fisher's Exact test)



Question 5 addresses the point, what change in HbA_{1c} is considered meaningful after treatment adjustment. Most professionals – again – consider 5 mmol/mol (0.5%-DCCT) as clinically relevant. There is no significant difference in answers between the different health care professionals (doctors, DSN and PCPN) but, again, there seems to be a kind of tendency to consider cut-off points rounded to whole or half percentage points.

Figure 5 Answers on question 6:

Again consider someone with T2DM and an HbA1c value of 9.0%. Treatment adjustments are made. How much increase in HbA1c value you would consider sufficient to allow the conclusion that glucose regulation has worsened?

(For clarity reasons only DCCT values are shown, p<0.001 Fisher's Exact test)

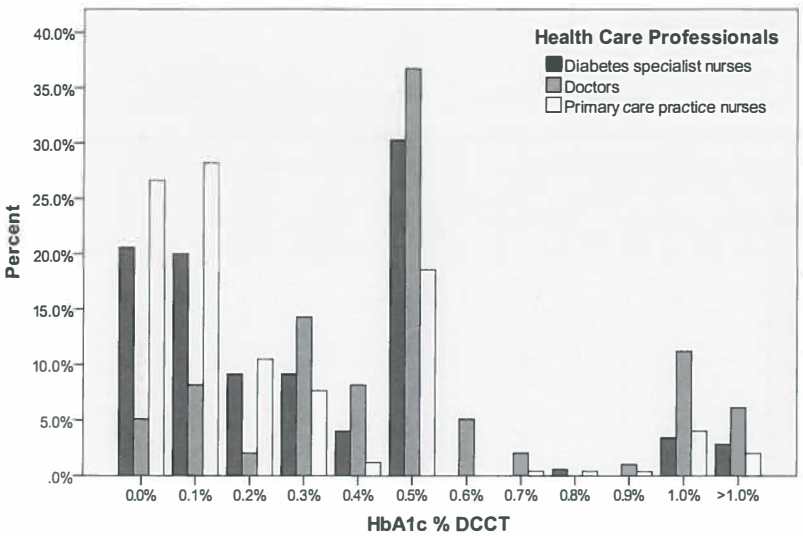
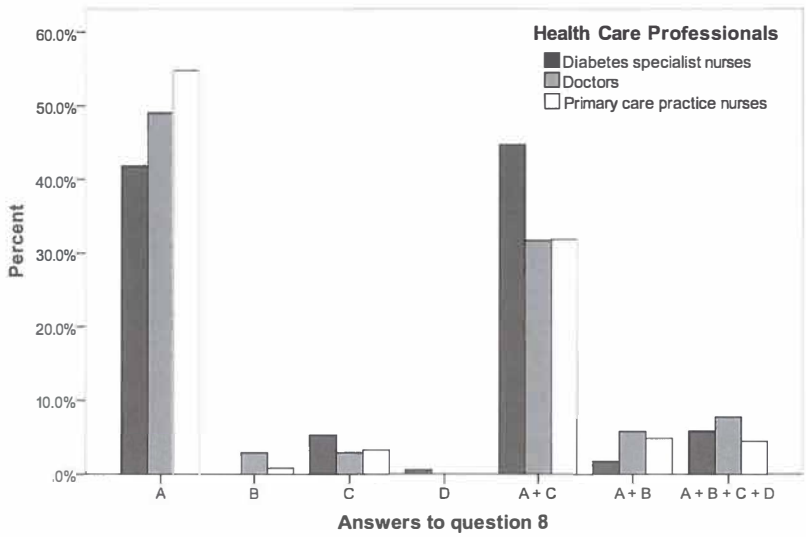


Figure 5 shows that, especially DSN and PCPN, are tend to conclude that glucose regulation has worsened even when the HbA_{1c} value was the same or slightly increased. The majority of the doctors stick to the clinically relevant change of 5 mmol/mol (0.5%-DCCT).

Figure 6 Answers on question 7:

I use the following test(s) to diagnose diabetes mellitus:
A: fasting glucose in laboratory
B: fasting glucose with point-of-care method
C: HbA1c measurement in laboratory
D: HbA1c measurement with point-of-care method

(p=0.02, Fisher's Exact test)



The majority of the health care professionals follow the guidelines for the diagnosis of diabetes based on a fasting glucose measured in a laboratory and approximately 35% is using HbA_{1c} in combination with fasting glucose for the diagnosis of diabetes.

Figure 7 Answers on question 8

At an HbA1c level of 7.0% DCCT I expect a reliability within a margin of:…

(For clarity reasons only DCCT values are shown, p<0.001 Fisher's Exact test)

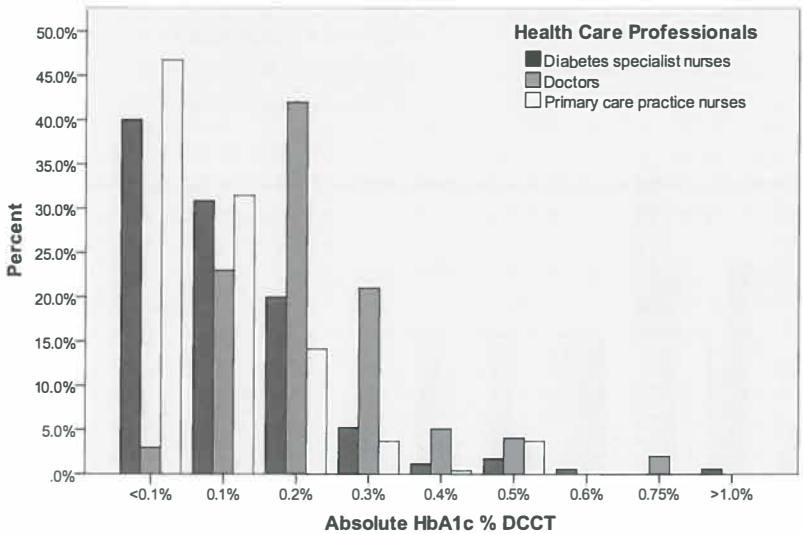


Figure 7 shows that most of the DSN and PCPN consider an HbA_{1c} value an absolute value and are not aware of the fact that every HbA_{1c} results know some uncertainty based on the analytical performance of the used HbA_{1c} method.

Figure 8 Answers on question 9:

At an HbA1c level of 9.0% DCCT I expect a reliability within a margin of:..

(For clarity reasons only DCCT values are shown, p<0.001 Fisher's Exact test)

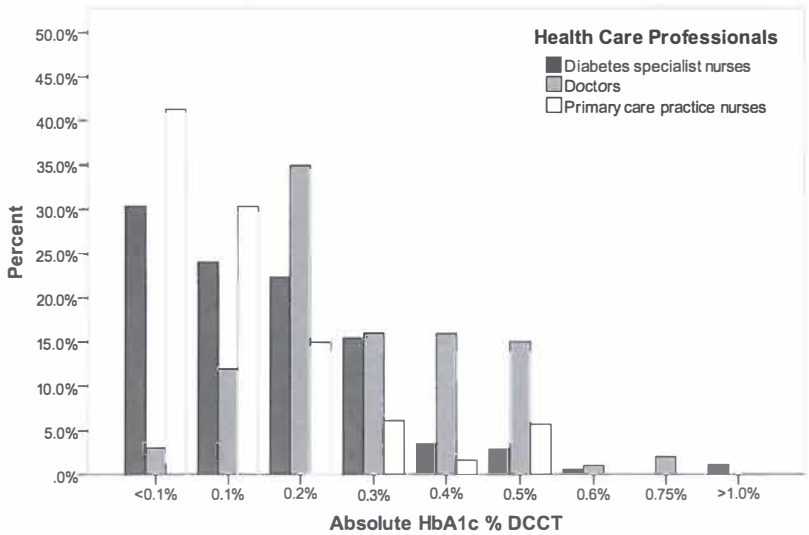


Figure 8 shows the same results as figure 7.

Discussion

Guidelines are necessary to define treatment modalities and treatment goals, as well as to assure quality in diabetes care management. When adhered to properly, they are meant to guarantee that every patient will be treated in more or less the same fashion. In the Netherlands there is an unique system, where the majority of the diabetes patients in primary care are seen and controlled by primary care practice nurses for most of the time[8]. These primary care practice nurses are trained to take over special tasks of the general practitioner and one of these tasks is the management of patients with diabetes. In general, when patients are difficult to manage or with other concomitant diseases, they will be referred to special diabetes secondary care centres. All health care professionals are supposed to work with the guidelines for the management of patients with diabetes. However, the results presented in this paper show that nurses are stricter in following protocols than doctors. This could partly be explained by the fact that most of the nurses consider an HbA_{1c} value as an absolute value and are not aware of the fact that every HbA_{1c} result know some uncertainty based on the analytical performance of the HbA_{1c} method used (Figure 7 and 8). As a consequence, nurses tend to consider treatment changes based on very small or even no difference in subsequent HbA_{1c} results. Figure 4 and 5 shows also that doctors and nurses interpret HbA_{1c} differently when concluding that there is a worsening or improvement of glycemic control. A decrease of at least 5 mmol/mol (0.5%-DCCT) or 11 mmol/mol (1.0%-DCCT) at an HbA_{1c} value of 75 mmol/mol (9.0%-DCCT) after adjustment of therapy, is considered sufficiently by all health care professionals to allow the conclusion that glucose regulation has improved. In contrast, a very small or no increase of HbA_{1c} is considered by most of the diabetes specialist nurses and primary care practice nurses as sufficiently to allow the conclusion that glucose regulation has worsened. These results were in line with the literature [9,10].

In general, guidelines consider a difference of 5 mmol/mol (0.5%-DCCT) as clinically significant. However, a recent study showed that the analytical performance of some HbA_{1c} methods is not accurate enough to sufficiently support treatment decisions in the management of patients with diabetes when differences in serial HbA_{1c} measurements amount to 5 mmol/mol (0.5%-DCCT) or less[5]. Another study showed that six of eight HbA_{1c} POC instruments do not meet the general accepted performance criteria[6]. Combining this with the outcome of this survey, we can conclude that most of the primary care practice nurses and diabetes specialist nurses may react on HbA_{1c} outcome variations based on the variability of the HbA_{1c} method used instead of the true changes in the degree of glucose control. As a consequence, this could lead to undue treatment changes with accompanying costs and/or inconvenience for the patient. Also several studies have confirmed that, especially for older patients, the benefit of lowering the HbA_{1c} value at every cost (patient inconvenience) is limited and may even lead to a higher mortality rate[3,11,12].

Mean HbA_{1c} of patients with diabetes in primary health care in the Netherlands is amongst the lowest in the world[8], and studies like the DCCT and the UKPDS showed very clearly that strict controlled patients have a lower risk on developing microvascular and macrovascular complications[1,2]. Therfore, there should be a

reasonable balance between treating the patient in as well as possible and feasible, and overmanaging of the patient.

To achieve this, we believe that every health care professional should be supplied with the information they need to interpret HbA_{1c} values properly. The reference change value (RCV), which is defined as the critical difference between two consecutive HbA_{1c} measurements representing a significant change in health status, might be a valuable tool[13,14]. The analytical CV (CVa) of the used HbA_{1c} method and the within person biological CV (CVw) are necessary to calculate the RCV (RCV (%) $= \sqrt{2} \times 1.96 \times \sqrt{[(CV_a)^2 + (CV_w)^2]}$). The analytical performance of different HbA_{1c} methods is ranging from poor (most of the POC instruments and some immunoassays) to state of the art (newer version cation-exchange HPLC)[4,5,15,16]. It is not realistic to assume that every health care professional knows the analytical performance of every HbA_{1c} method, not even the method used by his or her main providing laboratory.

Directors of laboratories or other decision makers are responsible for the choice of the HbA_{1c} method. This choice is based on many factors like analytical performance (which is hopefully the most important factor), sample throughput (commercial labs), costs per test, support of and contact with the manufacturer etc. The RCV makes clear what the impact is of bad performing methods and is hopefully a stimulant for directors of laboratories to choose for a method with acceptable analytical performance which allows changing therapy based on small changes in HbA_{1c} values.

More exchange of knowledge is necessary between clinical chemistry where the results are produced and diabetes medicine where the results are interpreted. The clinical chemist can help clinical decision making by providing healthcare professionals with the necessary information (RCV) to properly interpret HbA_{1c} results.

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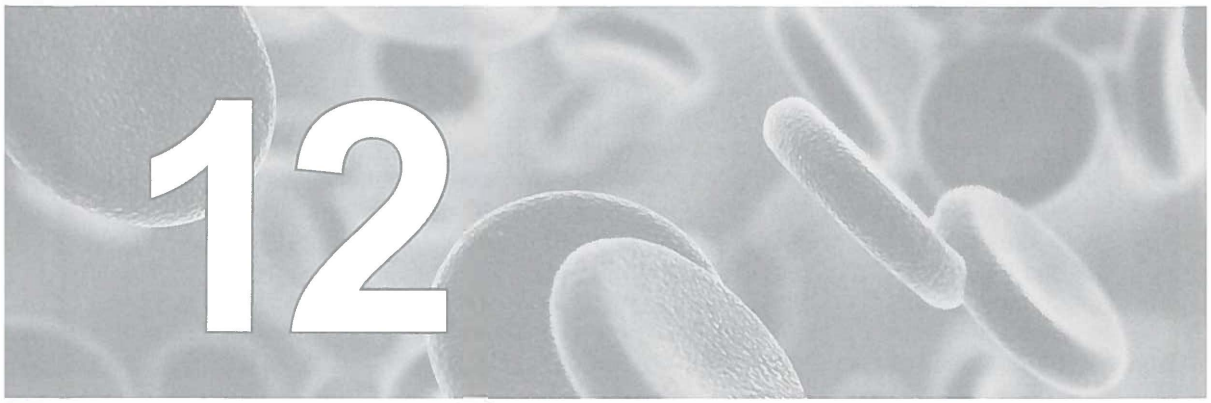

Summary, conclusions, recommendations and future perspectives

Summary

Since the discovery of the relation between increased concentrations of fast hemoglobin-fractions in patients with diabetes compared to such concentrations in subjects without diabetes by Samuel Rahbar and co-workers in 1969, glycated hemoglobin A1c (HbA_{1c}) has become a "golden standard" as one of the main parameters in the glucose management in patients with diabetes. Recently, HbA_{1c} has been advocated as a diagnostic marker for diabetes as a result of global standardisation of the HbA_{1c} assay, which further underlines the importance of HbA_{1c}. Currently, there are more than 30 methods available on the market with an analytical performance ranging from poor to precise enough to allow widespread use.

In general it is assumed, that when an HbA_{1c} method has a National Glycohemoglobin Standardization Program (NGSP) certification, the analytical performance is adequate. Given the fact, that even HbA_{1c} assays with poor analytical performance are certified, such an assumption can be challenged. In this thesis, the analytical performance of different HbA_{1c} methods is investigated with a focus on point-of-care (POC) HbA_{1c} instruments. Also the interpretation of HbA_{1c} values among different health care professionals was investigated.

Chapter 1 is a historical overview of literature data and personal views on HbA_{1c} in the management and diagnosis of diabetes mellitus. It covers the story of HbA_{1c} from the early beginning (discovery of fast moving hemoglobin fractions) till where are we now (more than 30 different methods on the market), including the world wide efforts directed towards standardisation of HbA_{1c}.
The analytical performance of different HbA_{1c} methods, including point-of-care instruments, is discussed, based on literature and external quality schemes, and proposed analytical goals (coefficient of variation <3.0% (based on IFCC values), coefficient of variation <2.0% (based on DCCT values), bias ≤ 2.0 mmol/mol (≤ 0.24% DCCT)).
In view of analytical performance of the HbA_{1c} method, we can conclude that considerable progress have been made, largely due to the efforts of the NGSP and the IFCC working group for the standardisation of HbA_{1c} in cooperation with manufacturers. However, both from the perspective of individual patients, and based on the required accurate performance when aiming to use HbA_{1c} as diagnostic parameter, we believe that the analytical performance of some HbA_{1c} methods is insufficient.

In **chapter 2** we describe the Hemoglobin A_{1c} determination in the A1C-Derived Average Glucose (ADAG) study. We also investigated whether off-line calibration with IFCC secondary reference material could improve the precision of the HbA_{1c} determination. The value assignment in the ADAG-study was carried out with four IFCC certified secondary reference methods with three different measurement principles. By using four different methods, the impact of the individual matrix effect on the ultimate result is minimized. Some samples yielded different results with a particular method. This, so-called, matrix effect is minimized by using the mean of the four methods. Also, information given by the Tosoh G7 method has led to the exclusion of samples with ageing or interference substances which would have

influenced the value determination if only one method, not free from interferences, was used for value determination.
Additional off-line calibration improved the 95% confidence interval between the four different HbA_{1c} methods at HbA_{1c} of 6.00% from ± 0.28% (5.72% - 6.28%) to ± 0.20% (5.80% - 6.20%) and at HbA_{1c} of 9.00% from ± 0.43% (8.57% - 9.43%) to ± 0.24% (8.76% - 9.24%). Also the coefficient of variation of the four methods used in this study after off-line calibration with secondary reference material, were all ≤1.9% as proposed in chapter 1.
We can conclude that the HbA_{1c} results used in the ADAG study were determined with the current lowest uncertainty technically feasible and as close as possible to the IFCC primary reference method by using four IFCC certified secondary reference methods and additional off-line calibration with IFCC secondary reference material to correct for insufficient performance of some assays.

Point-of-care (POC) HbA_{1c} instruments are used more frequently. So far, the consequences of the introduction of these new types of instruments with their specific characteristics have not been discussed thoroughly in the literature. Limited information is available regarding the analytical performance of POC instruments that measure HbA_{1c}. In addition, also for POC instruments it is not fully clear whether NGSP certification ensures the accuracy of every instrument used in the field. We evaluated eight different HbA_{1c} point-of-care instruments. The results of these studies are described in **chapter 3 to 6.**

The manufacturer of the A1CNow did not agree with the conclusions in the first study (**chapter 4**) noting that EDTA blood was used, which was in accordance with previous performed studies but not in accordance with the current manufacturer recommendations. Manufacturers of Quo-Test, Afinion and In2it have claimed that improvements were made to these methods since the original evaluation. Therefore, these four instruments were re-examined in either one or two different NGSP laboratories. Results are described in **chapter 5 and 6**.

The appropriateness of these studies lies in the fact that we used certified Clinical and Laboratory Standards Institute (CLSI) protocols and compared the results with 3 NGSP and IFCC secondary reference methods and with the mean of the three reference methods.

The coefficient of variation of the evaluated POC instruments ranged form 1.4% (Afinion) to 5.9% (Quo-Test). Only two instruments (DCA Vantage and Afinion) had an acceptable, but still not optimal, coefficient of variation of < 2.4% in the clinically relevant range.

Except for the InnovaStar, all investigated POC instruments were NGSP certified. In the original study only two POC instruments (DCA Vantage and Afinion) were able to pass the 2009 NGSP criteria with two different lot numbers compared with just one secondary reference method. In the most ideal situation, the methods should pass the NGSP criteria compared with different secondary reference methods and with different lot numbers. The method comparison results and the calculations of the NGSP certification showed significant differences in analytical performance between different reagent lot numbers for all HbA_{1c} POC instruments and were largest for the

Clover and the Quo-Test (differences between two lot numbers of approximately 0.85% DCCT). In this thesis, we were able to show that passing or failing the NGSP criteria depends on the choice of secondary reference method to compare with, and on which lot number was used. This became especially clear when we re-examined three of the previously investigated methods in either one or two different NGSP laboratories (**chapter 6**). Therefore, questions can be raised on the usability and meaning of this certification program. It should be noted that the NGSP requires that manufacturer certification is performed only once per year, and with only one reagent lot. The manufacturer is obliged to ensure the consistency among different lots. In this thesis we have demonstrated that an NGSP certification does not guarantee consistency among different reagent lots.

Not only the lot number dependency was a problem, also the bias with the secondary reference measurement procedures was a problem. Freedom from bias is critical because fixed cut off points are being used as targets for glycaemic control (e.g. HbA_{1c} <53 mmol/mol, <7.0% DCCT) and diagnosis of diabetes mellitus (≥48 mmol/mol, ≥6.5% DCCT). The bias found in our study ranged from -0.99% to +0.41% DCCT compared with one reference measurement procedure. If such biases were present and accepted in diagnostic testing, either tens of millions of people would be wrongly diagnosed with diabetes, or millions would wrongly remain undiagnosed.

In summary we can conclude that currently the majority of available POC testing devices for HbA_{1c} do not meet generally accepted analytical performance criteria, and may therefore significantly underestimate or overestimate the actual degree of Hb glycation. Until these analytical performance issues have been addressed properly, we recommend against the use of POC testing of HbA_{1c} as a tool influencing treatment decisions, or in the diagnosis and screening of pre-diabetes and diabetes. Our study showed that only the DCA Vantage and the Afinion can be used for monitoring of the patient, and then only under strict conditions (see recommendations).

In **chapter 7** we investigated the performance of a laboratory based method (Menarini/ARKRAY ADAMS A1c HA-8180V analyser for HbA_{1c}). The results of this investigation are in contrast to the evaluation results of most of the point-of-care instruments; performance of this analytical method was state of the art. The total coefficient of variation of the HA-8180 at low and high HbA_{1c} concentrations was 0.7% and 0.4%, respectively based on DCCT numbers. Trueness (bias) revealed a maximum deviation of 0.8 mmol/mol or 0.1% DCCT over the relevant analytical range. Linearity, carry-over and linear drift were excellent. There was no interference of labile- HbA_{1c}, carbamylated hemoglobin, icteric samples and variation in haematocrit did not affect HbA_{1c} outcome. Hemoglobin variants AS, AC and F did not affect HbA_{1c} outcome. However, HbA_{1c} can not be measured in samples with AE and AD, but these abnormalities were recognised with an abnormal chromatogram. The conclusion is that the HA-8180V performs at a consistently high level and is fit for any clinical application.

In **chapter 8** we ask attention for the analytical performance of different HbA_{1c} methods, including point-of-care instruments, when using these methods for the diagnosis of diabetes. We strongly recommend reporting the analytical performance

of the HbA_{1c} method in studies assessing the diagnostic value of HbA_{1c} in the diagnosis of diabetes mellitus. Furthermore, we think, that healthcare professionals should be provided with the same information to be able to properly interpret laboratory and point-of-care HbA_{1c} results.

Clinical biochemists can and should play a prominent role in this matter and should be encouraged to use HbA_{1c} methods with optimal analytical performance (no bias and a total coefficient of variation of < 3% (based on IFCC numbers), <2% (based on DCCT numbers)).

Chapter 9 also focuses on the potential role of point-of-care testing of glucose and HbA_{1c} in the diagnosis of pre-diabetes and diabetes. It gives an overview of the principles, pitfalls and analytical performance of glucose and HbA_{1c} point-of-care testing and summarizes the studies that have applied point-of-care testing of glucose and HbA_{1c} in the diagnosis of (pre-) diabetes.

The effectiveness of method standardisation and analytical performance of an HbA_{1c} method can be judged by examining external quality schemes. External quality schemes reveal the aggregated results of different methods. The mean bias of a method reveals the effectiveness of method standardisation and the inter-laboratory CV is a proxy for the precision of a method. On average, the analytical performance of most laboratory based methods seems adequate. Unfortunately, we do not know the analytical performance of individual laboratories using various methods for the determination of HbA_{1c}. Thanks to the whole-hearted cooperation of two External Quality Assurance Services (EQAS): the Stichting Kwaliteitsbewaking Medische Laboratoria (SKML) in the Netherlands and the Wetenschappelijk Instituut voor de Volksgezondheid (WIV) in Belgium, we were allowed to use the individual coefficient of variation results of 220 laboratories using various HbA_{1c} methods (**Chapter 10**). This external quality scheme is different in design in comparison to other external quality schemes. Most EQAS programs, including the College of American Pathologists (CAP) survey, use fresh pooled patient blood. The SKML uses 24 lyophilised interconnected samples (12 samples in duplicate) which have to be analyzed during the course of one year (one sample per fortnight). After one year, the precision, accuracy, linearity and deviation from IFCC primary reference method can be calculated. The precision (coefficient of variation) was used to calculate the Reference Change Value (RCV) of 220 individual laboratories using various methods. A coefficient of variation of <2.0% (based on DCCT numbers) is necessary to be able to meet the clinical significant difference of 5 mmol/mol (0.5%-DCCT) if a within person biological variation of 1.8% is taken into account. Sixty five percent of the laboratories had a coefficient of variation of <2.0%. This implies that 1 in 3 laboratories using various methods is not able to distinguish an HbA_{1c} result of 59 mmol/mol (7.5%-DCCT) from a previous HbA_{1c} result of 53 mmol/mol (7.0%-DCCT). If one takes a within person biological variation of 1.0% into account, in line with the data from Rohlfing et al, 21.8% of the laboratories using various HbA_{1c} methods are not able to distinguish an HbA_{1c} result of 59 mmol/mol (7.5%-DCCT) from a previous HbA_{1c} result of 53 mmol/mol (7.0%-DCCT). One of the remarkable findings was that 41.9% of the laboratories using immunoassays had a coefficient of variation >3.0% compared with only 10.4% of the laboratories using a high performance liquid chromatography (HPLC) based method.

Most diabetes care professionals rely (at least in part) on HbA_{1c} level to decide, whether treatment changes are to be advised to patients or not. As such, they presume HbA_{1c} measurements to be reliable and precise enough to allow such decisions. We noticed a gap in knowledge between clinical chemistry and the healthcare professionals. Therefore, to assess the daily routine regarding use of HbA_{1c} measurement techniques, expected precision of HbA_{1c}, and the magnitude of HbA_{1c} changes possibly eliciting treatment change advices, we surveyed a large group of diabetes care professionals regarding these aspects. In **chapter 11** we present the results of this survey. The survey showed that there is a difference in interpretation of changes in HbA_{1c} results between doctors and diabetes specialist nurses/primary care practice nurses. In general, nurses consider therapy changes based on very small changes in HbA_{1c}, whereas doctors preferably agree to the clinically relevant change of 5 mmol/mol (0.5% DCCT). Changing therapy based on small changes in HbA_{1c} (<5 mmol/mol, <0.5% DCCT) might lead to overmanagement of patient with diabetes, also due to the fact that the analytical performance of most of the HbA_{1c} methods is not precise and reliable enough to offer a well-founded rationale for such decisions.

Conclusion

The variation in analytical performance of different HbA_{1c} methods is huge, ranging from poor (most point-of-care instruments and some immunoassays) to state of the art (new version cation-exchange HPLC's). Health care professionals, especially diabetes specialist nurses and primary care practice nurses, expect better analytical performance than is possible for most HbA_{1c} methods and may therefore underestimate or overestimate the risk of diabetes.
The healthcare professionals should be provided with the information they need (Reference Change Value) to properly interpret laboratory and point-of-care HbA_{1c} results. The clinical biochemist can play a valuable role in this matter and should be encouraged to use HbA_{1c} methods with optimal analytical performance (no bias and a total coefficient of variation of <3% (based on IFCC numbers), <2% (based on DCCT numbers).

Recommendations for introduction of HbA_{1c} POC instruments

Although the use of POC HbA_{1c} instruments may have some negative consequences which need to be addressed, it is also important to keep in mind that obtaining HbA_{1c} results at the time of the patient's visit can contribute to the improvement of patient wellbeing and care. Currently, diagnosis and follow-up of people with diabetes is done in a variety of outpatient facilities, varying from primary care general practice offices to tertiary special diabetes care centres. Many patients have their blood drawn a week before they visit the physician to ensure that laboratory results are available for appropriate clinical action. By providing results rapidly following blood collection, POC instruments will minimize patient inconvenience by preventing the need for a laboratory visit, and possibly avoid an extra visit to the clinic. Studies have confirmed that immediate feedback of HbA_{1c} results improves glycaemic control in patients with type 1 and insulin-treated patients with type 2 diabetes mellitus [1-3].

Based on the experience in our hospital (Isala klinieken, Zwolle, The Netherlands) we recommend the following prerequisites for the introduction of an HbA_{1c} POC instrument:

1. HbA_{1c} POC instruments should fall under responsibility of the Central Laboratory.
2. Acceptable analytical performance (ideally: no bias, coefficient of variation <3.0% (based on IFCC numbers), <2.0% (based on DCCT numbers)). Validation of instrument by Central Laboratory.
3. Connectivity to the Central Laboratory for data management.
4. Education and training for users should be done by experienced POC coordinators (e-learning).
5. Only accredited users are allowed to use the instrument.
6. Internal and external quality control should be coordinated by the POC coordinator.
7. Ordering and control of reagent/cartridges will also be done by POC coordinator (check of new lot number!!).
8. Once a year HbA_{1c} on laboratory method.

References

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Future perspectives

State of the art Point-of-Care

In the past, special skills were necessary to run an HPLC instrument. Nowadays HPLC's have become easier to use. For example, the Tosoh G8 has advanced software which interprets the chromatogram. Results from samples which show a normal chromatogram and no other errors are sent directly to the laboratory information system. Only samples with a "problem" (abnormal chromatogram, technical problems, etc.) are shown in a worklist and should be assessed, and when necessary resolved by a technician. This means that, provided there is a good standard operation procedure and good support from the manufacturer/distributor, samples could be analyzed reliably by health care professionals in the diabetes care centre with supervision and support of a central laboratory. This could prove to be an example of clinical chemistry cooperating with health care professionals, thus facilitating patient centred care. In this way an HbA_{1c} result at the point of care can be achieved, using a state of the art instrument. If it is not possible to assist the health care professional in an optimal way when there is a problem with the instrument due to large distance between the laboratory and the diabetes care centre, one could choose for a POC instrument from Siemens (DCA Vantage) or Axis-Shield (Afinion). However, based on the results of this thesis, it is recommended to follow the terms for introduction stated on page 151 when choosing one of these instruments.

Lab on a Chip

Developments in nanotechnology are going very fast. It should come as no surprise that scientists and producers are already busy trying to develop an HbA_{1c} method which can be determined on a chip: HPLC on nano-level. The challenge for such a development is that the analytical performance of these chips should be equal or even better than laboratory based methods, otherwise it has no future. Scientists from different fields have to work together to make this a success. In the far future when such an approach has proven its value, it can be imagined that patients will determine their HbA_{1c} at home and that results will be shared with the health care professional, using an internet or mobile phone connection.

Future studies

Studies in the past have confirmed that introduction of new numbers can cause confusion and deterioration of glycaemic control. Therefore a new international study should be conducted to investigate the clinical implications of the implementation of the new IFCC numbers.

Also, the reference values with IFCC numbers should be investigated and established in a large study with healthy persons from different parts of the world and from different ethnic groups. A preliminary reference range study has been carried out in 2002 by utilising EDTA-washed red cells collected from a Danish population study (DiaRisk, Steno Diabetes Centre, Copenhagen, Denmark). The preliminary

reference range for HbA_{1c} as measured in this study using the reference methods was 33.3 ± 4.8 mmol/mol (mean ± 2 SD) or 4.8% to 5.6% DCCT. Currently, the reference values from the DCCT study (4.0% to 6.0%) have been translated with the master equation into IFCC numbers (20 to 42 mmol/ mol) which might not be correct. Establishment of proper reference values is also of eminent importance when considering using HbA_{1c} values for the diagnosis of diabetes.

Also, new criteria for the analytical performance of HbA_{1c} methods should formally be assesed. The master equation between the NGSP/DCCT method and the IFCC method (IFCC=10.93NGSP – 23.50) makes clear that the specificity of the Bio-Rex 70 method (used as reference method in the DCCT and UKPDS study) is significantly lower than the IFCC method. For example: 0.5% or 5.5 mmol/mol is considered to be clinically significant. Calculating the relative RCV at an HbA_{1c} value of 7.0% DCCT, gives a result of 7.1% (0.5/7.0=7.1%) or in IFCC numbers: 5.5/53=10.4%. These numbers are significantly different from each other but can be explained by taking into account the lower specificity of the Bio-Rex70 method (= intercept of -23.50): 5.5/53+23.50=7.1%!

Quite often health care professionals ask questions, because they see HbA_{1c} results of a patient, which do not correspond with glucose values. Sometimes this problem can be addressed by checking the glucose meter but most of the time the reason(s) for this incongruence cannot be found. Therefore, it is hypothesized, that there may be a different pace of glycation in different subjects, and possibly under different circumstances.
This "glycation gap" could be caused by several reasons:

1. Deglycation of hemoglobin. More research is necessary to investigate the deglycation pathway involving fructosamine 3-kinase.
2. The mean age of circulating red blood cells. At the moment, it is assumed that the life span of every individual red blood cell is more or less the same but a recent study has shown that this is not the case.
3. Genetic factors which may influence interindividual variation in levels of HbA_{1c}.
4. Interindividual differences in the transport of glucose across the membrane mediated by the GLUT1 transporter.

More research will be needed to shed some light on these possible interferences.

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Samenvatting, conclusies, aanbevelingen en toekomstperspectieven

Samenvatting

Sinds een verband ontdekt werd tussen verhoogde concentraties van snelle hemoglobine-fracties en de status diabetes door Samuel Rahbar en co-werknemers in 1969 is hemoglobine A_{1c} (HbA_{1c}) uitgegroeid tot een "gouden standaard". HbA_{1c} is één van de belangrijkste parameters voor de regulering van glucose in patiënten met diabetes. Onlangs is gepleit om HbA_{1c} te gebruiken als een diagnostische marker voor diabetes als gevolg van de wereldwijde standaardisatie van de HbA_{1c} test. Dit bevestigt verder het belang van HbA_{1c}. Momenteel zijn er meer dan 30 beschikbare methoden voor de analyse van HbA_{1c} op de markt met analytische prestaties, variërend van matig tot “state of the art”.

Sommige mensen nemen aan, dat wanneer een HbA_{1c} methode een Nationaal Glycohemoglobin Standardization Program (NGSP) certificering heeft, de analytische prestaties toereikend zijn. Gelet op het feit, dat zelfs HbA_{1c} methoden met slechte analytische prestaties zijn gecertificeerd, kan een dergelijke veronderstelling worden aangevochten. In dit proefschrift werden de analytische prestaties van verschillende HbA_{1c} methoden onderzocht met een focus op point-of-care (POC) HbA_{1c} instrumenten. Ook de interpretatie van de HbA_{1c} waarden onder verschillende groepen zorgverleners in de diabetes zorg, werd onderzocht.

Hoofdstuk 1 geeft een overzicht van de literatuur over HbA_{1c} in de tijd bij de behandeling van patiënten met diabetes en het stellen van de diagnose. Het is een overzicht van HbA_{1c} vanaf het prille begin (de ontdekking van snel bewegende hemoglobine fracties) tot nu (meer dan 30 verschillende methoden op de markt), met inbegrip van de wereldwijde inspanningen gericht op standaardisatie van HbA_{1c}. De analytische prestaties van verschillende HbA_{1c} methoden, inclusief point-of-care-instrumenten, wordt besproken op basis van literatuur en externe kwaliteitsprogramma’s, alsmede de op dit moment realistisch geachte analytische doelen te weten een variatiecoefficient <3% (gebaseerd op IFCC waarden) of een variatiecoefficent <2% (gebaseerd op DCCT waarden) en een bias ≤ 2,0 mmol/mol (≤ 0,24% DCCT)).
Met betrekking tot de analytische prestaties van de HbA_{1c} methoden, kunnen we concluderen dat er aanzienlijke vooruitgang is geboekt. Dit is grotendeels te danken aan de inspanningen van de IFCC werkgroep voor de standaardisatie van HbA_{1c} en de NGSP in samenwerking met fabrikanten. Echter, zowel vanuit het perspectief van individuele patiënten, en op basis van vereiste criteria, geloven wij dat de analytische prestaties van sommige HbA_{1c} methoden onvoldoende zijn om gebruikt te worden voor het stellen van de diagnose van diabetes.

In **hoofdstuk 2** is beschreven hoe de HbA_{1c} waarden zijn bepaald van de bloedmonsters in de “ A1C-Derived Average Glucose (ADAG)” studie. In deze studie is ook onderzocht of off-line kalibratie met IFCC secundair referentiemateriaal de precisie van de HbA_{1c} bepaling kan verbeteren. De HbA_{1c} waarde van de bloedmonsters in de ADAG-studie werd bepaald met vier IFCC-gecertificeerde secundaire referentiemethoden met drie verschillende meetprincipes. Door het gebruik van vier verschillende methoden, is de invloed van de individuele bloedmatrix op het uiteindelijke resultaat tot een minimum beperkt. Sommige bloedmonsters

geven namelijk met een bepaalde methode een afwijkend resultaat in vergelijking met andere methoden. Dit zogenaamde, matrix effect is geminimaliseerd door gebruik te maken van het gemiddelde van de vier methoden. Het chromatogram van de Tosoh G7 methode gaf nuttige informatie over eventuele interferenties (Hb-varianten en/of verouderd bloed) die van invloed kunnen zijn op bepaalde methoden. Deze informatie werd dan ook gebruikt om bloedmonsters uit te sluiten van de studie die anders een foutieve waarde hadden opgeleverd en niet waren opgemerkt indien maar één HbA_{1c} methode, niet vrij van interferenties, was gebruikt bij de waarde bepaling van de monsters in deze studie.
Extra off-line kalibratie verbeterde het 95% betrouwbaarheidsinterval tussen de vier verschillende methoden op een HbA_{1c} waarde van 6,00% van ± 0,28% (5,72% - 6,28%) tot ± 0,20% (5,80% - 6,20%) en op een HbA1c waarde van 9,00% van ± 0,43% (8,57% - 9,43%) tot ± 0,24% (8,76% - 9,24%). Ook de analytische variatie coëfficiënt (VC) van de vier methoden gebruikt in deze studie was na off-line kalibratie met secundaire referentiemateriaal <2%, zoals voorgesteld in hoofdstuk 1. De HbA_{1c} resultaten gebruikt in de ADAG studie zijn bepaald met de laagste onzekerheid wat momenteel logistiek en technisch gezien haalbaar is. Dit was mogelijk door gebruikt te maken van vier IFCC gecertificeerde secundaire referentiemethoden met drie verschillende meetprincipes en extra off-line kalibratie met IFCC secundair referentie materiaal.

Point-of-care (POC) HbA_{1c} instrumenten worden steeds vaker gebruikt. Tot dusver werden de gevolgen van de invoering van deze nieuwe soorten instrumenten met hun specifieke kenmerken niet grondig besproken in de literatuur. Beperkte informatie was beschikbaar over de analytische prestaties van HbA_{1c} POC instrumenten. Bovendien is, ook voor POC instrumenten, duidelijk dat een NGSP certificering vaak onvoldoende indicatief is voor de analytische prestaties van deze meters in de praktijk. Acht verschillende HbA_{1c} POC instrumenten zijn onderzocht. De resultaten van deze studies zijn beschreven in **hoofdstuk 3 tot 6.**

Vier POC instrumenten (Quo-Test, Afinion, In2it en A1CNow) zijn opnieuw geëvalueerd in één of twee verschillende NGSP laboratoria nadat verbeteringen waren aangebracht en/of de kalibratie was aangepast. De fabrikant van de A1CNow was het niet eens met de conclusies in de oorspronkelijke studie (hoofdstuk 4), omdat EDTA bloed was gebruikt voor de evaluatie (conform advies lokale distributeur en eerder uitgevoerde studies) wat niet in overeenstemming was met de aanbevelingen van de fabrikant zelf. In deze studie is voor de A1CNow methode heparine bloed gebruikt. Resultaten zijn beschreven in **hoofdstuk 5 en 6**.

Deze studies zijn uitgevoerd gebruik makend van gecertificeerde Clinical and Laboratory Standards Institute (CLSI) protocollen en de resultaten zijn vergeleken met 3 NGSP en IFCC secundaire referentiemethoden (individueel als ook met het gemiddelde van de drie referentiemethoden).

De variatie coëfficiënt van de geëvalueerde POC instrumenten varieerde van 1,4% (Afinion) tot 5,9% (Quo-Test). Slechts twee instrumenten (DCA Vantage en Afinion) hadden een acceptabel, maar nog niet optimale variatie coëfficiënt van <2,4% in het klinisch relevante gebied.

Alle onderzochte POC instrumenten waren NGSP gecertificeerd op de InnovaStar na. In de oorspronkelijke studie waren slechts twee POC instrumenten (DCA Vantage en Afinion) in staat om de 2009 NGSP criteria te halen met twee verschillende lotnummers in vergelijking met slechts één secundaire referentiemethode. In de meest ideale situatie, zouden de methoden de NGSP criteria moeten halen vergeleken met verschillende secundaire referentiemethoden en met verschillende lotnummers. De resultaten van de methode vergelijkingen en de berekeningen van de NGSP certificering toonden significante verschillen aan in de analytische prestaties bij gebruik van verschillende reagens lotnummers van alle HbA_{1c} POC instrumenten en waren de verschillen het grootst voor de Clover en de Quo-Test (verschillen tussen twee lotnummers van ongeveer 0,85% DCCT). In dit proefschrift wordt beschreven dat het halen of niet halen van de NGSP criteria afhankelijk is van welk lot nummer is gebruikt en welke secundaire referentiemethode wordt gebruikt om mee te vergelijken. Dit werd vooral duidelijk nadat drie van de eerder onderzochte methoden opnieuw onderzocht werden in één of twee verschillende NGSP laboratoria (**hoofdstuk 6**). Men kan zich dan ook afvragen wat het nut en de betekenis is van een dergelijk certificerings programma. Opgemerkt dient te worden dat de NGSP vereist dat een fabrikant éénmaal per jaar opgaat voor een certificering, uitgevoerd met slechts één reagens lotnummer. De fabrikant is verplicht om de variatie tussen de verschillende lotnummers te minimaliseren en te garanderen. Een NGSP certificering zegt alleen iets over het geteste lotnummer en geeft dus geen garantie voor de analytische prestaties van andere lotnummers (dit proefschrift).

Niet alleen lotnummer afhankelijkheid was een probleem, ook de afwijking (bias) met verschillende secundaire referentie methoden was een probleem. Geen afwijking hebben van de werkelijke waarde is cruciaal omdat vaste afkappunten worden gebruikt als doel voor de glycemische controle (bijv. HbA_{1c} <53 mmol/mol, <7,0%-DCCT) en de diagnose van diabetes mellitus (≥ 48 mmol/mol, ≥6,5%-DCCT). De bias gevonden in onze studie (hoofdstuk 4) varieerde van -0,99%-DCCT tot +0,41%-DCCT in vergelijking met één secundaire referentie methode. Indien dergelijke afwijkingen zouden worden aanvaard voor het stellen van de diagnose van diabetes, dan zouden tientallen miljoenen mensen ten onrechte worden gediagnosticeerd met diabetes, of miljoenen mensen zouden ten onrechte niet worden gediagnosticeerd.

Kortom, het merendeel van de beschikbare POC instrumenten voor HbA_{1c} voldoet niet aan algemeen aanvaarde analytische prestatie criteria. De werkelijke mate van Hb glycering kan daardoor aanzienlijk onder- of overschat worden. Indien de analytische problemen van deze POC instrumenten niet naar behoren wordt aangepakt, is het niet raadzaam om deze instrumenten te gebruiken voor de behandeling van patiënten met diabetes. Tevens is het niet raadzam om deze instrumenten te gebruiken als screening voor pre-diabetes en diabetes en voor het stellen van de diagnose van diabetes. Onze studie toonde aan dat alleen de DCA Vantage en de Afinion geschikt zijn voor het monitoren van patiënten onder strikte voorwaarden (zie aanbevelingen).

In **hoofdstuk 7** zijn de prestaties beschreven van een HbA_{1c} methode geschikt voor laboratoria (Menarini / ARKRAY ADAMS A1c HA-8180V). De resultaten van dit onderzoek zijn, in tegenstelling tot de resultaten van het POC onderzoek, zeer goed.

De totale variatie coëfficiënt van de HA-8180V bij een lage en een hoge HbA_{1c} concentratie was 0,7% en 0,4%, respectievelijk (gebaseerd op DCCT waarden). Juistheid (bias) was maximaal 0,8 mmol/mol of 0,1% DCCT over het klinisch relevante gebied. Lineariteit, carry-over en lineaire drift waren respectievelijk uitstekend en niet aanwezig. Labiel HbA_{1c}, gecarbamyleerd hemoglobine, icterische monsters en variatie in hematocriet had geen invloed op het HbA_{1c} resultaat. Ook de hemoglobine varianten AS, AC en F hadden geen invloed op HbA_{1c} resultaat. Daarentegen kan het HbA_{1c} van bloedmonsters met een AE en/of AD variant niet worden bepaald met de HA-8180. Deze Hb varianten worden gelukkig herkend door de software op basis van een afwijkend chromatogram. De conclusie is dat de HA-8180V presteert op een constant hoog niveau en is geschikt voor zijn klinische toepassing.

In **hoofdstuk 8** wordt aandacht gevraagd voor de analytische prestaties van de verschillende HbA_{1c} methoden, inclusief POC instrumenten, bij gebruik van deze methoden voor de diagnose van diabetes. Aanbeveling is om de analytische prestaties van de HbA_{1c} methode te vermelden in studies ter beoordeling van de diagnostische waarde van HbA_{1c} bij de diagnose van diabetes mellitus. Bovendien moeten de professionals in de gezondheidszorg worden voorzien van dezelfde informatie om HbA_{1c} waarden die bepaald zijn met een laboratorium methode of met een POC instrument goed te kunnen interpreteren.

Klinisch chemische laboratoria moeten worden aangemoedigd om HbA_{1c} methoden te gebruiken met voor dit moment optimale analytische prestaties (geen bias en een totale variatie coëfficiënt van <3% (gebaseerd op IFCC waarden), <2% (gebaseerd op DCCT waarden).

Hoofdstuk 9 richt zich ook op de mogelijke rol van point-of-care testen van glucose en HbA_{1c} bij de diagnose van pre-diabetes en diabetes. Het geeft een overzicht van de principes, valkuilen en analytische prestaties van glucose en HbA_{1c} point-of-care testen en een samenvatting van de onderzoeken die point-of-care testen van glucose en HbA_{1c} hebben toegepast in de diagnostiek van (pre-) diabetes.

De effectiviteit van de standaardisatie van HbA_{1c} en de analytische prestaties van een HbA1c methode kan worden beoordeeld door het bestuderen van de resultaten van externe kwaliteitsprogramma's. Externe kwaliteitsprogramma's geven de gemiddelde resultaten weer van verschillende methoden. De gemiddelde bias van een methode geeft de doeltreffendheid van de standaardisatie van de methode weer en de inter-laboratorium variatie coëfficiënt is een maatstaf voor de nauwkeurigheid van een methode. Gebaseerd op het gemiddelde van externe kwaliteitsprogramma's zijn de analytische prestaties van de meeste laboratorium HbA_{1c} methoden adequaat. Helaas was in het verleden niet bekend hoe de prestaties waren van de methoden in individuele laboratoria. Dankzij de medewerking van twee externe kwaliteitsprogramma's, Stichting Kwaliteitsbewaking Medische Laboratoria (SKML) in Nederland en het Wetenschappelijk Instituut voor Volksgezondheid (WIV) in België, kregen wij de individuele variatie coëfficiënt resultaten van 220 laboratoria die verschillende methoden gebruikten voor het bepalen van HbA_{1c} (**hoofdstuk 10**). De SKML/WIV rondzending is verschillend in ontwerp in vergelijking met andere externe kwaliteitsprogramma's. De meeste externe kwaliteitsprogramma's, waaronder het

College van American Pathologists (GAP) programma, maken gebruik van vers gepoold patiënten bloed. De SKML maakt gebruikt van 24 gevriesdroogde bloed monsters die onderling met elkaar verbonden zijn (12 bloedmonsters in duplo). Deze bloedmonsters moeten worden geanalyseerd in de loop van een jaar (één monster per twee weken). Na een jaar, kan de precisie, nauwkeurigheid, lineariteit en de afwijking van de IFCC primaire referentiemethode worden berekend. De precisie (variatie coëfficiënt) werd gebruikt om de "Reference Change Value" (RCV) te berekenen van 220 individuele laboratoria. Een variatie coëfficiënt van <2,0% (gebaseerd op DCCT waarden) is nodig om een klinische significant verschil van 5 mmol/mol (0,5%-DCCT) te kunnen aantonen indien men ervan uit gaat dat de biologische variatie van HbA_{1c} 1,8% is. Vijfenzestig procent van de laboratoria hadden een variatie coëfficiënt van <2,0%. Dit houdt in dat 1 op de 3 laboratoria die verschillende HbA_{1c} methoden gebruiken, niet in staat is om een HbA_{1c} resultaat van 59 mmol/mol (7,5%-DCCT) te onderscheiden van een vorig HbA_{1c} resultaat van 53 mmol/mol (7,0%-DCCT). Houdt men rekening met een biologische variatie van 1,0%, in overeenstemming met de gegevens van Rohlfing et al, dan is 21,8% van de laboratoria niet in staat om een HbA_{1c} resultaat van 59 mmol/mol (7,5%-DCCT) te onderscheiden van een vorig HbA_{1c} resultaat van 53 mmol/mol (7,0%-DCCT). Een opmerkelijke bevinding was dat 41,9% van de laboratoria die gebruik maakten van een immunoassays voor het bepalen van HbA_{1c}, een variatie coëfficiënt > 3,0% had in vergelijking met slechts 10,4% van de laboratoria die een HPLC methode gebruikten voor het bepalen van HbA_{1c}.

De meeste zorgverleners in de diabeteszorg vertrouwen (althans gedeeltelijk) op een HbA_{1c} waarde om te beslissen of zij patiënten moeten aanraden om de behandeling te veranderen of niet. Als zodanig, veronderstellen zij dat HbA_{1c} resultaten betrouwbaar en nauwkeurig genoeg zijn om zulke beslissingen te kunnen nemen. Wij merkten een kloof in kennis op tussen klinisch chemische laboratoria en de zorgverleners in de diabeteszorg. Daarom ondervroegen wij verschillende groepen zorgverleners in de diabeteszorg m.b.t. de dagelijkse routine betreffende het gebruik van HbA_{1c}, verwachte precisie van de HbA_{1c} methode, en wat het verschil in HbA_{1c} waarde moet zijn in vergelijking met een vorige HbA_{1c} waarde om te doen besluiten de behandeling te veranderen. **In hoofdstuk 11** staan de resultaten beschreven van dit onderzoek. Uit de enquête kwam een verschil in interpretatie van HbA_{1c} resultaten naar voren tussen artsen en diabetes verpleegkundigen/praktijkondersteuners. Gemiddeld genomen overwegen verpleegkundigen/praktijkondersteuners de behandeling te veranderen gebaseerd op zeer kleine veranderingen in HbA_{1c} waarden, terwijl de meeste artsen bijvoorkeur zich vast houden aan de klinisch relevante verandering van 5 mmol/mol (0,5% DCCT). Verandering van therapie gebaseerd op kleine veranderingen in HbA_{1c} (<5 mmol/mol, <0,5% DCCT) kan leiden tot overmanagement/overbehandeling van de patiënt met diabetes, mede gezien het feit dat de analytische prestaties van de meeste HbA_{1c} methoden niet nauwkeurig en betrouwbaar genoeg zijn om een dergelijke beslissing te rechtvaardigen.

Conclusie

De variatie in de analytische prestaties van verschillende HbA_{1c} methoden is enorm, variërend van slecht (de meeste point-of-care instrumenten en een aantal immunoassays) tot "state of the art" (nieuwe versie kationuitwisselings HPLC's). Professionals in de gezondheidszorg, in het bijzonder diabetes verpleegkundigen en praktijkondersteuners, verwachten betere analytische prestaties dan mogelijk is voor de meeste HbA_{1c} methoden en kan dus onder- of overschatting van de risico's van diabetes tot gevolg hebben.
De professionals in de gezondheidszorg dienen te worden voorzien van informatie om laboratorium en point-of-care HbA_{1c} resultaten goed te kunnen interpreteren. De Reference Change Value (RCV) zou een waardevolle toevoeging kunnen zijn. Het klinisch chemisch laboratorium kan hierbij een waardevolle rol spelen en moet worden aangemoedigd om HbA_{1c} methoden te gebruiken met voor nu geldende optimale analytische prestaties (geen bias en een totale variatiecoëfficiënt van <3% (gebaseerd op IFCC waarden), <2% gebaseerd op DCCT waarden).

Aanbevelingen voor de invoering van HbA_{1c} POC instrumenten

Hoewel het gebruik van POC HbA_{1c} instrumenten een aantal negatieve gevolgen kan hebben die moeten worden aangepakt, is het ook belangrijk om in gedachten te houden dat het verkrijgen van HbA_{1c} resultaten op het moment van het bezoek van de patiënt aan de arts, kan bijdragen aan de verbetering van de patiënt m.b.t. welzijn en zorg. Tijdens de diagnose en follow-up van mensen met diabetes worden deze patiënten met een verscheidenheid aan poliklinische faciliteiten geconfronteerd, variërend van eerstelijnszorg bij de huisarts tot tertiairzorg in speciale diabetes zorgcentra. Veel patiënten hebben hun bloed laten afnemen in de week voordat ze naar de arts gaan om ervoor te zorgen dat de resultaten van laboratoriumonderzoek beschikbaar zijn voor een juiste medische actie op het moment van bezoek aan de arts. Door het verstrekken van resultaten snel na de bloedafname met een POC instrument, kan een extra bezoek van de patiënt aan een laboratorium of bloedafnamepost worden voorkomen. Tevens hebben studies bevestigd dat onmiddellijke feedback van HbA_{1c} resultaten de glycemische controle verbetert van patiënten met type 1 en type 2 patiënten die behandeld worden met insuline [1-3].

Gebaseerd op ervaringen in de Isala klinieken raden wij de volgende voorwaarden aan voor de invoering van een HbA_{1c} POC instrument voor zowel binnen de ziekenhuis muren als daar buiten:

1. HbA_{1c} POC instrumenten moeten vallen onder de verantwoordelijkheid van het Centraal Laboratorium.
2. Aanvaardbare analytische prestaties (idealiter: geen bias, variatie coëfficiënt <3% (gebaseerd op IFCC getallen), <2% (gebaseerd op DCCT getallen)). Validatie van het instrument door Centraal Laboratorium.
3. Connectiviteit met het Centraal Laboratorium voor data management.
4. Onderwijs en opleiding voor de gebruikers moet worden gedaan door ervaren POC coördinatoren (e-learning).
5. Alleen geaccrediteerde gebruikers zijn toegestaan om het instrument te gebruiken.
6. Interne en externe kwaliteitscontrole moeten worden gecoördineerd door de POC-coördinator.
7. Bestellen en controle van het reagens/cartridges moet worden gedaan door POC coördinator (controle van nieuwe lotnummer!).
8. Eenmaal per jaar HbA_{1c} meten op een laboratorium methode.

Referenties

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2. Ferenczi A, Reddy K, Lorber DL. Effect of immediate haemoglobin A1c results on treatment decisions in office practice. Endocr Pract 2001;7:85-8.
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Toekomstperspectieven

State of the art Point-of-Care

In het verleden waren speciale vaardigheden nodig om analyses uit te voeren met een HPLC-instrument. Tegenwoordig zijn HPLC systemen gemakkelijker in gebruik geworden. De Tosoh G8 bijvoorbeeld, heeft geavanceerde software die het chromatogram voor de analist interpreteert. De analyses van bloedmateriaal die een normal chromatogram tonen en geen andere technische fouten, worden direct verzonden naar het laboratorium informatie systeem. Alleen bloedmonsters met een "afwijking" (abnormaal chromatogram, technische problemen, enz.) worden getoond in een werklijst en moeten worden beoordeeld, en indien nodig, aan nader onderzoek worden onderworpen door een analist. Dit betekent dat, mits er een goede standard operating procedure (SOP), een goede ondersteuning van de fabrikant/distributeur en begeleiding van het centaal laboratorium is, bloedmonsters betrouwbaar kunnen worden geanalyseerd door diabetes verpleegkundigen in een diabeteszorg centrum. Dit zou een heel mooi voorbeeld kunnen zijn waarbij een klinisch chemisch laboratorium optimaal samenwerkt met zorgverleners waardoor gemakkelijker patiënt gerichte zorg kan worden aangeboden. Op deze manier zou een POC HbA_{1c} resultaat kunnen worden verkregen met een "state-of-the-art" instrument. Indien het niet mogelijk is om de zorgverlener op een optimale manier te helpen als er een probleem is met het instrument als gevolg van een te grote afstand tussen het laboratorium en het diabeteszorg centrum, kan men kiezen voor een POC instrument van Siemens (DCA Vantage) of Axis-Shield (Afinion). Echter, gebaseerd op de resultaten van dit proefschrift, is het raadzaam om de voorwaarden voor de invoering van HbA1c POC instrumenten, vermeldt op bladzijde 162, op te volgen.

Laboratorium op een chip

Ontwikkelingen in de nanotechnologie gaan razendsnel. Het mag dan ook geen verrassing zijn dat wetenschappers en producenten al bezig zijn om een HbA_{1c} methode te ontwikkelen op een chip: HPLC op nano-niveau. De uitdaging voor een dergelijke ontwikkeling is dat de analytische prestaties van deze chips gelijk moeten zijn, of zelfs beter, dan laboratorium gebaseerde methoden, anders hebben ze waarschijnlijk geen toekomst. Wetenschappers uit verschillende vakgebieden moeten samenwerken om dit tot een succes te maken. In de nabije toekomst, wanneer een dergelijke methode zijn waarde heeft bewezen, kan eraan gedacht worden om patiënten hun HbA_{1c} thuis te laten bepalen en de resultaten door te laten sturen naar de beroepsbeoefenaar in de gezondheidszorg, met behulp van internet of een mobiele telefoonverbinding.

Toekomstige studies

Studies in het verleden hebben bevestigd dat de invoering van nieuwe waarden kan leiden tot verwarring en verslechtering van de glykemische controle. Een nieuwe internationale studie zou moeten worden opgezet om de klinische implicaties van de implementatie van de nieuwe IFCC waarden te onderzoeken.

Ook moeten de HbA_{1c} referentiewaarden (met IFCC waarden) worden onderzocht en opnieuw vastgesteld in een grote studie met gezonde personen uit verschillende delen van de wereld en uit verschillende etnische groepen. De referentiewaarden zijn in een kleine studie in 2002 vastgesteld onder de Deense bevolking (DiaRisk, Steno Diabetes Centrum, Kopenhagen, Denemarken).
De referentiewaarde, zoals gemeten in dit onderzoek met behulp van de referentiemethode was 33,3 ± 4,8 mmol/mol (gemiddelde ± 2 SD) of 4,8% tot 5,6% DCCT. Op dit moment zijn de referentiewaarden van de DCCT studie (4,0% tot 6,0%) vertaald met de "master equation" naar IFCC waarden (20 tot 42 mmol/mol). Vaststelling van de juiste referentiewaarden is ook van eminent belang bij de overweging om HbA_{1c} waarden te gebruiken voor de diagnose van diabetes.

Ook moeten formeel de nieuwe criteria voor de analytische prestaties van HbA_{1c} methoden worden vastgesteld. De "master equation" tussen de NGSP/DCCT methode en de IFCC methode (IFCC = 10,93NGSP – 23,50) maakt duidelijk dat de specificiteit van de Bio-Rex70 methode (gebruikt als referentie methode in de DCCT en UKPDS-studie) aanzienlijk lager is dan de IFCC methode. Bijvoorbeeld: 0,5% of 5,5 mmol/mol wordt beschouwd als klinisch significant. De berekening van de relatieve total error op een HbA_{1c} waarde van 7,0% DCCT, leidt tot een resultaat van 7,1% (0,5/7,0 = 7,1%) of in IFCC nummers: 5,5/53 = 10,4%. Deze getallen zijn significant verschillend van elkaar, maar kunnen worden verklaard door de lagere specificiteit van de Bio-Rex70 methode (= intercept van -23,50): 5,5/53 +23,50 = 7,1%!

Regelmatig stellen beroepsbeoefenaren in de gezondheidszorg vragen over HbA_{1c} resultaten van een patiënt die niet overeenstemmen met de glucose waarden van de patient over de bijbehorende periode. Soms kan dit probleem worden opgelost door het controleren van de glucose meter, maar meestal kan er geen verklaring voor worden gevonden. Eén hypothese is dat glycering in een ander tempo plaats vindt in verschillende patiënten en onder andere omstandigheden. Ook binnen de patiënt kunnen de omstandigheden in de loop der tijd wijzigen.
Dit "glycerings verschil" kan op verschillende wijzen worden veroorzaakt nl.:

1. Deglycatie van hemoglobine. Meer onderzoek is nodig om de deglycerings route inzichtelijk te krijgen. Fructosamine 3 kinase speelt waarschijnlijk een rol in deze route.
2. De gemiddelde leeftijd van circulerende rode bloedcellen. Op dit moment wordt aangenomen dat de levensduur van de rode bloedcellen van ieder individu min of meer hetzelfde is, maar een recente studie heeft aangetoond dat dit niet het geval is.
3. Genetische factoren die van invloed kunnen zijn op de interindividuele variatie in het niveau van HbA_{1c}.
4. Interindividuele verschillen in het transport van glucose over het membraan gemedieerd door de GLUT1 transporter.

Meer onderzoek zal nodig zijn om enig inzicht te krijgen in deze mogelijke interferenties.

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Sabrina Schilling, we first met when you were working for Bio-Rad. We did a study together and there was a kind of synergy between us. We both are very driven and enthusiastic. You switched from job (as I predicted) and again you were responsible for HbA_{1c}. Almost every year we see each other at the AACC and we always find time to have dinner with each other. Having dinner with you feels like having dinner with friends. Thank you for your friendship and thank you for being my supporter.

Henriëtte Venenberg, John en Anita Weijel, dankjewel dat ik bij jullie mijn verhaal kwijt kon wanneer ik er even helemaal klaar mee was!

Mam, bedankt dat je mij tijd gaf om te schrijven door je te ontfermen over het "strijkgoed". Sorry, dat ik de afgelopen jaren niet zoveel tijd met je heb kunnen doorbrengen omdat ik "altijd en eeuwig" met dat proefschrift bezig was terwijl ik wist dat je na het overlijden van papa redelijk vaak alleen zat. Ik hoop dat je nog vaak met mij, Merle en Tom een weekje wilt uitwaaien op één van de eilanden.

Merle, Tom en Jan Herm, jullie hebben het meest moeten afzien terwijl ik bezig was met het schrijven van het proefschrift. Bijna elk vrij uurtje heb ik de laatste jaren besteed aan het studeren en schrijven en dat ging ten koste van jullie. Tom, je maakte mij dit vrij duidelijk door te zeggen: mama, moet je nu weer werken? en dan

zei ik altijd: ja, Tom, voor niets gaat de zon op en voor de rest moet je hard werken. Merle, jij was altijd zo lief. Je zei vaak tegen mij: mam, je mag ook wel eens even iets voor jezelf doen hoor. Je hoeft niet altijd te schrijven en zo hard te werken! Jan Herm, ik weet dat je het verschrikkelijk vindt dat ik je hier noem maar ik doe het lekker toch! Je hebt mij altijd door dik en dun gesteund. Zonder jouw steun, zowel geestelijk (door te relativeren) als ook praktisch (door dingen van mij over te nemen), had ik het nooit gered. Lieve schatten, heel erg bedankt!

Curriculum Vitae

Erna Lenters-Westra is op 3 oktober 1965 als jongste van drie kinderen geboren te Nijverdal (gemeente Hellendoorn). In 1983 behaalde zij haar H.A.V.O. diploma aan het College Noetsele te Nijverdal. Met veel plezier heeft ze de laboratorium school te Hengelo, afdeling medisch, studierichting klinische chemie doorlopen. Zij heeft stage gelopen op het laboratorium van ziekenhuis de Weezenlanden en werd na haar stage aangenomen als medisch analiste op hetzelfde laboratorium. Na twee jaar als routine analiste te hebben gewerkt werd zij analiste met specialisatie bloedtransfusie. Op deze afdeling heeft zij, mede met andere collega's, verscheidene nieuwe technieken geïntroduceerd en opgezet. In 1995 werd zij research analist op het gebied van HbA_{1c} met het oog op de internationale standaardisatie plannen voor HbA_{1c}. In 2009 is zij cum laude afgestudeerd in Biologie en Medisch Laboratoriumonderzoek aan de Saxion Hogeschool te Enschede. Hierna is zij begonnen aan haar formele traject tot promotie. Zij geeft regelmatig presentaties in binnen en buitenland en is consultant voor een aantal wereldwijd actieve diagnostica fabrikanten. Daarnaast is zij recent toegetreden tot de advisory board van één van deze bedrijven. Erna is getrouwd met Jan Herm Lenters en heeft een dochter Merle en een zoon Tom.