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Hemoglobin A(1c) Reporting Units and Diagnostic Cut-Offs in Relation to International Recommendations

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Abstract: Purpose. In this account were studied the use of units and cut-off limits for hemoglobin A(1c) in worldwide clinical laboratory practice and the quality assurance ranges of hemoglobin A(1c) by calculating the target limits from the values of Labquality Ltd. and ERLGH. Methods. The use of HbA(1c) units and the diagnostic limits for diabetes were examined using e-mail and letter inquiries to 37–51 societies of laboratory medicine (mainly clinical chemistry) sent from 2009 to 2017. The parametric statistical programs of Labquality Ltd., SPSS® 13.0, and MS Excel 2013 (Microsoft® Co., Cambridge, MA, USA) were used. Results. The mean values of the Finnish quality control organization Labquality Ltd. and the corresponding values from the HbA(1c) queries as to the percentage and mmol/mol SI units (IFCC) were used. The IFCC system for hemoglobin A(1c) is slowly but constantly gaining acceptance in Europe, but remains quite rare outside Europe where the percentage results were correspondingly lower. The mean round values of Labquality Ltd. and the corresponding mean values of the European Reference Laboratory for Glycohemoglobin (ERLGH) showed equal ranges for calculation of the target values with ± 6% intervals for the percentage results and ± 8% intervals for the mmol/mol results. Conclusions. To avoid confusion, the overall use of mmol/mol as a single unit for HbA(1c) may be the best option when the IFCC system has been accepted worldwide. The target values can be calculated equally well from the mean values of Labquality Ltd. and the ERLGH values in terms of both per percentage and mmol/mol units.

Keywords: Diabetes, HbA(1c), IFCC Recommendation, Methods Quality Assurance, Target Limits, Units

1. Introduction

There are 5.5 million inhabitants in Finland, of which about half a million suffer from diabetes mellitus (DM), and the number is constantly rising [1]. DM mainly affects the nervous, muscle, kidney, and ocular tissues by changing glucose metabolism [1, 2]. Previously DM was diagnosed by glucose concentration in the blood or urine. Later in the 1960s, measurement of the hemoglobin A(1c) fraction by qualitative or quantitative assays began when Trivelli & al. published the first quantitative assay of HbA(1c) [3, 4]. Numerous methods have then been developed for HbA(1c) measurements [2, 4-7]. Weykamp & al concluded that the high variation between the methods and the laboratories was substantially corrected by a new calculation of the primary results [5].

In the United States and Canada in the 1970s, the results of HbA(1c) analyses varied extensively between methods and laboratories. As a result, working groups (WG) of the Diabetes Control and Complications Trial (DCCT) were set up with the National Glycohemoglobin Standardization Program (NGSP). This NGSP/DCCT program was subsequently expanded to standardize the HbA(1c) assays using a liquid chromatography as the DCCT/NGSP reference [7].

Later in the 1990s, the International Federation of Clinical Chemistry (IFCC) organized working groups to achieve standardization of all types of assays for HbA(1c). Reference standards and a principle for a standardized method were developed for international use [8, 9]. A reference system for the international standardization of HbA(1c) measurements in the form of a reference laboratory network was organized [10]. These recommendations were developed for international use,

as proposed by the IFCC in 2004 [11].

Furthermore in 2010, the American Diabetes Association (ADA) announced the possibility to select a HbA1c value of 6.5% for the diagnosis of DM [12]. But with a fixed diagnostic limit of HbA(1c), the methods must be highly precise to ensure proper clinical practice [13, 14].

In this paper, the methods, the queries concerning HbA(1c) units, the relation of the old percentage unit to the new mmol/mol unit, and the precision of the target requirements for the HbA(1c) assays are described based on the quality control rounds of Labquality Ltd. and those of ERLGH [15].

2. Methods

The annual HbA(1c) rounds of Labquality Ltd. were performed four to six times per year using two native EDTA blood samples, one close to the level of HbA(1c) at the diagnostic level recommended by the ADA, namely 6.5% (48 mmol/mol), and the second typically at a moderately elevated HbA(1c) level [2, 12, 15]. Only the results from the native EDTA blood samples were used for this study as their variation is smaller than that of commercial liquid samples.

The EDTA samples for quality control (QC) assays were drawn from two volunteers in the morning of sample collection, mixed, divided into 0.5 ml portions and sent to the office of Labquality Ltd. by airmail [15]. In the office, the samples were further distributed and sent to the participants on the same day.

The common Finnish analytical methods used for HbA(1c) by Labquality Ltd. are presented in Table 1 for rounds 3/1994 and 6/2017 [15]. The methods in round 6/2017 were: two liquid chromatography (Bio-Rad D-10 and Variant, Hercules, CA, USA; Tosoh G, Tosoh Bioscience Division, Tokyo, Japan), two enzymatic (Abbott Architect C, Abbott Diagnostics, IL, USA; HemoCue® Glucose 201+ System, Ängelholm, Sweden), one capillary electrophoresis (Capillarys 3 Tera, Sebia, Lisses, France), and multiple immunochemical methods (Axis-Shield Afinion, Alere Technologies AS, Oslo, Norway; Beckman Coulter, Inc., Pasadena, CA, USA; Roche Tina-quant®, Roche Diagnostics, Rotkreuz, Switzerland; Siemens Advia and DCA 2000, Diamond Diagnostics, Holliston, MA, USA; Thermo Fisher Scientific, Konelab, Espoo, Finland) manufactured for HbA(1c). In addition, from 2011 to 2012, the enzymatic method of Diazyme Laboratories (Poway, CA, USA) was used before those of Abbott [16].

Table 1. *Methods used in the surveys of Labquality Ltd. in round 3/1994 for percentage results and in round 6/2017 for percentage and mmol/mol results.*

Round 3/1994				**Round 6/2017**			
GHbA1c%	**Kpl**	**KA**	**CV&**	**HbA1c%**	**Kpl**	**KA**	**CV%**
Ciba-Cornic HPLC	5	5.36	2.10	Abbott enzymatic	1	6.86	
Diamat Bio-Rad HPLC	20	5.52	5.60	Axic Shilds Afinion IA	4	6.70	2.80
Kyoto Daichii HPLC	4	5.64	6.50	Beckman Coulter	2	6.60	2.40
Pharmacia MonoS FPLC	18	5.24	10.70	Beckman Coulter LX&ck	1	6.40	
Schimadzu HPLC	2	5.58	5.70	Eurolyser 700	1	7.35	
HPLC others	3	5.45	18.40	Hemoque	2	7.35	1.10
Bio-Rad minicolumn	3	5.80	9.10	HPLC Bjo-Rad D-10	2	6.75	1.10
Beckman electrophoresis	5	4.46	8.60	HPLC Tosoh HPLC	14	6.77	1.30
Abbott IMz	16	5.32	6.00	Roche Tina-quant IA	1	6.70	
Ames DCA 2000	30	5.08	2.70	Serbia capillary electr	2	6.85	1.20
Boeringer Tina-quant	17	5.02	6.70	Siemens Advia	2	6.90	0.00
Dako EIA	3	4.87	11.60	Siemens DCA 2000 LA	4	7.33	12.10
ALL	126	5.20	8.50	Thermo Konelab EIA	8	6.79	3.00
				ALL	44	6.80	2.10
GHbA1%							
Bio-Rad Diamat HPLC	6	6.64	4.50	HbA1c mmol/mol			
HPLC others	2	6.80	3.90	Abbitt enzymatic	1	51.50	
GHb%				Axis Afinion	4	49.75	3.90
Affinity others	2	6.67	7.00	Beckman Coulter	2	48.80	3.60
				Beckman Coulter Syncro	1	50.60	
				Eurolyser 700	1	46.00	
				Hemoque	2	56.50	1.40
				HPLC Bjo-Rad D10	2	50.55	1.40
				HPLC Buo-Rad Variant	1	50.00	
				HPLC Tosoh	28	50.58	1.60
				Roche c-Tina-quant IA	5	49.56	2.50
				Roche Tina-quant IA	1	54.00	
				Serbia capillary electr	2	51.50	1.60
				Simens Advia Analysers	2	52.00	0.00
				Siemens DCA 2000+	7	52.00	2.10
				Thermo Konelab EIA	13	50.55	3.20
				ALL	72	50.70	2.70

Questionnaires concerning the use of HbA(1c) units and later on, the acceptance of the fixed diagnostic limit for DM were sent between 2009 and 2017 mainly by e-mail but also by mail to 37–51 societies of laboratory medicine (mainly clinical chemistry) in Europe as well as a few elsewhere.

In terms of the annual rounds of Labquality Ltd. [15], the HbA(1c) values for percentage results from 1994 and for mmol/mol results from 2010 are presented in Table 1. The reference methods of ERLGH for HbA(1c) were available for all rounds from 1997 a*n*d were used in the comparison using Menarini HA-8160 HPLC ion exchange chromatography and Primus HPLC affinity chromatography [17]. In Finland, the mean value of the surveys of Labquality Ltd. was used as the target value for the participants since the start of the glycohemoglobin rounds in 1985 [15]. Since 1994, the number of participants has been over 100, enabling proper statistical calculations. Starting from 1997, the HbA(1c) reference values of ERLGH were used in the comparison and the survey participants were informed of them.

In 1994, 59% of the participants in the HbA(1c) rounds were Finnish, but by 2017, the percentage was down to 43%. Thus, the company today is more international as a QC organization than before. In addition, in 1994, 60% of the HbA1(1c) analyses were performed using liquid chromatography, but in 2017 this figure had decreased to 29% (Table 1). There was thus a continuous transition from chromatography to immunochemistry [2, 15].

Statistics

The mean values, standard deviations (SD), and coefficients of variation (CV%) of the replies were calculated using the parametric statistical methods of Labquality Ltd., SPSS® 13.0 (SPSS Inc., Chicago, IL, USA), and MS Excel 2013 (Microsoft® Co., Cambridge, MA, USA) [15]. The mean value ± 2*SD contains 95.6% of the laboratories participating in the rounds.

3. Results

The EDTA blood samples were sent to the participating laboratories on the day of handling the samples. Since 1994, transfer problems have been experienced in only three surveys and these were excluded from the calculations.

3.1. Methods Used in the Rounds of Labquality Ltd

He methods used for the HbA(1c) assays of Labquality Ltd. are presented in the paragraph describing the materials and methods of the rounds in 3/1994 and 6/2017 and listed in Table 1 [15]. There were small but insignificant differences between the method groups (percentage or mmol/mol) in consecutive rounds. It was important to study the mean values of the rounds in terms of the target value for percentage results starting from the beginning of the glycohemoglobin surveys in 1985. After 1997 when the HbA(1c) reference values of ERLGH became available, the mean values of the rounds of Labquality Ltd. were compared to those of ERLGH, measured from the same parallel samples [15]. Figure 1 shows an excellent, continuous agreement between the values ($p < 0.001$), and therefore, the mean values of the rounds for HbA(1c) were used as the target values.

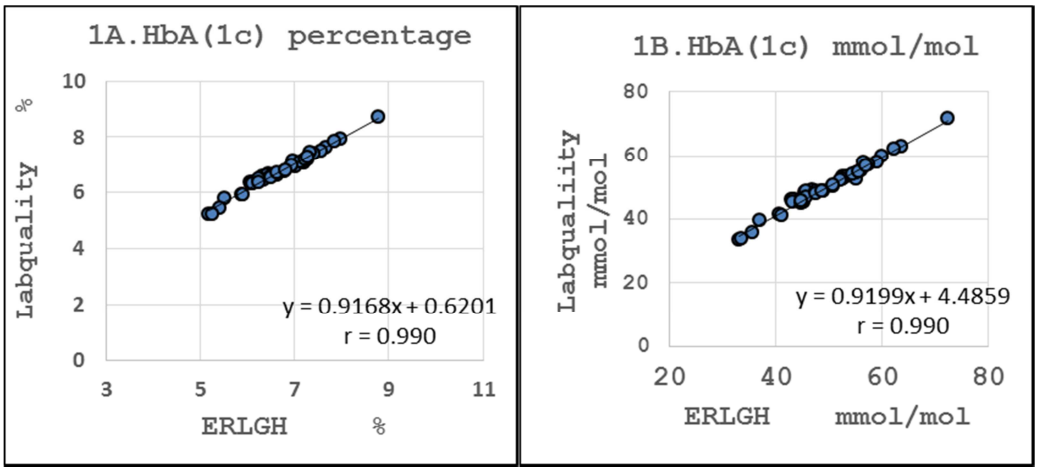

Figure 1. *Relationship of the quality control values of Labquality Ltd. and the parallel control values of European Reference Laboratory for Glycohemoglobin as percentage results (1A, mean 6.3%) and as mmol/mol results (1B, mean 46.5 mmol/mol) from 2010 to 2017 (r = 0.990, p < 0.001).*

3.2. Questionnaires to the Societies of Laboratory Medicine

Several questionnaires have been sent to societies of laboratory medicine starting from 2009. The replies after the data collection from 2009 to 2017 are presented in Table 2, indicating the use of HbA(1c) units [2]. The number of answers increased as of 2009 as compared to previous years (e.g. 59.5% in 2009, 78.4% in 2011, 80.3% in 2014, and 82.4% in 2017), but the increase was fairly similar from year to year. Starting from 2014, the recommendations of the societies for the use of the ADA diagnostic limit value for HbA(1c) in the diagnostics of DM (HbA(1c) > 6.5% or ≥ 48 mmol/mol) were requested, and the results are presented in Table 2 [12].

Table 2. *Summary of the replies to the questionnaires sent to the societies of laboratory medicine (mainly clinical chemistry) concerning the use of units and the acceptance of the diagnostic cut-off limit of HbA(1c) for diabetes.*

Country	HbA(1c)	Percentage	Parallel	Only	Dg limit
	name	only	% and mmol/mol	mmol/mol	6.5%/48 M
Germany	HbA(1c)	Yes	1.1.2009	1.1.2010	Yes
The Nethetlands	HbA(1c)	Yes	2009-2010	1.1.2011	Yes
Sweden	HbA(1c)	Yes	1.9.-31.12.2010	1.1.2011	Yes
Great Britain	HbA(1c)	Yes	1.6.2009-30.9.2011	1.10.2011	Yes
Check Rebublic	HbA(1c)	Yes	2010	1.1.2012	Yes
Italy	HbA(1c)	Yes	From1.1.2011	1.10.2012	Yes
Denmark	HbA(1c)	Yes	From 1.8.2008	1.1.2013	Yes
Ireland	HbA(1c)	Yes	From 1.7.2010	16.1.2012	Yes
Hungry	HbA(1c)	Yes	From 1.4.2011	1.4.2013	Yes
Australia	HbA(1c)	Yes	From July 2011	July 2013	Partly
Belgium	HbA(1c)	Yes	From 1.6.2011	2012	Yes
New Zealand	HbA(1c)	Yes	From July 2011	July 2013	Yes
Finland	HbA(1c)	Yes	From 3.3.2010	1.1.2016	Yes
Norway	HbA(1c)	Yes	2018	1.9.2018	Yes
Chile	HbA(1c)	Yes	1.4.2011	No	Yes
Estonia	HbA(1c)	Yes	From 1.1.2012	No	Yes
Croatia	HbA(1c)	Yes	2012	No	Yes
France	HbA(1c)	Yes	From 2009	No	No
Greece	HbA(1c)	Yes	2012	?	?
Iceland	HbA(1c)	Yes	1.1.2016	?	Yes
Israel	HbA(1c)	Yes	2010	?	?
Lithuania	HbA(1c)	Yes	From 15.4.2011	No	?
Poland	HbA(1c)	Yes	From 2013	?	Yes
Serbia	HbA(1c)	Yes	From 1.9.2009	?	Yes
Slovenia	HbA(1c)	Yes	2011	?	?
Slovakia	HbA(1c)	Yes	From 13.6.2012	?	No
Spain	HbA(1c)	Yes	Yes (partly)	?	Yes
Turkey	HbA(1c)	Yes	From 2012	?	Yes
Replies without mmol/mol			Replies		Number
EU	Non EU		Total questions		51
8	5		percenrage only		13
No reply at all			percentage & mmol/mol		15
ÉU	Non-EU		mmol/mol only		14
4	5		No reply at all		9
Ilkka Penttklä	12.6.2018		Accepted diagnostgic	2014	2017
	Kuopio		limit for diabetes	22	37

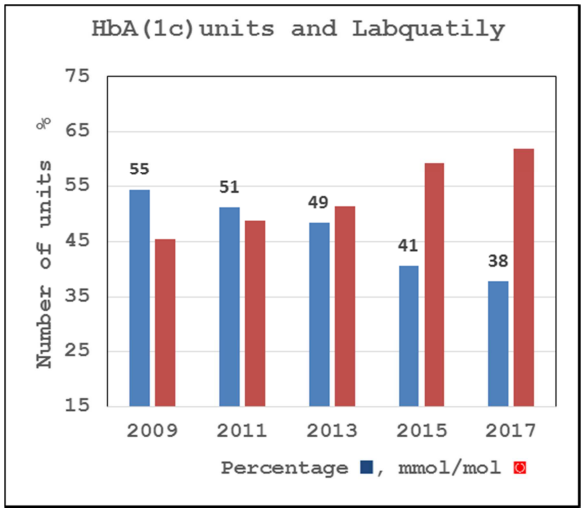

Figure 2. *Ratios of HbA(1c) users of Labquality Ltd. from 2009 to 2017 in terms of percentage units and mmol/mol units. The decrease in percentage users was significant and as well as the increase in mmol/mol users ($r = 0.951$, $p < 0.001$).*

The change in the use of units was calculated from the annual rounds of Labquality Ltd. from 2010 onwards when the mmol/mol units for HbA(1c) became available from the ERLGH [15]. As seen in Figure 2, there is a continuous, significant shift from the percentage results to the mmol/mol results from 2010 to 2017 ($r = 0.950$, $p < 0.001$).

3.3. Quality Assurance for the HbA(1c) Assays

The variation coefficients of HbA(1c) in the percentage reports of laboratories participating in the annual rounds of Labquality Ltd. have improved since 1994 from 8.1% to 3.2% in 2017 (no. 93, $r = 0.910$, $p < 0.001$) [15]. The CV% for mmol/mol after the 2010 replies decreased significantly from 6.0% to 4.3% (no. 47, $r = 0.701$, $p < 0.001$). Thus, the CV decrease for both percentage and mmol/mol results almost reached the lowest accepted value, 3.0% and 4.0% [2].

The mean values of Labquality Ltd. from 2009 to 2017 correlated well with the corresponding ERLGH values in terms of percentage results (6.61% versus 6.60%) and mmol/mol results (50.1 mmol/mol versus 50.0 mmol/mol) [15]. The correlations were significant for both the percentage values ($r = 0.388$, $p < 0.01$) and the mmol/mol values ($r = 0.702$, $p < 0.001$).

3.4. Target Values for HbA(1c) Assays

In terms of the target values in Finland, on 1 January 2015, Labquality Ltd. accepted new reference limits for HbA(1c): ± 6.0% for percentage results and ± 8.0% for mmol/mol results around the target values, instead of the earlier limit of ± 10% for both units (Table 3) [15]. These ranges, calculated either from the mean values of Labquality Ltd. or from the ERLGH values with roughly equal limits, also comply with the limits published earlier for percentage results and for mmol/mol results [2, 12, 15, 19, 20].

4. Discussion

The Finnish Society of Clinical Chemistry (FSCC) and the Finnish societies for diabetes research and treatment agreed in 2009 to begin HbA(1c) analysis according to the IFCC recommendation as of 3 March 2010 [10]. Consequently, the results for HbA(1c) were expressed both in the earlier percentage units and in the IFCC mmol/mol units. After five years of accumulating experience, the FSCC decided that parallel results had been used long enough. From 1 January 2016 onwards, the FSCC recommended that HbA(1c) results are given only in mmol/mol units [21]. Many other societies, such as ones in Germany, have also previously shifted from percentage units to mmol/mol units when reporting their results for HbA(1c) [2, 22].

Since 2009, multiple queries to European societies of laboratory medicine and various other societies outside Europe concerning the use of the old NGSP/DCCT percentage units and the IFCC mmol/mol units were sent. In 2011, the use of a fixed diagnostic limit for HbA(1c) was also introduced to the diagnosis of diabetes and added to the questionnaires starting from 2014 [2, 15].

Use of the mmol/mol system is slowly but constantly increasing in Europe, but is still less common outside Europe [2]. Germany was the first to accept the mmol/mol unit only for their HbA(1c) reports, followed by many other European countries (Table 2) [22]. However, use of the IFCC mmol/mol system is not common in non-European countries despite the proposal of mmol/mol as the only logical unit for HbA(1c) assays [23].

The annual rounds in Figure 2 show how the utilization of percentage units as opposed to mmol/mol has clearly decreased constantly in the HbA(1c) reports of Labquality Ltd. [15]. This change is a significant process that is mainly limited to Europe and is rare outside Europe.

Table 3. *Calculation of the target values for HbA(1c) from the mean values of Labquality Ltd. and the ERLGH values were made from the round 6/2017. The correlations for percentage units (r = 0.992) and for mmol/mol units (r = 0.989) were highly significant (p < 0.001) between Labquality Ltd. and ERLGH results.*

HbA(1c) results: percentage

Source	**ERLGH**		**Labquality Ltd.**		**ERLGH**
Value	**Value**	**Mean±SD**	**Mean±2SD**	**Mean±6%**	**6.81±6%**
No.44	6.81	6.80±0.14	6.52-7.04	6.39-7.21	6.40-7.22
r		0.992			

HbA(1c) results: mmol/mol

Source	**ERLGH**		**Labquality Ltd.**		**ERLGH**
Value	**Value**	**Mean±SD**	**Mean±2SD**	**Mean±8%**	**50.9±8%**
No.72	50.9	50.7±1.36	48.0-53.4	46.6-54.8	46.8-55.0
r		0.989			

In order to study the QC level of HbA(1c) analyses, the assay results obtained from the annual rounds of Labquality Ltd. were examined by calculating the variation of the results as the CV% [2, 15]. The mean round CV% decreased in the last seven years from about 6.0 to 4.3 in terms of the mmol/mol results ($r = 0.702$, $p < 0.001$) and from 4.0% to about 3.2% in the percentage results ($r = 0.388$, $p < 0.01$). The mean CV% for the percentage results decreased from 7.9% to 3.2% from 1994 to 2017 ($r = 0.900$, $p < 0.001$) [2]. These findings for HbA(1c) are highly comparable to ones published earlier in terms of percentage results and in terms of mmol/mol results [2, 18-20, 25]. While the mean results from the rounds of Labquality Ltd. are close to the values of ERLGH (Table 3), the mean round HbA(1c) value was selected for the target values, and each laboratory participating the survey could select the value they use as the target value [15].

Correlations between the IFCC method and the NGSP/DCCT methods were published by Hoelzel & al., and the equations between the IFCC and DCCT methods were: HbA(1c) (mmol/mol) = 10.93 * HbA(1c) (%) – 23.50 and HbA(1c) (%) = 0.0915 * HbA(1c) (mmol/mol) + 2.15 [11]. These equations allow the results to be reliably estimated, as shown by the HbA(1c) rounds of Labquality Ltd. compared to the results measured by ERLGH [15]. This is highly important when considering the fixed HbA(1c) limit of the ADA in diagnosing DM [12]. The reported limit of 6.5% corresponds to 48 mmol/mol in the IFCC system [11]. The queries indicated that most countries/societies recommend the use of the 6.5% or 48.0 mmol/mol limits in the diagnosis of DM, and the practice is becoming more widespread over time (Table 2).

The quality assurance limits of Labquality Ltd. for HbA(1c), mean ± 6% for percentage results and mean ± 8% for mmol/mol results, correspond well to the limits published previously [15, 18, 19, 22], which also are in line with the findings of Weykamp & al. in that the quality requirements are different for the NGSP/DCCT and the IFCC system [24]. In addition, Lenters-Westra and English point out in their article that the CV% of HbA(1c) using NSGH methods should be below 2.0 [26]. Their material was based on the results of

reference laboratories as to the low limit that cannot be reached from the universal QC reports, such as from Labquality Ltd. [15].

It is concluded that in each round either the mean value of the round of Labquality Ltd. or the ERLGH value can equally well be utilized as the target value when they correspond significantly with each other [15].

The mmol/mol system, recommended by the IFCC for HbA(1c) with the new mmol/mol unit, as well as parallel reporting with both percentage and mmol/mol units are slowly gaining acceptance in Europe, but rare outside Europe. Use of the diagnostic cut-off limit of the HbA(1c) value is still not fully established, although it is slowly increasing.

The authors also hope that the use of the IFCC mmol/mol unit for HbA(1c) would gain worldwide acceptance to enable the comparison of results from different studies and to lessen confusion, as only one unit would then be used.

Abbreviations

DCCT, DM, EDTA**,** HbA(1c), IFCC, NGSP, SI.

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Conflict of Interest

All the authors confirm do not have any possible conflicts of interest.

All authors declare that they have no competing interests.

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