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MINIREVIEWS

Methods, units and quality requirements for the analysis of haemoglobin A_{1c} in diabetes mellitus

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Abstract

The formation of glycohemoglobin, especially the hemoglobin A_{1c} (HbA_{1c}) fraction, occurs when glucose becomes coupled with the amino acid valine in the β-chain of Hb; this reaction is dependent on the plasma concentration of glucose. Since the early 1970s it has been known that diabetics display higher values of HbA_{1c} because they have elevated blood glucose concentrations. Thus HbA_{1c} has acquired a very important role in the treatment and diagnosis of diabetes mellitus. After the introduction of the first quantitative measurement of HbA_{1c}, numerous methods for glycohemoglobin have been introduced with different assay principles: From a simple mini-column technique to the very accurate automated high-pressure chromatography and lastly to many automated immunochemical or enzymatic assays. In early days, the results of the quality control reports for HbA_{1c} varied extensively between laboratories, therefore in United States and Canada working groups (WG) of the Diabetes Controls and Complications Trial (DCCT) were set up to standardize the HbA_{1c} assays against the DCCT/National Glycohemoglobin Standardization Program reference method based on liquid chromatography. In the 1990s, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) appointed a new WG to plan a reference preparation and method for the HbA_{1c} measurement. When the reference procedures

were established, in 2004 IFCC recommended that all manufacturers for equipment used in HbA_{1c} assays should calibrate their methods to their proposals. This led to an improvement in the coefficient of variation (CV%) associated with the assay. In this review, we describe the glycation of Hb, methods, standardization of the HbA_{1c} assays, analytical problems, problems with the units in which HbA_{1c} values are expressed, reference values, quality control aspects, target requirements for HbA_{1c}, and the relationship of the plasma glucose values to HbA_{1c} concentrations. We also note that the acceptance of the mmol/mol system for HbA_{1c} as recommended by IFCC, *i.e.*, the new unit and reference ranges, are becoming only slowly accepted outside of Europe where it seems that expressing HbA_{1c} values either only in per cent units or with parallel reporting of percent and mmol/mol will continue. We believe that these issues should be resolved in the future and that it would avoid confusion if mmol/mol unit for HbA_{1c} were to gain worldwide acceptance.

Key words: Diabetes; Hemoglobin A_{1c}; Glycohemoglobin; Glucose; International Federation of Clinical Chemistry and Laboratory Medicine; Reference values; Quality assurance; Recommendation; Target limits

Core tip: The aim of this review is to clarify methods, units, quality requirements, reference and cutoff limits for hemoglobin A_{1c} (HbA_{1c}) and ratio of blood glucose/HbA_{1c} on the basis of the results from Finnish quality control surveys by comparing them to the literature. The HbA_{1c} surveys of Labquality Ltd. (Helsinki, Finland) were started in 1986 by using two fresh EDTA-blood samples. From 1994, the number of the participating laboratories had risen to 139, of which 75 were Finnish and 64 from five other countries. In 2014, the number of the participating laboratories was total 214, 141 were Finnish and 73 from 13 other countries.

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INTRODUCTION

Fisher[1] synthetized a molecule named fructosamine in 1889. In the 1950s and 60s, it was reported that carbohydrate residues could become attached to hemoglobin (Hb)[2,3]. In 1967 Holmquist and Schroeder[4] observed that glucose bound to Hb and Rahbar[5] revealed that the electrophoretic fraction containing glucose was higher in blood samples from diabetic subjects than in healthy subjects. Subsequently, Shapiro *et al*[6] described that many carbohydrate components including glucose could be bound to Hb and that glucose was most efficiently bound to the β-chain of Hb. After Trivelli *et al*[7] published their quantitative measurement of Hb fractions in human blood, the analysis of HbA_{1c} was recognized as being a very important parameter in the assessment of diabetic patients.

In the early years, the results of HbA_{1c} analysis varied extensively between methods and laboratories[8-12]. Therefore during the 1980s, in United States and Canada working groups (WG) of the Diabetes Controls and Complications Trial (DCCT) were set up; these were originally incorporated within a multicenter, randomized clinical trial designed to compare treatments of insulin dependent diabetes mellitus in the National Glycohemoglobin Standardization Program (NGSP), but subsequently this activity was expanded to standardize the HbA_{1c} assays to the DCCT/NGSP reference method of liquid chromatography[9].

Later in the 1990s, the International Federation of Clinical Chemistry (IFCC) decided to develop a reference system for the international standardization of HbA_{1c}/glycohemoglobin measurements[13], which became the basis of the reference laboratory network for HbA_{1c}. In addition, IFCC set up another WG on Standardization for HbA_{1c} (1994/1995) in order to develop a primary standard[14] and reference method[15] for worldwide use in the HbA_{1c} measurement. These were the reasons why from the 1990s, IFCC organized WG to promote the standardization of all types of assays for HbA_{1c}. The adoption of these recommendations achieved substantial improvements in the analytical aspects and clinical significance of HbA_{1c}[16,17] so that today reference standards and standardized methods are in widespread use.

Further in 2010, the American Diabetes Association (ADA) emphasized the important role of HbA_{1c} in the diagnosis of diabetes, setting an analytical cutoff limit value of 6.5% for HbA_{1c} values[18] if they were expressed in percentage terms. Recently Hanas and John[19] reported the conclusions of the International Consensus Committee 2013 Update that HbA_{1c} results should be reported by clinical laboratories worldwide in Système Internationale (SI) units (mmol/mol - no decimals) and the corresponding NGSP units (% - one decimal) and recommended strongly to the editors of scientific journals that submitted manuscripts should report HbA_{1c} values in both SI (IFCC) and NGSP/DCCT units.

This review provides a brief summary of the reaction of glucose with Hb and the possible techniques available for analyzing HbA_{1c}. It also reviews the quality control, requirements of the target limits and considers issues related to the units and cutoff limits for HbA_{1c} in relation to the recommendations of IFCC and ADA.

GLYCATION OF Hb

Glucose is the major soluble carbohydrate and it can combine with different protein molecules in blood and

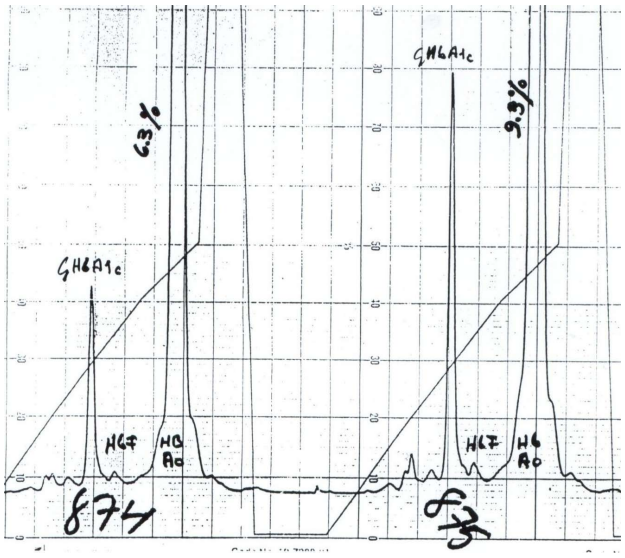

Figure 1 Fractionation of blood hemoglobin by an automated Mono S™ HR5/5 cation exchange column from Pharmacia with malonate buffer (0.01 mol/L, pH 5.7, 11 min, room temperature) for normal and diabetic human blood samples is illustrated as a chromatogram (Penttilä *et al*[10]).

tissues depending on the glucose concentration. In blood, Hb is one of these proteins and the β-chain of Hb is the main target of glucose[6]. Glucose is initially non-enzymatically bound to the amino acid valine on the β-chain of Hb *via* the formation of a reversible aldimine moiety, which then becomes rearranged into the irreversible ketoamine form. The structure of this ketoamine is similar to that of fructosamine[6]. This phenomenon occurs during the whole lifetime of erythrocytes (120 d) and thus the content of the ketoamine in Hb correlates with the age of the erythrocytes and the value of HbA_{1c} normally represents a mean value reflecting a time period starting from about three months before sampling.

By analyzing Hb components in blood, Trivelli *et al*[7] demonstrated that the small fraction A_{1c} was glycated more than the other small fractions A_{1a} and A_{1b} and that the amounts of this A_{1c} fraction were clearly higher in the blood of diabetic patients than in the normal subjects. The fractionation of Hb by a Mono S™ HR5/5 cation exchange column from Pharmacia with 0.01 mol/L malonate buffer for normal and diabetic human blood samples is illustrated in Figure 1[20].

METHODS OF HbA_{1c} ASSAYS

The first methods for the HbA_{1c} measurements were simple electrophoretic or ion-exchange mini-column chromatography assays, but they were rather soon replaced by many automated techniques, *e.g.*, different liquid chromatography techniques [ion-exchange chromatography (HPLC or FPLC) or affinity chromatography][8-12,20-22] as seen in Tables 1 and 2. The NGSP/DCCT organizations in United States and Canada selected the automated Bio-Rex 70 liquid

Table 1 The methods commonly used for analyzing hemoglobin A_{1c} according to the surveys conducted by Labquality Ltd. in 1994

Numerical summary from a diabetic EDTA-blood sample 1/1994				
Analyte/method group	Mean	SD	CV%	Number
GHbA1c %				
Ion exchange Ciba Corning	8.7	0.4	4.3	4
Ion exchange Diamat Biorad	9.7	0.8	8.4	15
Ion exchange Kyoto Daichii	8.4	0.3	3.4	3
Ion exchange Pharmacia MonoS	9.0	0.8	8.7	17
Ion exchange Shimadzu	10.7	0.1	0.7	2
Ion exchange others	9.5	0.8	7.9	5
Ion exchange minicolumn Biorad	9.7	0.8	8.1	4
Electrophoresis Beckman	9.7	0.8	7.8	8
Affininity method Abbott Imx	8.4	0.6	7.5	15
Affininity method Abbott Vision	8.8	0.4	4.8	8
LaInIA Ames DCA 2000	9.1	0.2	2.6	29
TinIA Boehringer	9.1	1.0	10.4	15
Immunochemical others	8.8	1.7	19.3	2
All method groups	9.2	0.8	8.9	127
$GHbA_1$				
Ion exchange Ciba Corning	11.8	0.3	2.4	2
Ion exchange Diamat Bio-Rad	12.1	0.4	3.2	7
Ion exchange others	11.5	-	-	1
All methods groups	12.0	0.4	3.3	10
GHb				
Affinity method Abbott Imx	13.4	0.4	2.7	2
Affinity method mini col Isolab	10.3	1.4	13.7	2
All method groups	11.8	2.0	16.5	4

HbA_{1c}: Hemoglobin A_{1c}.

chromatography assay as the reference method for the HbA_{1c} measurement[9]. It should be stressed that this equipment has not been available for many years. However, in addition to the NGSP/DCCT system, there were other different assay techniques used for standardization and these had their own standards, *e.g.*, in Japan (NGH) and in Sweden (FPLC with a Mono S column). These methods could be compared to each other and to the new IFCC procedure by using the appropriate master equations[23].

In 1988, an automated immunoassay method for the epitope assays of proteins was developed[24] and then utilized in the assay of HbA_{1c}. These immunoassay or enzymatic methods have replaced the chromatography methods previously used in clinical laboratories so that today only about 20% to 40% of methods for HbA_{1c} are based on liquid chromatographic techniques[16,25]. The numbers of immunoassays for HbA_{1c} based on many different principles are continuously increasing but these techniques usually have a higher inter-laboratory CV% than can be attained with liquid chromatography or enzymatic methods[16,22,25-27]; some of the most important immunoassay methods are listed in Tables 1 and 2.

STANDARDIZATION OF HbA_{1c}

As noted before, there were marked inter-laboratory differences in the quality assurance results before standardization procedures were adopted[8-12]. It was

Table 2 The methods commonly used for analyzing hemoglobin A_{1c} according to the surveys conducted by Labquality Ltd. in 2015

	Numerical summary from a diabetic EDTA-blood sample 5/2015					
Analyte/method group	**Mean**	**SD**	**CV%**	**Min**	**Max**	**Number**
HbA_{1c}, mmol/mol						
Abbott Arhitect enzymatic	53.64	2.45	4.6	48.4	32.1	6
Axis-Shield Afinion	54.70	2.17	4.0	51.0	58.0	44
Beckman Coulter	55.2					1
Hemoque	51.4	3.98	7.7	46.0	55.0	5
HPLC Bio-Rad D-10	55.00					1
HPLC Bio-Rad Variant	56.00	3.21	5.7	54.0	58.0	2
HPLC Tosoh	56.42	1.23	2.2	35.6	59.8	33
Roche Cobas c Tina-Quant	54.76	2.56	4.7	33.0	57.0	13
Roche Cobas Integra	55.0					1
Rocgw Tina Quant	56.00					1
Siemens Advia Centaur	57.93					1
Siemens DCA 2000+ and Vant	55.05	1.96	3.6	51.0	58.0	33
Thermo Scientific Konelab	54.44	2.63	4.8	48.0	58.4	24
All	55.05	2.17	3.9	32.1	59.8	165
HbA_{1c}						
Abbott Arhitect enzymatic	7.20	0.00	0.0	7.2	7.2	3
Axis-Shield Afinion	7.17	0.21	2.9	6.9	7.5	44
Beckman Coulter	7.2					1
Hemoqe	6.88	0.25	3.7	6.6	7.2	6
HPLC Bio-Rad D-10	7.2					1
HPLC Bio-Rad Variant	7.5					1
HPLC Tosoh	7.34	0.13	1.8	7.0	8.8	23
Roche Cobas c Tina-Quant	7.29	0.12	1.6	6.7	7.4	7
Siemens Advia Centaur	7.45			5.8	7.0	1
Siemens DCA 2000+ and Vant	7.19	0.18	2.6	6.8	7.5	31
Thermo Scientific Konelab	7.13	0.25	3.5	6.6	7.5	26
All	7.20	0.21	2.9	6.6	8.8	143

HbA_{1c}: Hemoglobin A_{1c}.

also reported that by incorporating an extra sample, against which the primary results could be recalculated, and significantly smaller CV% values could be obtained[9,16,17].

After the appearance of the IFCC WG for HbA_{1c}, a reference system was soon organized in 1996[13], with the goal of achieving standardization of HbA_{1c} analysis; this formed the basis for the worldwide reference laboratory network to help clinical laboratories in their HbA_{1c} measurements. The reference preparations which represented the primary and secondary standards of HbA_{1c} and HbA0 were produced in 1998[14] followed in 2002 by the reference method for the specific measurement of HbA_{1c}[15]. The final measurements in the reference method were based on the assay of N1-deoxyfructosyl-hemoglobin[28]. The assay consisted of a primary fractionation of the sample by affinity chromatography followed by analysis utilizing either HPLC/electrospray mass spectrometry or HPLC/capillary electrophoresis. The primary and secondary standards and the reference methods and the guidelines about names and units were then introduced to be adopted worldwide by all manufacturers making equipment for analysis of HbA_{1c} and also for clinical laboratories[13,23,25,29].

The standardization protocols about HbA_{1c} have been published by many societies of laboratory medicine in their own languages, *e.g.*, ADA in United States[18,29], DGKL in Germany[30], NEQAS in United Kingdom[31], SiBioC in Italy[32], EQUALIS in Sweden[33], Finnish Society of Clinical Chemistry (FSCC) in Finland[34], *etc*. With respect to the standardization procedures, it should be noted that according to Weykamp *et al*[35] the units and standardization protocols for the NGSP/DCCT and IFCC procedures are different for NGSP/DCCT and IFCC results and thus the reference values and quality control requirements cannot be of the same size.

ANALYTICAL PROBLEMS OF HbA_{1c} ASSAYS

The typical problems[36] which interfere with the HbA_{1c} assays, are attributable to hyperbilirubinemia, hypertriglyceridemia, leukocytosis and many Hb variants. In addition, certain physiological and pathological characteristics such as gestational stage, age, race, presymptomatic type 1 diabetes, malaria, iron deficiency, bleedings, transfusions, splenectomy, kidney failure, alcohol abuse, and some drugs may affect the HbA_{1c} results. The HbA_{1c} results may be too high in some cases and too low in others (hemolysis, pregnancy).

If erythrocytes have a short life-time, *e.g.*, in hemolytic anemias, this may decrease the HbA_{1c} results. Some abnormal forms of Hb cause erroneous results

Table 3 Summary of the questionnaires about the use of the hemoglobin A_{1c} units sent to 35-51 societies of laboratory medicine (mainly clinical chemistry) and returned by 28.12.2014

Year	2009	2011	2013	2014
Queries sent	35	37	47	51
Only % unit	16	14	15	17
Parallel units, % and mmol/mol	4	9	12	11
mmol/mol unit only in use	([1])	6	11	13
No reply	15	8	9	10

[1]Germany was the first country which adopted the exclusive use of mmol/mol unit for HbA1c from 1.1.2010. The questionnaires have been dispatched by e-mail, telefax and mail mainly to Europe and to a small extent outside of Europe from 2009 to 2014.

(HbF, HbS, HbC, HbD, HbE, *etc*.) depending on the assay type[37-39]. Furthermore, iron deficiency anemia as well as iron deficiency without anemia may induce elevated HbA_{1c} values compared with controls even though blood glucose levels are normal[39].

Many of these errors can be very difficult to detect, especially when using immunochemical assays. With some systems such as with liquid chromatography, the erroneous results can be visualized in the chromatograms, and these assays are commonly used for comparison[23,35-40]. However, errors can also be due to the problems in the action or response of insulin which are not directly related to the methods being used to assay HbA_{1c}[36].

UNITS OF HbA_{1c} IN LABORATORY PRACTICE

In 2009, prior to the use of the new IFCC system with the accepted name and unit for HbA_{1c}, questionnaires about which units should be used were sent to the European societies of laboratory medicine and some other societies outside of Europe (mainly in clinical chemistry)[40]. Germany was the first country to adopt mmol/mol exclusively in the daily laboratory practice (Deutsche Vereinte Gesellschaft für Klinische Chemie und Laboratoriums-medizin); this was inaugurated at the start of 1.1.2010. Germany was followed later in 2011 by The Netherlands, Sweden and the United Kingdom**.** Then gradually the number of mmol/mol reports of HbA_{1c} increased up to 13 in 2014, representing 25% of all replies from 51 queries. During these years, there was also an increase in the numbers of laboratories (from 9 to 12) reporting HbA_{1c} values in parallel units, *i.e.*, in both % and mmol/mol. By 2014, a minority of responders (24%) stated that they were using only the mmol/mol whereas nearly every other respondent (49%) stated that values were being expressed not only in mmol/mol but also as % values (Table 3). However, by the end of 2014, ten societies had not responded to the questionnaire, although the e-mail, telefax and mail addresses were taken from the annual catalogues of IFCC.

On the other hand, in the period from 2010 to 2015, the users of % values in the HbA_{1c} surveys conducted by Labquality Ltd.[41] have gradually but significantly decreased (from 54% to 40%).

The Finnish experience is an example of the difficulty to obtain acceptance of the molar unit recommended by IFCC for expressing HbA_{1c}. In 2009, the FSCC recommended that clinical laboratories should report the HbA_{1c} results in parallel, *i.e*., as mmol/mol and % values according to the recommendations[23]. In 2011, the FSCC proposed that the HbA_{1c} values should be provided only in mmol/mol. Nonetheless, the issue has not fully resolved due to the opposition from physicians treating diabetics. Thus the following compromise was agreed: In the future, the laboratories will report the HbA_{1c} values in parallel units, *i.e*., as mmol/mol and % although the measurements in many laboratories have originally been calculated in mmol/mol[40].

In Finland, one out of five university hospital districts (Pirkanmaa and Kanta-Häme) stopped reporting the % values for HbA_{1c} from April 2014; in that hospital the HbA_{1c} values are only being reported in mmol/mol[40]. It is possible that some other Finnish hospital districts will come to the same decision in the near future. In September 2014, the board of FSCC established a new working group to discuss with physicians and with non-laboratory societies and as well as with other interested parties about the issues related to the determination of HbA_{1c}. During the summer of 2015, FSCC decided to recommend that from 1.1.2016 the HbA_{1c} values should no longer be expressed as % units; hopefully this recommendation will prove acceptable.

QUALITY ASSYRANCE AND TARGET LIMITS OF HbA_{1c}

The QC surveys utilize common statistical methods such as the coefficient of variation (CV%)[16-18]. On the basis of these and the standard deviation (SD) values, the new target limits have been calculated for the QC-surveys of HbA_{1c} around the mean and the reference values expressed as either % or mmol/mol values. This means that within the range mean ± 2SD 95.6% of all results are within the acceptable limits and these correspond to the recent findings of Hyltoft Petersen and Lee[42]. Furthermore, the new report of Nielsen *et al*[43] about the value of HbA_{1c} in the classification of diabetes highlights the importance of the exact and precise measurement of HbA_{1c}.

As mentioned earlier, there was a remarkably high variation in the values being obtained with the different methods being used in laboratories all around the world[8-12]. Weykamp *et al*[11] were one of the first groups who described the dramatic improvement that could be obtained in the CV% of the HbA_{1c} results of either normal or diabetic subjects when the primary results were recalculated by incorporating an extra sample with

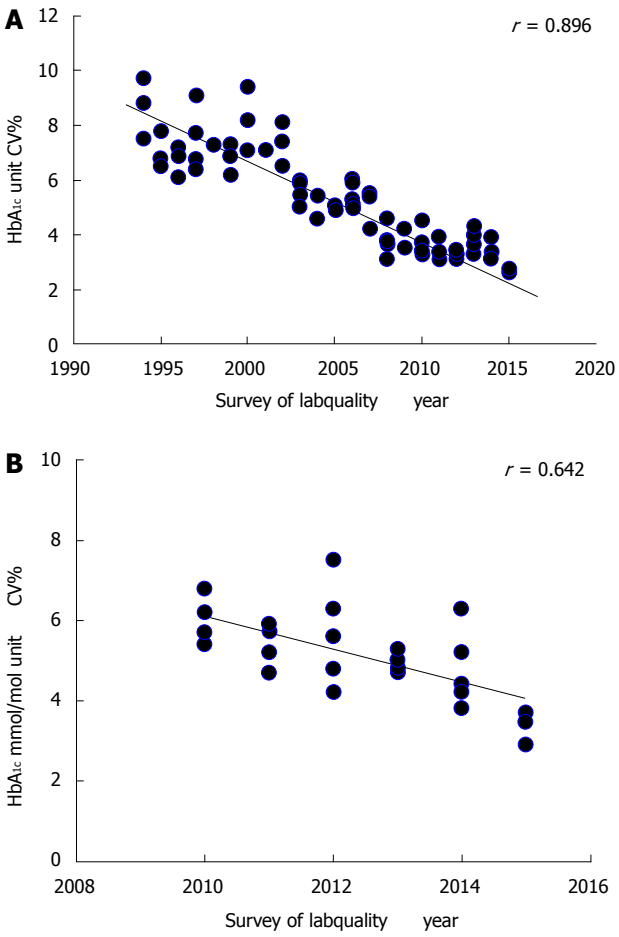

Figure 2 The total variations of the glycohemoglobin results have been presented both in % and in mmol/mol units from the surveys of Labquallity Ltd. from 1994 to 2015. In the part A have been presented the total variations of the glycohemoglobin in % units and in the part B the total variations of HbA_{1c} in mmol/mol units.

a known HbA_{1c} value. They had reviewed the corrected results from 110 laboratories using 21 different methods.

In Finland, the glycohemoglobin quality assurance surveys of Labquality started in 1986 with two fresh native EDTA-blood samples, one at the normal level and the second at the diabetic level, which were sent in each survey to all participants[41]. The mean values of the surveys were used as the target values and the acceptable ranges were ± 10% around the mean values. Figure 2A displays the significant improvement in the HbA_{1c} % values (r = 0.896) from 1994 to 2015 expressed as CV% values, they correspond well to the earlier publications[16,17]. Similarly in Figure 2B from 2009 when the results were reported in mmol/mol units, a further improvement in the CV%s was observed (for results expressed as mmol/mol) (r = 0.642). The mean ± SD values and CV% have been calculated from the surveys of Labquality.

In the most recent survey (2015) the range mean ± 2SD indicates that 95% of all results are within these limits[9,44,45]. For example, in December 2014, at the HbA_{1c} level of 6.77%, near the cut-off limit of ADA of 6.5%[18], the SD value was 0.21 for % units and that for mmol/mol units at the level of 50.8 mmol/mol the corresponding SD value was 2.1 mmol/mol. Thus the calculated acceptable limits with the old range of mean HbA_{1c} 6.77% ± 10% were from 6.09% to 7.48% and with the new narrower limit dating from 1.1.2015 the limits with mean ± 6% were from 6.36% to 7.18%. The latter range corresponds well with that of 6.77% ± 0.21% with the CV of 3.26% for the survey (0.21/6.77 × 100%). If one takes the mean ± 2SD of all results, then the range would be 6.35% to 7.19% in that survey and these would be clearly inside the old range of 10% and similar to the new limits from Labquality. Correspondingly in the same survey of December 2014 for mmol/mol values, the mean HbA_{1c} value was 50.8 ± 2.1 mmol/mol and the range for mean ± 2SD extended from 46.6 to 55.0 mmol/mol being very close to the new mmol/mol limit from Labquality as mean ± 8%, *i.e.*, from 46.7 to 54.9 mmol/mol with the CV% of 4.13% (2.1/50.8 × 100%). In the future, the target limits for HbA_{1c} of Labquality may be made more demanding. The latest Finnish results that in 2015 the CV% values for HbA_{1c} have reached a sufficiently low level (Figure 2) to be comparable to others in the literature both for % and mmol/mol results[9,16-18,46,47].

From the survey conducted in 2002, the HbA_{1c} values of the EDTA blood samples for the % results were also analyzed by the European Reference Laboratory for Glycohemoglobin (ERLGH)[48]. It was found that there was an almost perfect correlation between the mean HbA_{1c} % values of Labquality and the values HbA_{1c} % values of ERLGH (r = 0.997). The same phenomenon was also seen for the mmol/mol results from 2010 when comparing the mean values of Labquality and those of ERLGH (r = 0.973). Since the mean values of the surveys conducted by Labquality are in practice around the same size as the ERLGH values, thus one may be utilized as target values for mmol/mol The findings correspond well with the earlier reports[43,44] despite the fact that there are differences in both units and standardization programs between the NGSP/DCCT and IFCC procedures[35]. The new target limits of the HbA_{1c} results in 2015 for % results are ± 6% and for mmol/mol results ± 8% around the target value and agree well to those reported by Little *et al*[17], Weykamp *et al*[49] and Lindblad and Nordin[50]. In addition, on the basis of CAP surveys, Little *et al*[17] have reported that at the normal HbA_{1c} levels (6%-7%) the target value described in % units and with ± 6% around the reference value may be good enough for the diagnosis and follow-up of the treatment of diabetes. Lindblad and Nordin[50] reported that when near to the critical level of HbA_{1c} of 48 mmol/mol, the maximum allowable difference from the target value should be less than 3.5 mmol/mol, which corresponds to a CV% of 7.3%.

The Uppsala Meeting for Quality Specifications in Clinical Laboratories in early 1990s had one session devoted to the quality of HbA_{1c} measurements. The Danish clinical chemists[51,52] proposed that a change of

Table 4 The relationships between the hemoglobin A_{1c} units as % values to the mmol/mol values are presented at different levels of hemoglobin A_{1c}

HbA_{1c}	HbA_{1c} (%)	HbA_{1c} (mmol/mol)
Reference limits	4.0-6.0	20-42
Diagnosis limit	6.5	48
Treatment limits, adults	7.0	53
children < 6 yr	7.5	69
children 6-12 yr	8.0	64
children 13-19 yr	8.5	58
Poor diabetic balance	9.0	75
Very poor diabetic balance	12.0	108

Equarions from the NGSP/DCCT mean values to the IFCC units: % = 0.095 × mmol/mol + 2.15; mmol/mol = 10.93 × % - 23.5. The table also shows the most important equations to convert the results described in % units of NGSP/DCCT into the values of IFCC expressed as mmol/mol units[23]. HbA_{1c}: Hemoglobin A_{1c}; NGSP/DCCT: National Glycohemoglobin Standardization Program/the Diabetes Control and Complicatioins Trial Research Group; IFCC: International Federation of Clinical Chemistry.

1.0% from the measured % HbA_{1c} value might suggest that the treatment could be necessary whereas a change of 2.0% would demand that treatment should be initiated. This degree of accuracy is necessary that clinicians could feel confident with their decisions to initiate what could well be life-time therapy. These findings are in accordance with the earlier reports[8,16,20] and with our present findings. On the other hand, recently in 2015, Weykamp *et al*[45] reported that when using mmol/mol units, the calculated total allowable error could be 4.2 mmol/mol. The HbA_{1c} results of current Labquality surveys conducted in 2015, *i.e*., the mean values of 50.8 ± 2.1 mmol/mol are within this value.

On the other hand, a single laboratory should be able to perform HbA_{1c} % measurements with a total analytical variation (within and between series) from between 1.4% to 3.0% when using liquid chromatography[10,20,21,26], this variation due to the analytical procedure is about 4.5 times lower than the measured biological variation of this parameter[42,53]. Furthermore, also the differences (errors) between frequent measurements should be as small as possible to ensure reliable follow-up of the treatment of those patients in a stable diabetic state[16,17,23,31]. After the proposal from ADA[18] to use a diagnostic cutoff limit of HbA_{1c} for diabetes, this change demanded that the accuracy of the analytical performance of HbA_{1c} had to be better than before the setting of this fixed limit, a fact that has also been criticized.

REFERENCE VALUES OF HbA_{1c}

The commonly used reference intervals for HbA_{1c} in % DCCT/NGHP units have been from 4.0% to 6.0%[16,17,20,49,54]; the corresponding values for mmol/mol have been from 20 to 42 mmol/mol[18,50] as presented in Table 4. The exact values somewhat vary around those in the table but nonetheless are rather close to those published earlier[16,17,20,49,50,54]. In addition, the commonly applied reference values of HbA_{1c} in published reports expressed as per cent and mmmol/mol are normally stated in the laboratory manuals all around the world as are also the important limits for diagnosis and treatment of diabetes; these manuals also provide the master equations to convert between the system in use and the procedure of IFCC[23,30-35].

HbA_{1c} AND PLASMA GLUCOSE

The commonly used limit of plasma glucose of 7.0 mmol/L[8,9,17-19] has been widely accepted when there is a need to diagnose diabetes or to monitor the treatment of the patients. Many physicians treating diabetics like to compare the actual plasma glucose values to the HbA_{1c} values and consider that they are assessing some kind of balance of diabetes. However, it was not until 2002 when Rohlfing *et al*[55] collected enough published data to be able to devise an equation describing the well-established ratio between blood mean HbA_{1c} and plasma glucose, *i.e*., (PG/HbA_{1c}): Plasma glucose (mmol/L) = [1.98 × HbA_{1c}(%)] - 4.29 (n = 1439, r = 0.82). However, when inspecting their proposal, it is quite evident that at the same HbA_{1c} value, *e.g.*, 8.0%, the plasma glucose concentration may maximally vary from 6 to 15 mmol/L and thus the results are not precise, especially at the higher blood HbA_{1c} levels[49]. In addition, in subjects with impaired glucose tolerance (plasma glucose value from 5.6 to 6.4 mmol/L or blood HbA_{1c} from 5.7 to 6.4 mmol/mol) the 2 h oral glucose tolerance test may be as good or better at revealing both the insulin sensitivity and disturbance in glucose metabolism than can be achieved with a single value of fasting plasma glucose[56-60]. These are the reasons why many laboratories do not calculate the mean PG/HbA_{1c} ratios but report glucose and HbA_{1c} results separately[41]. It has also argued that in fulminant (acute) type I diabetes, the specificity of the analysis of HbA_{1c} may be doubtful low when compared to the analysis of glycated albumin with a significantly higher specificity[59]. This may have a significant role to make a selection of the analyze type in diagnosing of acute diabetes or prediabetes. It is also important that plasma glucose and blood HbA_{1c} levels should be assessed in an accredited laboratory if the values are to be used for diagnosis or screening of diabetes[18,19,23,58-62].

HbA_{1c} AND SOME OTHER ASPECTS

Finally, it has been pointed out that in both men and women elevated levels of blood HbA_{1c} increase the risk of developing cardiovascular disease[63]. In addition, as described in the chapter of analytical problems, many other diseases may affect the HbA_{1c} results and cause difficulties for the clinicians. Some diseases other than cardiovascular are also associated with HbA_{1c}[62,63], *e.g.*, diseases altering iron metabolism.

The point-of care instruments (POC) are being continuously introduced for the analysis of HAb_{1c}[25,64] but there are still many issues associated with their use[64-66]: (1) they have not been universally recommended for the diagnosis of diabetes according to the guidelines[17-19,30]; (2) many POC users are not participating in the quality control programs of their home country; and (3) the POC analyses are difficult to standardize. In the future, in conjunction with the further development of these methods, especially with the adoption of reliable quality systems, POC analyses may achieve real breakthroughs[25].

CONCLUSION

This assessment of HbA_{1c} analytical procedures and values indicates that a considerable improvement has occurred during the past 30 years with respect to both their the precision and accuracy and these improvements are still on-going as reflected in reduced assay CV% values. The immunoassay techniques have replaced many chromatographic procedures during this time period.

During 2014/2015, the reports from quality assurance systems have confirmed the marked improvement for the quality of HbA_{1c} measurements irrespective of whether the results have been expressed in % or mmol/mol units. The acceptance of the mmol/mol system recommended by IFCC for HbA_{1c} and the new unit and reference ranges are only becoming slowly accepted outside of Europe where it seems that the parallel reporting for HbA_{1c} will continue. The use of a diagnostic cutoff limit for the HbA_{1c} value is still not finalized.

The reference analyses from the mean values of the survey results may be used as target values in both % and mmol/mol units assuming that the number of the participating laboratories is high enough to be statistically satisfactory.

The authors also hope that the use of the mmol/mol unit for HbA_{1c} can gain worldwide acceptance as this would make it much easier to compare results from different studies and remove the possibility of confusion when units are converted from one form to the other.

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