Hemoglobin A1c units, diagnostic limits and quality aspects updated.

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Abstract

In this report the use of the units, diagnostic limits and the quality aspects of hemoglobin A1c (HbA1c) were studied in worldwide clinical laboratory practice. In addition, the quality aspects were calculated for the target values from the surveys of Labquality Ltd. The use of HbA1c units and the diagnostic limits for diabetes were examined using e-mail and mail inquiries to 37 or 51 societies of laboratory medicine (mainly clinical chemistry) from 2009 to 2019. The parametric statistical programs of Labquality Ltd. and commercial programs of SPSS® 13.0 and MS Excel 2013 (Microsoft® Co., Cambridge, MA, USA) were used.

The IFCC system for HbA1c as mmol/mol unit slowly but constantly gaining acceptance in Europe, but remains quite rare outside Europe whereas the DCCT/NGSP system as a per cent unit was reduced. The mean round values of Labquality Ltd. and the corresponding values of the European Reference Laboratory for Glycohemoglobin (ERLGH) were calculated for the target values with ±6% intervals for the per cent results and ±8% intervals for the mmol/mol results. To avoid confusion, the overall use of mmol/mol unit only for HbA1c by the IFCC system could be the best one in the future.

Introduction

In the 1960s, measurement of the hemoglobin A1c fraction (HbA1c) by qualitative or quantitative assays began [1,2,3]. In the seventies Trivelli & al. [4] published the first genuinely quantitative assay for HbA1c showing that more carbohydrates are bound to the HbA1c fraction in diabetics than in non-diabetics. Since then numerous methods have been developed for HbA1c measurements [1,2,4-7] which caused a markedly high variation between laboratories and countries. This was clearly shown by Weyksmp & al. (5) with their quality control round for HbA1c of 111 laboratories and 21 different methods, that the
originally high variation between the methods and the laboratories was substantially corrected when using a third control sample for a new calculation of the results.

However, in the United States and Canada from the 1970s there it was shown that the results of HbA1c analyses varied extensively between methods and laboratories. As a result, working groups (WG) of the Diabetes Control and Complications Trial (DCCT) were set up; these were originally incorporated in a multicentre, randomised clinical trial designed to compare treatments of insulin-dependent diabetes mellitus in the National Glycohemoglobin Standardisation Program (NGSP). This DCCT/NGSP program was subsequently expanded to standardise the HbA1c assays using the liquid chromatography as the DCCT/NGSP reference [7].

In the 1990s, the International Federation of Clinical Chemistry (IFCC) organised working groups to achieve standardisation of all types of assays for HbA1c. For these the reference standards [8] and a standardised method [9] were developed for international use. Also, the reference system for the international standardization of HbA1c measurements in the form of a reference laboratory network was organised [10]. These recommendations were developed for the international use proposed by IFCC [11].

In addition, the American Diabetes Association (ADA) announced in 2010 the possibility to select a specific fixed value for the diagnosis of DM, namely an HbA1c value of 6.5% (12). This proposal involved the assumption that when using a fixed limit of HbA1c for diagnostic purposes, the methods used must be highly accurate and precise to ensure proper clinical practice [13].

For the present paper the queries were updated to the societies of laboratory medicine about the worldwide use of the units and diagnostic limits for HbA1c (1,14). The queries concerning HbA1c units, the diagnostic cut off limits and the precision and accuracy requirement for the HbA1c assays were calculated and
presented. The results of the quality control rounds from the Finnish quality control organisation Labquality Ltd. [15] and the values of the European Reference Laboratory for Glycohemoglobin (ERLGH) were utilised for the mean values of the rounds [15-17].

Methods

Questionnaires concerning the use of HbA1c units from 2009 to 2019 (1,14,16,17) and later, on the utilisation from 2014 of the fixed diagnostic limit of ADA (12) for diabetes were sent mainly by e-mail but also by mail to 37 or 51 societies of laboratory medicine (mainly clinical chemistry) in Europe as well as some outside of Europe (Table 1). The e-mail and mail addresses for the societies were taken from the latest list of the IFCC on July 2019.

The annual HbA1c rounds of Labquality Ltd. (15) were started in 1987 by performing from three to six times per year using two native EDTA blood samples from two volunteers [1,15], one close to the level of HbA1c at the diagnostic level recommended by the ADA [12] as 6.5 % (48 mmol/mol). Parallel control samples from 1997 were sent to ERLGH) in the Netherlands (17) for comparison with the values of Labquality Ltd. (15).

From the rounds of Labquality Ltd. only the results from the native EDTA blood samples were used for the calculations. The EDTA samples for control (QC) assays were drawn from two volunteers on the morning of the sample collection, mixed, divided into 0.5 ml portions and sent to the office of Labquality Ltd. by airmail. From the office, the samples were then sent to participants during the same day. The HbA1c reference values of ERLGH were compared to the corresponding values of Labquality Ltd. and informed to the participants. Since
1994, the results only from three surveys were excluded for the calculations due to the sample transfer problems.

In the 1990s, two-thirds of the participants in the HbA1c rounds of Labquality Ltd. were Finnish, but in late 2000s the percentage was down to about one-third. In the same time, liquid chromatography as the principal method (60%) was reduced to one-third of the methods the most common one now belonging to immunochemistry.

2.1 Statistics

For the calculations of the mean values, standard deviations (SD), and the coefficients of variation (CV%) of the replies the parametric statistical the methods of Labquality Ltd. [15] were used, the SPSS® 13.0 program of SPSS (SPSS Inc., Chicago, IL, USA) and the MS Excel 2013 program (Microsoft® Co., Cambridge, MA, USA) and also the Scientific Tables of Documenta Geigy (6th Edition 1962, J.A.Geigy, Basel, Switzerland). The mean ± 2*SD values thus, containing 95.6% of the laboratories participating in every round were used for calculations and the reports.

Results

In Table 1 a summary is presented of the replies to the questionnaires from the societies up to August 15, 2019. Earlier report were presented in 2015 (1), 2017 (14) and 2019 (16). The use of the mmol/mol unit only (r=0.836, p<0.05) and the parallel units for HbA1c were slowly increased up to 2017 after which no change occurred (r=0.962, p<0.001). The use of the per cent unit only was correspondingly clearly decreased (r=0.974, p<0.001). Table 2 contains the detailed a summary of the replies of societies for the groups A to C while eight societies in the group D have not responded all.
During the period of the questionnaires, the units for HbA1c from the rounds of Labquality Ltd. [15] were studied for a comparison to the corresponding ERLGH values (1). As seen in Figure 1, the practice of mmol/mol users was significantly increased during the period from 2009 to 2019 with a corresponding decrease of per cent users (no. 49, r=0.990, p<0.001). It must be pointed out that in the surveys of Labquality LTD. [15] that most of the participants originate from Europe.

Before the calculation of the target values the quality aspects of the results for the HbA1c methods were studied as the coefficient of variation (CV%) from the replies as per cent and mmol/mol users using the results of Labquality LTD. [15], from 2010 to 2019 while the mmol/mol values for HbA1c cent were available. The mean CV% of the mmol/mol was reduced from 6.03 % in 2010 to 3.17±9.35 % in 2019 (no. 49, r= 0.771, p<0.001). On the other hand, the CV% of per cent values was decreased from 4.4 % to 2.33±0.06 %. Correlations of the mean HbA1c values of Labquality Ltd. [15] between the per cent values (no. 49, 3.36±0.91) and mmol/mol values (no. 49, 4.40±1.03) the from rounds was highly significant (r=0.783, p<0.001) corresponding earlier reports [18-20]. Thus, both units may equally be used to follow up on the quality of the methods, while being very close.

Table 3 shows the calculated target values for per cent and mmol/mol of Labquality Ltd. [15] for HbA1c as well of the corresponding values of ERLFG from the round 5/2018 as an example. The very close agreement and the correlation of the mean values of per cent and mmol/mol results were noted when calculated from the surveys of Labquality LDT. from 2010 to 2019 (no 49, r=0.995, p<0.001). The new target limits were calculated from the round values of the earlier rounds of Labquality Ltd. [15] and accepted as the mean ±6 % values for per cent and the mean ±8 % for mmol/mol values from January 2016.
The limits correspond well with the CV% values with the ERLGH as ±6% and ±8% from 2016 instead of earlier ±10 % limits for both units.

Discussion

The Finnish Society of Clinical Chemistry (FSCC) and the Finnish Societies for Diabetes Research and Treatment agreed in 2009 that, for the reports of HbA1c of Labquality Ltd. [15] in Finland, the mmol/mol values of HbA1c should also be added corresponding to the recommendation of the IFCC [10] from March 3 2010 [21]. Consequently, the reports for HbA1c were expressed in parallel units in per cent and mmol/mol. After five years of accumulating experience, the FSCC decided that parallel results had been used long enough and from January 1 2016 onwards only the mmol/mol unit for HbA1c was recommended [16].

Since 2009 multiple queries have been sent to European Societies of Laboratory Medicine and some other societies in and outside of Europe concerning the use of the old DCCT/NGSP per cent unit and the new mmol/mol for HbA1c recommended by IFCC (10). In 2011, the use of a fixed diagnostic limit of ADA [12] for HbA1c to diagnose diabetes was added in Finland in 2014 and then to the questionnaires [1,14,16].

The use of the mmol/mol system of IFCC only and the parallel reports in mmol/mol and per cent also are slowly and constantly increasing in Europe, but still less common outside of Europe as seen in Table 1 [14,16]. Germany [22] was the first country that accepted the mmol/mol unit only for their HbA1c reports Table 2), followed then by other European countries. However, the use of the IFCC mmol/mol system has not universally accepted by non-European societies despite the IFCC proposal that the mmol/mol is the only logical unit for use in HbA1c assays [23].
Thus the decrease of CV% for per cent values nearly reached the lowest practical value below 3.0 % and of mmol/mol below 4.0 %, which can be reached in large quality control surveys such as in Labquality Ltd. The CV% results correspond well to the findings of Weykamp & al. (24) that the quality requirements for the HbA1c units are different.

In order to study the QC level of HbA1c analyses, the assay results obtained from the annual rounds of Labquality Ltd. [15] were examined by calculating the variation of the results as the CV% [1]. The mean round CV% of the rounds decreased in the last seven years from 6.1% to 3.17 % in terms of the mmol/mol results (r = 0.702, p < 0.001) and from 4.0 % to 2.33 % of the per cent results (r = 0.388, p < 0.01). The mean CV% for the per cent results decreased from 8.1% to 2.33 % from 1994 to 2019 (r = 0.900, p < 0.001). These findings for HbA1c are highly comparable to earlier publications in terms of the per cent results [18,20] and in terms of the mmol/mol results [2,19,25] for HbA1c corresponding well to the findings of Weykamp & al. [24].

The mean results from the rounds of Labquality Ltd. [15] were very close to the values of ERLGH (Table 3). For the target values, the mean round HbA1c values were selected while the mmol/mol and per cent values were almost identical with the corresponding ERGH values (Table 3). Thus each laboratory participating in surveys can chose the value they will use as their target value.

Correlations between the IFCC methods and the DCCT/NGSP methods were published by Hoelzel & al. [11], and the equations between the IFCC and DCCT methods were: HbA1c (mmol/mol) = 10.93 * HbA1c (%) – 23.50 and HbA1c (%) = 0.0915 * HbA(1c) (mmol/mol) + 2.15. These equations allow the results to be reliably estimated, as shown in the HbA1c rounds of Labquality Ltd. [15] compared to the results measured by ERLGH. This is highly important when considering the fixed HbA1c limit of the ADA [12] in diagnosing DM. The
reported limit of 6.5% corresponds to 48 mmol/mol in the IFCC system. The queries indicated that the most countries/societies recommend the use of either 6.5 % or 48.0 mmol/mol limits in the diagnosis of DM, and this practice is being more widespread over time (Table 2).

From the quality assurance results of Labquality LTD. [15] the precision limits for HbA1c, the mean value ±6 % for per cent results and mean ±8 % for mmol/mol results correspond well to the limits published previously [18,19,22], which also are in line with the findings of Weykamp & al. [24] in that the quality requirements are different for the DCCT/NSGH and the IFCC systems. The decrease of CV% for per cent values reached the lowest possible value below 3.0 % and for the mmol/mol result below 4.0 % (16,25,26). Recently, Lenters-Westra and English [26] in their article pointed out that the CV% of HbA1c by using NSGH methods should be below 2.0. Their material was based on the results from the reference laboratories (10) which kind of limit cannot be reached from the large quality control reports, such as Labquality Ltd. [15.], which correspond well to other recent findings [27,28].

Conclusions

The mmol/mol system, recommended by the IFCC for HbA1c 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 are rare outside Europe. The use of the diagnostic cut-off limit of the HbA1c value is still not fully established, although it is slowly increasing.

In each round either the mean value of the round of Labquality Ltd. [15] or the corresponding ERLGH value can equally well be utilised as the target value as they correspond significantly with each other.
The authors also hope that the use of mmol/mol unit for HbA1c of IFCC would gain worldwide acceptance to enable the comparison of results from different studies and to lessen confusion, where only one unit would then be used.

**Abbreviations**

DCCT, DM, EDTA, HbA1c, IFCC, NGSP

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lingua for revising the English language.

**Legends to the figures and tables**
Figure 1. Relationship of the quality control values of the rounds of Labquality Ltd. and the parallel control values of the European Reference Laboratory for Glycohemoglobin as per cent results (mean 6.3%) and as mmol/mol results (mean 46.5 mmol/mol) from 2010 to 2019 (r = 0.990, p < 0.001).
Table 1.

<table>
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<tr>
<th>Year</th>
<th>2009</th>
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<th>2014</th>
<th>2017</th>
<th>2019</th>
<th>2019 %</th>
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<td>51</td>
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<td>41</td>
<td>42</td>
<td>43</td>
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<td>17</td>
<td>13</td>
<td>14</td>
<td>27.5</td>
</tr>
<tr>
<td>per cent &amp; mmol/mol</td>
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<td>9</td>
<td>11</td>
<td>15</td>
<td>15</td>
<td>29.4</td>
</tr>
<tr>
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<td>(*)</td>
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<td>13</td>
<td>14</td>
<td>14</td>
<td>27.5</td>
</tr>
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<td>8</td>
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</tr>
</tbody>
</table>

Table 1. Summary of the enquiries for HbA1c from 2009 to 2019 by e-mail and/or mail. The enquiries concerning the use of the units were sent to the societies of laboratory medicine (mainly clinical chemistry) in Europe and other societies outside of Europe. The summary of the replies was collected on August 15 2019.

*The first society that chose the mmol/mol unit was Germany starting the mmol/mol only replies at January 1, 2010.
Table 2. Summary of the replies to the questionnaires sent to the societies of laboratory medicine (mainly clinical chemistry) concerning the use of per cent and mmol/mol units in HbA1c analyses in daily reports and the acceptance of
the diagnostic limit of HbA1c as of 2019 in the diagnosis of diabetes. The whole summary of the replies was collected on August 15, 2019.

### Table 3

<table>
<thead>
<tr>
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<th>ERLGH</th>
<th>Labquality Ltd.</th>
<th>ERLGH</th>
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</thead>
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<tr>
<td><strong>HbA1c from the round 5/2018</strong></td>
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<td></td>
</tr>
<tr>
<td><strong>HbA1c in per cent unit</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Source</td>
<td>Value</td>
<td>Mean±SD</td>
<td>Mean±2SD</td>
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<tr>
<td>No. 44</td>
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<td>6.52-7.04</td>
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<tr>
<td></td>
<td>0.992</td>
<td></td>
<td>6.40-7.22</td>
</tr>
<tr>
<td><strong>HbA1c in mmol/mol unit</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Source</td>
<td>Value</td>
<td>Mean±SD</td>
<td>Mean±2SD</td>
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<tr>
<td>No. 72</td>
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<td>48.0-53.9</td>
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<tr>
<td></td>
<td>0.989</td>
<td></td>
<td>47.8-55.0</td>
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</table>

**Table 3.** Calculation results of the target values for HbA1c from the mean values of Labquality Ltd. and the corresponding values of ERLGH from the round 5/2018. The correlation between the mean
values of Labquality Ltd. and the value of ERLGH was 0.990 for per cent results (p<0.001) and 0.989 for mmol/mol results (p<0.001).

References


