Evaluation of the Radiometer Whole Blood Glucose Measuring System, EML 105

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Summary: The performance of a new glucose electrode system from Radiometer was tested using two EML™105 analyzers (Radiometer Medical A/S, Copenhagen, Denmark). Results were very precise (both analyzers reported CV = 1.0% at a glucose concentration of 13.4 mmol/l). Comparison of methods was performed according to the NCCLS EP9-T guideline. Patients glucose results from both analyzers were lower compared with the results obtained with a Hitachi 911 (Boehringer Mannheim, Mannheim, Germany). There was no haematocrit dependency of relevance.

Introduction

Recently, Radiometer introduced an electrode system for the quantitative measurement of whole blood glucose. The electrode consists of an inner electrode with a platinum anode and a silver/silver chloride reference cathode, which is placed in a removable jacket with a multi-layer membrane. The 20 µm thick multi-layer membrane consists of three layers; an outer, low-porous, diffusion-limiting, polyester layer which exposes the second layer, containing immobilized glucose oxidase, to a lower glucose concentration than is present in the sample and a third layer consisting of cellulose acetate, which holds back compounds of relative molecular mass of M_r ≥ 100.

The electrode is located in an EML105 analyzer. The whole blood sample is undiluted and no lysis of erythrocytes takes place. Calibration of the glucose electrode is performed automatically at user-preset time intervals. Before each measurement or calibration the zero current is measured and corrected. No internal standard is used for correction for possible drift of the glucose electrode. The manufacturer claims that there is no haematocrit dependence.

We made an analytical evaluation of the new glucose electrode using two EML105 analyzers.

Materials and Methods

Two identically equipped EML105 analyzers were used including calibrating solutions (an aqueous standard glucose solution 10.0 mmol/l, and an aqueous solution without glucose), Qualicheck™ 4 control, lotnr. 1: level 1 (11.7—14.4 mmol/l), and level 3 (1.9—2.9 mmol/l) were from Radiometer Medical A/S, Copenhagen, Denmark. The glucose electrode was calibrated at 4-hour intervals.

Imprecision

Each of two different levels of Qualicheck 4 glucose control was analyzed three times a day for 25 days.

Linearity and recovery

A range of glucose concentrations were prepared using saline solution: 5, 10, 20, 30, 33.3, 40, 50, 60, 80 and 100 mmol/l. Each concentration was measured twice after a new electrode membrane

had been installed. At the end of the electrode membrane guarantee period of one month, the experiment was repeated.

The method described by Burnett (1) for quantitatively assessing linearity consists of fitting the data pairs to a polynomial of the form

\[ y = a_0 + a_1x + a_2x^2 \]

and testing the value of \( a_2 \) thus obtained to see if it differs significantly from zero.

Comparison of methods

Experiments were performed according to the NCCLS EP9-T guideline (2). A total of 50 heparinized whole blood samples from patients were measured in duplicate on the two EML105 analyzers. After centrifugation of the whole blood samples, plasma glucose measurements were performed in duplicate on the two EML105 analyzers. Subsequently, measurements were performed in duplicate on a Hitachi-911 analyzer (Boehringer Mannheim, Mannheim, Germany) with a glucose dehydrogenase method.

Results and Discussion

Imprecision

Imprecision results of each EML105 analyzer reported at concentration (CV %) were: 13.4 mmol/l (1.0%), 2.4 mmol/l (3.2%) and 13.4 mmol/l (1.0%), and 2.4 mmol/l (3.0%), respectively.

Linearity

The linearity of the newly mounted membranes were: 40 mmol/l and 60 mmol/l, respectively. At the end of the electrode membrane lifetime, the linearity is decreased to 30 mmol/l (EML105 analyzer 1) and 33.3 mmol/l (EML105 analyzer 2). Radiometer claims that linearity exists up to 30 mmol/l.

The highest glucose control concentration (11.7—14.4 mmol/l) available from Radiometer is insufficient for checking linearity, which decreases during the electrode membrane lifetime.

Comparison of methods

The results of the correlations according to the EP9-T guideline were calculated using an orthogonal regression with Hitachi 911 plasma results on the x-axis and EML105 whole blood results on the y-axis (fig. 1 and fig. 2). Means of the 50 patient whole blood duplicates on EML105 analyzers 1 and 2 were 7.16 and 7.14 mmol/l, respectively. The results show that glucose measurements on the EML105 analyzers produced lower results than those on the Hitachi 911. The 10.0 mmol/l glucose calibrator of the EML105 measured as sample resulted in 9.47 mmol/l (n = 24). This calibration solution is led to the glucose electrode via a path different from the sample path. This phenomenon clarifies probably the origin of the difference between the two EML105 analyzers and the Hitachi
Fig. 1 Comparison glucose EML-105 system 1 versus glucose Hitachi 911.
\[ n = 50; y = 0.961 x - 0.03; s_x = 0.08 \text{ mmol/l} \]
Mean difference \( X_i - Y_i = 0.32 \text{ mmol/l} \)
Standard deviation of the differences \( X_i - Y_i = 0.19 \text{ mmol/l} \)

Fig. 2 Comparison glucose EML-105 system 2 versus glucose Hitachi 911.
\[ n = 50; y = 0.965 x - 0.08; s_x = 0.11 \text{ mmol/l} \]
Mean difference \( X_i - Y_i = 0.34 \text{ mmol/l} \)
Standard deviation of the differences \( X_i - Y_i = 0.24 \text{ mmol/l} \)

The EML105 software has an option for correction for slope and intercept in order to adjust the results to other methods.

Imprecision calculated from the duplicates of patients whole blood sample measurements on both EML105 analyzers was divided into 3 ranges, low, medium, and high. The respective concentrations were mean (CV\%): low \( n = 17, 4.74 (0.8\%) \) and 4.74 (0.8\%); medium \( n = 17, 6.91 (0.7\%) \) and 6.86 (0.7\%); high \( n = 16, 9.99 (0.6\%) \) and 9.98 (0.5\%).

The influence of the sample haematocrit on the glucose measurement was evaluated using the same set of patient samples as used for the comparison of methods. The mean (± SD) haematocrit of the patients' whole blood samples was 0.30 (± 0.08) 1/l with a lowest and highest value of 0.18 and 0.43 1/l, respectively. Using an orthogonal regression analysis for comparing EML105 plasma (\( x \)) glucose values with EML105 whole blood (\( y \)) glucose values the following results were obtained for EML105 system 1:

\[ y = 0.980 x + 0.03 \text{ and } s_y = 0.08 \text{ mmol/l} \]
Mean difference \( X_i - Y_i = 0.12 \text{ mmol/l} \)
Standard deviation of the differences \( X_i - Y_i = 0.16 \text{ mmol/l} \)

For EML105 system 2:
\[ y = 0.987 x + 0.00 \text{ and } s_y = 0.08 \text{ mmol/l} \]
Mean difference \( X_i - Y_i = 0.16 \text{ mmol/l} \)
Standard deviation of the differences \( X_i - Y_i = 0.17 \text{ mmol/l} \).

These results indicate that there is no dependency on haematocrit of relevance.

We conclude that the new Radiometer glucose electrode provides reliable and accurate results.

Acknowledgements

We are grateful to Dr. G. Kokholm, Radiometer Medical A/S, Copenhagen, Denmark, for reading this manuscript.

References

A Re-Evaluation of Glycohaemoglobin Standardisation: The Italian Experience with 119 Laboratories and 12 Methods

Andrea Mosca, Renata Paleari, Alessandra Trapolino, Fabio Capani, Gianfranco Pagano and Mario Plebani

Materials and Methods

Study design

From April to June, 1995, four samples consisting of two lyophilized calibrators (CAL 1 and CAL 2) and two quality-assurance lyophilized samples (SAM 1 and SAM 2), one with a low and one with a high HbA1c content in each pair, were mailed to 119 laboratories throughout Italy. The laboratories were asked to measure the HbA1c content in triplicate, by assaying the materials within a normal routine run.

Samples and calibrators

Blood samples from subjects homozygous for Hba, with normal urea concentrations and no acetylsalicylate medication were collected in the presence of EDTA. Two lots of the lyophilized materials (CAL 1 and SAM 1, both with a normal HbA1c content) were prepared from outdated blood collected from our local blood transfusion centre. An additional lot of lyophilized material (CAL 2, with a high HbA1c content) was prepared from blood obtained from a diabetic patient who regularly underwent therapeutic bleeding for secondary polycythaemia. The fourth lot of lyophilized material (SAM 2, with high HbA1c content) was prepared from pooled haemolysates from 10 informed consent diabetic patients, during their regular check-up at the antidiabetic centre. Patients’ samples used in this study were collected in accordance with the ethical requirements of our institutions.

The lyophilized materials were prepared as follows. After plasma removal, red blood cells were washed three times with 9.0 g/l NaCl solution and dialysed overnight at + 5 °C against the same saline solution. The erythrocytes were then haemolysed by adding cold distilled water to obtain a 16 g/l haemoglobin solution. Solid sucrose was then added to the haemolysate to give a final 50 g/l concentration. The resulting haemoglobin solution was centrifuged immediately at 20,000 g for 30 min to remove any cellular stroma. A clear supernatant was separated and dispensed in small aliquots (2 ml), into 5 ml glass vials. Finally, the vials were lyophilized and stored at + 4 °C. For reconstitution of the lyophilized samples, 300 μl of distilled water was added to each vial. Ten minutes standing at room temperature was recommended before their use. The reconstituted materials, which had a total haemoglobin concentration in the range of undiluted blood samples, had to be handled in exactly the same way as patient specimens.

After preparation, the materials were studied by HPLC separation (methods are listed later), spectrophotometric analysis in the 300–700 nm region and by measuring MetHb concentration. HPLC results (profiles and areas) as well as absorption spectra were found to be similar to those obtained with fresh blood samples. The MetHb content was less than 2% in all samples. In order to test the stability of the lyophilized materials, samples were stored at...
different temperatures (−20 °C, +4 °C, +25 °C, +37 °C) and assayed periodically. The samples stored at −20 and +4 °C were found to be quite stable over 12 months of storage. During the storage period no significant changes were found in HbA1c concentrations, and no anomalous peaks corresponding to Hb ageing components were revealed by HPLC analysis. Furthermore, no significant increase in MetHb levels was detected. The HbA1c concentrations assayed by HPLC with an L-9100 Hitachi system in two samples with normal and high HbA1c content, stored at +25 °C and +37 °C, were the following: day 0, normal content: 4.2−4.3%; high content: 9.1−9.1%; day 14 (+25 °C), normal: 4.4−4.5%; high: 9.2−9.2%; day 49 (+25 °C), normal: 4.1−4.1%; high: 8.8−8.8%; day 14 (+37 °C), normal: 4.3−4.3%; high: 8.9−9.0%; day 49 (+37 °C), normal: 3.7−3.7%; high: 8.8−8.8%. Stability of the materials after reconstitution was also quite satisfactory, since storage for over seven months at −20 °C, fifteen days at +4 °C and three days at +37 °C did not alter HbA1c values, as determined by HPLC.

Analytical methods

The following methods (code, number of participants using the method) were used by the participants, according to manufacturers' instructions:

Affinity chromatography methods

Abbott Vision (code A, used by 16 laboratories) and Abbott IMx (code B, used by 5 laboratories; Abbott Diagnostica, Rome, Italy).

HPLC and LPLC methods

Bio-Rad Diamat (code C, 27 laboratories) and Bio-Rad Variant (code C, 3 laboratories; Bio-Rad Laboratories, Segrate, Milan, Italy); Menarini HA 8110 and 8121 (code E, 34 laboratories; A. Menarini, Florence, Italy); Kontron systems 300 and 400 (code F, 5 laboratories; Kontron Instruments, Milan, Italy); Hitachi system L-9100 (code C, 5 laboratories; Merck Bracco, Milan, Italy); Instrumentation Laboratory system Glycollab (code H, 2 laboratories; Instrumentation Laboratory, Milan, Italy); Beckman system Gold (code I, 1 laboratory; Beckman, Milan, Italy); Waters test (code I, 1 laboratory; Waters Associates, Milford, MA).

Except for the Instrumentation Laboratory method (a low pressure liquid chromatography system, LPLC) all the other methods used were HPLC.

Immunochemical methods

Bayer DCA 2000 test (code L, 3 laboratories; Bayer, Milan, Italy); Boehringer hemoglobin Alc Tina-quant test (code M, 12 laboratories; Boehringer Mannheim, Milan, Italy).

Electrophoretic methods

Helena REP-glyco test (code N, 4 laboratories; Helena laboratories, Assago, Milan, Italy).

Data evaluation

Evaluation without calibration

Intra-laboratory variation was calculated as the mean of the CV obtained from the replicates reported for each of the lyophilized samples, SAM 1 and SAM 2. Where only two replicates were available per sample, we calculated the CV by the method based on the differences of duplicates. The mean intra-laboratory variation, per method and overall, was calculated as the overall mean of the CV from individual laboratories.

Inter-laboratory variation, defined as the variation arising from the results obtained by different laboratories using the same method, was evaluated for the two quality assurance samples SAM 1 and SAM 2. For each sample the CV of all results obtained by the participant using the same method were calculated.

Inter-method variation, defined as the variation arising from systematic differences between methods, was evaluated from the data for SAM 1 and SAM 2, by averaging the means of all results obtained by laboratories using the same method. The inter-method variation was expressed in terms of CV, calculated by the means of the various methods.

Overall inter-laboratory variation was calculated for the two samples SAM 1 and SAM 2, from the means reported from each laboratory, for all laboratories.

Evaluation with calibration

The data obtained from 12 laboratories using HPLC methods with a good HbA1c peak resolution (3 users of method C, 3 users of method D, 5 users of method F, 1 user of method I) were used to assign the HbA1c percentages to the calibrators. From the 32 values for each of the calibrators, we obtained the following data (means ± SD, min-max range): 4.36 ± 0.74%, 2.8−5.2% (CAL 1) and 11.98 ± 0.85%, 9.9−13.3% (CAL 2). Therefore, for each laboratory we constructed a calibration curve with the assigned HbA1c percentages on the x-axis and the reported values on the y-axis, and we corrected all the values relative to SAM 1 and SAM 2 by using calibration curves, as described elsewhere (10). Intra-laboratory, inter-laboratory, inter-method and overall variations were recalculated using the calibrated results. The HbA1c values recalculated after calibration are reported here as standardised HbA1c.

Statistical analysis

Kurtosis, asymmetry and Kolmogorov-Smirnov's tests were used to determine whether distributions were Gaussian. Means of duplicate assays were used for evaluating between-run reproducibility. Groups were compared by Student's t test for unpaired observations. Values of P < 0.05 were considered statistically significant. All calculations were performed with the aid of a commercial statistical program (Labstat.400, S.I.Bio.C and Bio-Rad Laboratories, Segrate, Milan, Italy).

Results

Typical HPLC recordings, obtained with the Bio-Rad Variant system, of blood samples and of our proposed lyophilized haemolysates are shown in figure 1. The retention times for HbA1c were 1.10 min in all the chromatograms. The total areas, closely correlated to the total haemoglobin concentration of each sample, were 6.2 × 10⁶, 2.5 × 10⁶, 2.9 × 10⁶ and 1.8 × 10⁶ arbitrary units, respectively. Similar data (not shown here) were obtained with the other two lyophilized haemolysates (SAM 1 and SAM 2), analysed together with the same blood samples shown in figure 1 by four different HPLC techniques (methods D, E, F and G). In conclusion, the lyophilized haemolysates were found to be similar to blood samples with regard their peak to profiles and peak areas in HPLC chromatography.

The cheapest way of delivering the lyophilized materials is via the postal service. From the questionnaire distributed to the participants we found that the materials were delivered within 12 ± 9 days (mean ± SD) from the dispatch date. Most of the participants analysed the samples within two weeks of receiving them, while eight of the 119 laboratories performed the assays one month after the date of arrival of the materials, or later.

Intra-laboratory variation

Table 1 reports the minimum, maximum and mean intra-laboratory CV values (range columns). The total number of laboratories (bottom of tab. 1) was 114 instead of 119, because five laboratories (three of them using method A, one method B and one method E) made single measurements. Therefore, intra-laboratory variation relative to these laboratories was not calculated.
Technical note

Fig. 1  HPLC recordings from analysis of
(a) a normal blood sample,
(b) a sample from a diabetic patient and
(c, d) the lyophilized haemolysates with (c) low and (d) high
HbA1c content.

Intra-laboratory variation is higher for affinity chromatography
than for other methods. However, even for the most popular
HPLC methods, we registered some cases of CV > 3.5%. In
particular, the results of one participant using the Bio-Rad Dia-
mat showed a CV of 5.3%, while one of the Menarini users
reported results with a CV of 7.3%. All the remaining users of
these methods worked with a CV of < 3.5%. Also one partici-
pant using the Boehringer immunoenzymatic method obtained
an intra-laboratory CV > 3.5% (i.e. 5.9%). Calibration did not
significantly change the intra-laboratory variation (tab. 1, col-
umns 4 vs. 6, and 5 vs. 7).

In summary, most of the participants obtained an intra-laboratory
variation of less than 3.5%. However, 14 out of 114 laboratories
(12.3%) showed a CV greater than 3.5%, and nine (7.9%) obtained
CV > 5.0%.

Tab. 1  Intra-laboratory variation.

<table>
<thead>
<tr>
<th>Method</th>
<th>Code</th>
<th>No. of laboratories</th>
<th>Without calibration</th>
<th>With calibration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Range mean</td>
<td>CV (%)</td>
</tr>
<tr>
<td>Affinity chromatography</td>
<td>A</td>
<td>13</td>
<td>4.2</td>
<td>1.7-8.6</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>4</td>
<td>4.9</td>
<td>2.0-7.9</td>
</tr>
<tr>
<td>HPLC</td>
<td>C</td>
<td>27</td>
<td>1.4</td>
<td>0.6-5.3</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>3</td>
<td>0.7</td>
<td>0.3-1.0</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>33</td>
<td>1.5</td>
<td>0.1-7.3</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>5</td>
<td>2.6</td>
<td>1.0-3.8</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>5</td>
<td>1.5</td>
<td>0.4-4.3</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>2</td>
<td>2.4</td>
<td>2.2-2.7</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>3</td>
<td>0.8</td>
<td>0.5-1.5</td>
</tr>
<tr>
<td>Immunoassay</td>
<td>L</td>
<td>3</td>
<td>1.7</td>
<td>1.1-2.1</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>12</td>
<td>2.4</td>
<td>1.3-5.9</td>
</tr>
<tr>
<td>Electrophoresis</td>
<td>N</td>
<td>4</td>
<td>1.6</td>
<td>0.4-3.3</td>
</tr>
<tr>
<td>All methods</td>
<td></td>
<td>114</td>
<td>2.0</td>
<td>0.1-8.6</td>
</tr>
</tbody>
</table>

Affinity chromatography: A (Abbott IMx), B (Abbott Vision);
HPLC: C (Bio-Rad Diamat), D (Bio-Rad Variant), E (Menarini), F
(Kontron), G (Merck-Hitachi), H (Instrumentation Laboratories),
I (other);
Immunoassay: L (Bayer), M (Boehringer);
Electrophoresis: N (Helena).
### Tab. 2  Inter-laboratory variation. Mean per method glycohaemoglobin concentration (HbA\textsubscript{1c}) and coefficients of variation (CV) expressed as %, are reported in the absence of (without) and after (with) calibration.

<table>
<thead>
<tr>
<th>Method(a)</th>
<th>Low HbA\textsubscript{1c} sample</th>
<th>High HbA\textsubscript{1c} sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without calibration</td>
<td>With calibration</td>
</tr>
<tr>
<td></td>
<td>HbA\textsubscript{1c} (%)</td>
<td>CV (%)</td>
</tr>
<tr>
<td>A</td>
<td>6.6</td>
<td>11.4</td>
</tr>
<tr>
<td>B</td>
<td>5.3</td>
<td>24.3</td>
</tr>
<tr>
<td>C</td>
<td>5.7</td>
<td>8.8</td>
</tr>
<tr>
<td>D</td>
<td>5.9</td>
<td>2.6</td>
</tr>
<tr>
<td>E</td>
<td>4.5</td>
<td>10.5</td>
</tr>
<tr>
<td>F</td>
<td>4.4</td>
<td>10.1</td>
</tr>
<tr>
<td>G</td>
<td>4.4</td>
<td>16.2</td>
</tr>
<tr>
<td>H</td>
<td>6.0</td>
<td>10.8</td>
</tr>
<tr>
<td>I</td>
<td>4.2</td>
<td>20.9</td>
</tr>
<tr>
<td>L</td>
<td>5.9</td>
<td>2.6</td>
</tr>
<tr>
<td>M</td>
<td>5.8</td>
<td>14.4</td>
</tr>
<tr>
<td>All methods</td>
<td>5.4</td>
<td>18.7</td>
</tr>
</tbody>
</table>

\(a\) See legends to tab. 1.

**Inter-laboratory variation, per method**

This was evaluated on the basis of data obtained on samples SAM 1 and SAM 2 before and after correction with the calibration curve. The variations, expressed in terms of CV, are reported in table 2 (columns 3, 5, 7 and 9). Caution should be used in interpreting the inter-laboratory CV of method H, since this method was used by only two participants.

Without calibration most methods showed inter-laboratory CV > 10%. With the low HbA\textsubscript{1c} content sample, only the Bio-Rad Variant and the Bayer DCA 2000 analysers had CVs < 3.5%. With calibration, almost all methods showed substantial reductions in their CV. Only in the case of the Bayer test did we note an increase in the CV after calibration, mostly with respect to the low HbA\textsubscript{1c} sample, from 2.6% to 6.2%. In the remaining cases, reductions in the CV were marked, with a less prominent decrease for the Boehringer and Helena methods.

**Inter-method variations**

Figure 2, produced from the data of the means reported in table 2, shows a comparison between the 12 methods, before and after calibration. Without calibration the percentages were dispersed along the diagonal of the graph, as in a typical Youden plot in the presence of a systematic error. With calibration (right), the inter-method variation is markedly reduced, inter-method CV decreasing from 16.3% to 5.6%, and from 15.3% to 4.2%, respectively.

**Overall variation**

The overall effect of calibration for 119 laboratories that analysed the low and high HbA\textsubscript{1c} samples is illustrated in figure 3. Without calibration (bottom) the values obtained were distributed broadly, with partial overlap between the data relative to the two samples. Global CV were around 18% (tab. 2, bottom line). After calibration (fig. 3, top) the distribution curves were narrower and the two samples were clearly resolved from each other. The overall CV decreased to 7.4% and 5.4% for the low and high HbA\textsubscript{1c} samples, respectively (tab. 2, bottom line).

**Discussion**

External quality assessment schemes for the performance of glycohaemoglobin analysis in Italy show a large degree of variabili-

![Fig. 2 Mean HbA\textsubscript{1c} percentages measured by 12 methods in 119 laboratories in samples with low (x-axis) and high (y-axis) HbA\textsubscript{1c} content without (left) and with correction with calibration (right).]
Another important issue concerns the possible matrix effect of the lyophilized materials. In our study the effect of standardisation was calculated from results of lyophilized samples calibrated with lyophilized calibrators, according to the model previously proposed by Weykamp (10). In a more recent paper, Weykamp demonstrated that calibration with lyophilized calibrators is also effective for whole blood glycohaemoglobin results (11), thus excluding any possible matrix effect of the lyophilized materials (due, for instance, to the elimination of the labile pre-A1c fraction during the preparation of the lyophilized materials). However, the denaturation of whole-blood samples during storage and transport was evident in some cases mentioned in Weykamp’s second study (11) and seemed to limit to use of whole-blood samples in survey programmes and in multicentre studies. For this reason we decided not to use whole-blood samples in our study.

Our results for standardisation of data from the Abbott methods differ from those reported by Weykamp (11). Weykamp excluded Abbott IMx and Vision results because of artifacts ascribed to matrix effects. In our experience we are not sure whether our lyophilized samples are not compatible with the HbA1c assays of Abbott IMx and Vision. In the case of the IMx users, a mean value of 6.6% in the low HbA1c sample (1.2% higher than the mean of 5.4% of all methods; see tab. 2) was reported. After calibration, 4.7% (0.3% lower than the mean of all methods), was reported with a total change of 1.5% with respect to the mean. Moreover, in the high HbA1c sample a mean value of 11.3% (2.1% higher than the mean of 9.2% of all methods) was found and, after calibration 9.2% (0.3% higher than the mean of all methods) was calculated, with a total change of 1.8% with respect to the mean. In the case of Vision users the changes with respect to the means were very small. In conclusion, after calibration the results of Abbott IMx and Vision clustered, as for other methods. Therefore we think that our procedure of lyophilization did not introduce any matrix effect, because lyophilization did not raise the measured HbA1c over the original whole-blood samples. The comparability study presently underway will probably clarify this aspect.

From a clinical point of view it could be argued that even with calibration there is considerable spread between values (i.e. 4.4–5.3% for the low sample and 8.2–9.5% for the high sample). This kind of dispersion is of the same order as that found by Weykamp (4.6–5.5% and 8.6–10.3%, excluding the Abbott methods; i.e. (11)) and still not satisfactory for medical needs. As a matter of fact, a 1% difference in HbA1c represented a sizable difference in risk for diabetic complications, according to the Diabetes Control and Complication Trial (DCCT) study (3). Therefore, in order to further reduce bias, the following may be pursued:

(a) value assignment to calibrators could be performed separately per group of methods;
(b) the number of methods could be restricted.

The first choice is not practical, because value assignment to calibrators should be ideally performed by an absolute glycohaemoglobin method calibrated with primary standards. In order to limit the number of methods actually used to measure glycohaemoglobin, some restriction criteria have to be defined. The method’s reproducibility could be one of these criteria, and, for instance, it could be decided to abandon those methods not meeting the recommended intra-laboratory CV of < 5%, or the more desirable clinical limit of < 3.3%. In addition, since the standardisation involves a two-point or a three-point calibration, it could be also decided to abandon these methods not suitable for calibration with more than one point. It is relevant to note that presently only a minority of the commercial HPLC systems are able to support a two point calibration. In conclusion, it is difficult to suggest definitive criteria to further restrict bias among standardised HbA1c results. We think that the only way to achieve international standardisation soon will be to adopt the recent proposal of the International Federation of Clinical Chemistry working group on standardisation of HbA1c (19), which involves calibration by the manufacturer.

In conclusion, we confirmed previous findings (10, 11) showing the HbA1c determinations can be calibrated using lyophilized haemolysates. In addition, our study provides information on:

- Whether intra-laboratory or within or between methods (13, 14).
- More recent results from 10 laboratories using only HPLC methods (Bio-Rad Diamat, Menarini 8110 and 8121) (15) demonstrated that intra-laboratory variations were small (CV ≤ 2%) in 83% of cases. However, analysis of two samples with low and high HbA1c percentages gave mean inter-laboratory CV values of 36.2% and 29.5%. These findings are in agreement with those obtained from previous trials in Italy (15).
- Our findings for intra-laboratory variation are in agreement with those obtained from previous trials in Italy (15). Unfortunately, those obtained from previous trials in Italy (15) are still not satisfactory for medical needs. As a matter of fact, a 1% difference in HbA1c represented a sizable difference in risk for diabetic complications, according to the Diabetes Control and Complication Trial (DCCT) study (3). Therefore, in order to further reduce bias, the following may be pursued:

(a) value assignment to calibrators could be performed separately per group of methods;
(b) the number of methods could be restricted.

The first choice is not practical, because value assignment to calibrators should be ideally performed by an absolute glycohaemoglobin method calibrated with primary standards. In order to limit the number of methods actually used to measure glycohaemoglobin, some restriction criteria have to be defined. The method’s reproducibility could be one of these criteria, and, for instance, it could be decided to abandon those methods not meeting the recommended intra-laboratory CV of < 5%, or the more desirable clinical limit of < 3.3%. In addition, since the standardisation involves a two-point or a three-point calibration, it could be also decided to abandon these methods not suitable for calibration with more than one point. It is relevant to note that presently only a minority of the commercial HPLC systems are able to support a two point calibration. In conclusion, it is difficult to suggest definitive criteria to further restrict bias among standardised HbA1c results. We think that the only way to achieve international standardisation soon will be to adopt the recent proposal of the International Federation of Clinical Chemistry working group on standardisation of HbA1c (19), which involves calibration by the manufacturer.
(a) a new detailed protocol for the preparation of such haemolysates;

(b) the standardisation of other HPLC (from Kontron, Merck and instrumentation Laboratories) and electrophoresis methods (from Helena) that are quite scattered in Mediterranean countries and not yet submitted to standardization with lyophilized calibrators.

Moreover, we think that possible differences among different preparations of lyophilized haemolysates proposed as reference materials (i.e. those proposed by Weykamp and those used in this study) could explain discrepancies concerning the matrix effect on the Abbott methods. Another larger inter-laboratory trial with six batches of samples and calibrators is currently under development in Italy and it will be useful for further ascertaining the matrix effect, if present. In such a study the value assignment to the calibrators will be performed either by only one HPLC method or, if available, by the recently proposed IFCC reference method (20). In this way we will restrict the range of value assignment and we will be able to definitely test the suitability of Abbott methods. In the meantime, all of our samples are also available to other groups upon request, in the interests international cooperation in glycohaemoglobin standardisation.

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