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Survey and assessment of the actual state of routine measurement of glycohaemoglobin/GHb by commercial methods: warning to the users and the providers[1]

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Abstract

As the clinical availability of glycohaemoglobin/GHb measurement increases, so does the need for comparable and accurate values among different laboratories and different methods. At least there should be comparability, i.e., commutability or feasibility of providing comparable results from different assays in different laboratories. A clinical joint study on insulin therapy, a survey of the actual inter-laboratory differences in GHb measurement among 41 institutions and an assessment of 11 assay methods for the determination of GHb were performed using commercial calibrators and fresh blood samples. Data on the actual state of inter-laboratory and inter-assay differences of observed values were useful for comparing results among facilities. The recommendation of the Japan Diabetes Society to measure only the stable GHb component and to correct the GHb percentage by two-point calibration with assigned values, was effective but not sufficient. Even after correction, 8 out of 11 methods still remained of little practical use because of their large relative errors. Inter-method differences among 11 available assay methods were great even after correction and depended on not only the methods but the samples used for the determination. The performance of some methods or instruments used are only poor at distinguishing the stable glycated haemoglobin itself. Some alternative measurement system with comparability, commutability and precision should be established. An urgent and worldwide problem to remove inter-laboratory differences in the measurement of GHb needs to be solved. Users in clinical practice must recognize these problems, and, before supply, the providers should check their method and keep records that are readily traceable. © 1997 Elsevier Science B.V.

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1. Introduction

In spite of the importance of GHb/glycohaemoglobin levels for the long-term assessment of blood glucose control in diabetics, there have been difficulties in comparing results among labo-

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ratories because of great inter-laboratory differences in the measured values. Recently, some new assay systems of immuno- and affinity-methods for GHb have begun to be provided for daily measurement and there is apprehension that these might magnify the inter-laboratory differences. To avoid such apprehension it is important to know the actual state of routine measurement of GHb and the quality of the instruments which have been used or will be used for the determination.

In the diverse measurements of glycated haemoglobin, many studies for comparability and commutability have been conducted; these include reports on the unacceptable fluctuation of the results [1–8], reports on the fractional separation of glycated haemoglobin [9–16] and reports on the inter-laboratory differences and the feasibility for providing comparable results by calibration [17–25]. For the commutable determination of glycated haemoglobin as an index of glyceamic control in diabetic patients, the authors of many reports [4,5,11,12] have attempted to measure irreversibly-glycated haemoglobin itself excluding interfering components.

Haemoglobin A1c/HbA1c fraction which had been chromatographically separated by a Bio-Rex 70 column [26,27] and measured for glyceamic control was successfully confirmed in 1981 [11] to consist of at least six components including stable glycated haemoglobin (St-A1c), two labile glycated haemoglobins (L-A1c), foetal haemoglobin (HbF) and others. More recently, it has became possible to separate St-A1c in the HbA1c fraction nearly pure [13] and to measured routinely St-A1c in the presence of interfering components by precise HPLC methods with an SP-NPR column within 4 min [14,15] and within 1.8 min [16]. However, the results from these HPLC methods are 1–2% lower than those measured values of the same samples by the conventional methods which are generally used in clinical laboratories; the HPLC methods have not prevailed for daily use unfortunately, despite claims emphasizing the advantages of measuring GHb as St-A1c for accurate determination. In the surveys for the actual state of inter-laboratory differences in the measurement of GHb, many authors confirmed the difficulty of directly comparing observed values among different laboratories [17–19], they also showed the feasibility of calibration and the availability of assigned calibrators for improving compatibility and precision of the results [20–22]. The Committee on Inter-laboratory Standardization of HbA1c Determination of the Japan Diabetes Society (JDS) recommended a standardized procedure, i.e., measuring only the stable HbA1c component and correcting the HbA1c percentages thus obtained with the assigned value of the calibrators (JDS-correction) [24].

Glycohaemoglobin/GHb has been mostly measured by a HPLC method in Japan, whereas the actual inter-laboratory differences of measuring GHb are too great especially in the measurement of fresh blood compared with lyophilized samples [18]. For the universal assessment of the glyceamic state of patients with diabetes, it is difficult directly to compare the observed values among laboratories. In the course of planning the first meeting on 7 September 1994 of the Study Group on Insulin Therapy of Children for the intensive insulin therapy of IDDM trial, it was necessary first to confirm whether information on the actual state of measuring GHb in the laboratories of the group was available and whether some measuring systems were needed for the trial among different laboratories. In the data gathered from the questionnaire to about 100 qualified diabetologists in Japan, a change in the GHb value of not more than 3.3–3.9% has been evaluated as an acceptable technical limit [24]. For the clinical use of GHb the permitted range of inter-laboratory differences must be not more than the evaluated range.

For the confirmation and the anticipated needs in the future, it was decided first to make a survey in August 1994 for the actual state of inter-laboratory difference in GHb measurement of those laboratories that would offer to take part in this trial, and second to conduct a joint study for the assessment of the assay methods of GHb which might become available and of which providers would accept cooperation with the authors.

2. Experimental

2.1. Study design

The study was planned to be performed in 41 laboratories including 26 university hospitals and 15 general hospitals throughout the country. The reference laboratory for this study was that of Pathobiological Medicine, Pharmaceutical Institute, Keio University in Tokyo. This laboratory has performed. GHb analysis by an ion-exchange HPLC method of KO40 as a tentative reference method.

For the assessment of inter-laboratory differences, the authors delivered to the laboratories a set of the JDS master calibrator (two assigned lyophilized erythrocyte haemolysates) and five kinds of commercial calibrators (lyophilized erythrocyte haemolysates); each laboratory was asked to prepare about ten fresh blood samples from individual diabetics in their own institution for GHb measurement and to send the authors' laboratory 1 ml of each blood sample under ice cold for the measurement by KO40. All the laboratories were requested to measure GHb levels by their own method once for each blood sample and six times for each calibrator and to send the results to this laboratory.

For the assessment of assay methods of GHb, eleven methods including six HPLCs, one affinity- and four immuno-methods were evaluated. To the respective providers were sent fresh or deep frozen blood samples (20–425 samples depending on the provider's assistance), a set of the JDS master calibrator and two to five kinds of calibrators; each provider was asked to measure GHb levels in all these samples by their own method once for blood samples and six times for each calibrator and to send the results to this laboratory. All the samples measured by the respective assay method were determined by KO40 for the cross-checking.

The institutions which offered to take part in this trial were the following 26 university hospitals and 15 general hospitals. University hospitals were: Asahikawa Medical College, Hokkaido University, Tohoku University, Gunma University, Chiba University, Saitama Medical School, National Defense Medical College, University of Tokyo, Nihon University, Tokyo Women's Medical College Diabetes Center, Tokyo Women's Medical College, Keio University, Nihon Medical School, Kitasato University, Yokohama City University, Yamanashi Medical University, Shinshu University, Niigata University, Hamamatsu University School of Medicine, Kanazawa University, Kyoto Prefectural University of Medicine, Osaka Medical College, Osaka City University, Okayama University, Ehime University, The University of Tokushima and Kumamoto University. General hospitals were: Sapporo City Hospital, Tonann Hospital, Ibaragi Children's Hospital, Kanto Central Hospital, Musashino Nisseki Hospital, Kanagawa Children's Hospital, National Sanatorium of Mie, Kobeseiko Hospital, Kobe West City Hospital, Fukuoka Children's Hospital and Kurobe City Hospital.

Eleven methods were assessed in this experiment including KO32 ion-exchange HPLC which was developed and had been used as the authors, field method [14]; L-9100™ ion-exchange HPLC (HI, Hitachi, Tokyo); HS-10™ ion-exchange HPLC (JO, Jookoo, Tokyo); Valiant™ ion-exchange HPLC (BI, Bio-Rad Labs, Tokyo); HLC723-GHB III™ ion-exchange HPLC (TO, Tosoh, Tokyo); HA-8131™ ion-exchange HPLC (KY. Kyoto Dai-ichi Kagaku); DCA2000™ immunoassay (DC, Miles-Sankyo, Tokyo); Riki-Tech™ immunoassay (BM, Boeringer Mannheim GmBH, Tokyo); Rapidia-Auto™ immunoassay (FU, Fujirebio, Tokyo); COBAS™ immunoassay (RO, Japan Roche, Tokyo); and IMX™ affinity binding assay (DI, Dainabot, Tokyo). For the three methods of HI, DC and DI, the samples were measured in the authors, laboratory according to the manufacturer's instructions.

2.2. Materials for calibration and assessment

For the quality assessment of measuring GHb, fresh blood samples and seven calibrators were used. For the blood samples used for this experiment, laboratories were asked to withdrew samples from individual diabetics in their own institutions and to treat as mentioned above. Lyophilized erythrocyte haemolysates were supplied from the manufacturers; these were provided

as commercial calibrators for the routine measurement of GHb by the HPLC method. Four out of seven calibrators were two different lots from Kokusai-shiyaku, Kobe (SC1L, SC1H) and (SC3LSC3H); the remaining three calibrators were from Bio-Rad Labs, Tokyo (SC2L, SC2M, SC2H).

A set of two assigned calibrators of JDS made of lyophilized erythrocyte haemolysates (MCL = 5.5%, MCH = 10.5%) were used for the correction of observed values and for the assignment of secondary calibrators. These were kindly given by the Committee of JDS. For some particular GHb assay methods, the calibrator was supplied by the manufacturer.

2.3. Tentative reference method

A HPLC method of KO40 that had been developed for the determination of St-A1c [15] was used as a tentative reference method for this trial. The HPLC system comprised a chromatograph of Nano-Space (Shiseido), an SP-NPR column (TSK gel Glyco His ϕ 4.6 × 35 mm, Tosoh) kept at 25°C and an auto-sampler with a sample-holder kept at 5°C.

Of the sample 8 μl was injected on to the column every 5 min using the auto-sampler and the effluent was monitored at 415 nm; the relative concentration of St-A1c was calculated with an integrating recorder.

Two kinds of mobile phases were used for the elution, (A) 20 mM MES-HEPES containing 0.01% NaN_3 (pH 5.20), and (B) 20 mM MES-HEPES containing 0.01% NaN_3 and 0.55 M NaCl (pH 5.20). Linear gradient elution was performed at a flow-rate of 1.0 ml min^{-1}. The concentration of B% was changed as follows: after the equilibration of the column with 21% for one min, 21–22% from the time of sample injection to 0.6 min, 22% for 1.6 min, 22–80% from 1.6–3.6 min, 80% for 4.0 min and reduced to 21% until 4.1 min.

Blood samples submitted for the routine determination of GHb were kept below 4°C; the samples were either analyzed within a week after being withdrawn from the patient or were stored in a stoppered tube below −75°C until use. Just before the analysis, blood samples were lysed with fifty times volume of haemolytic reagent, 5 mM HEPES containing 0.01% NaN_3 (pH 7.0); the mixture was centrifuged at 12 000 rpm for 5 min and the supernatant was used for analysis. Lyophilized erythrocyte haemolysates were dissolved just before the analysis according to the manufacturer's directions and injected.

This HPLC method enables GHb to be measured nearly pure excluding interfering components: labile glycated haemoglobins, HbF, carbamylated haemoglobin and acetylated haemoglobin. Fig. 1 is a typical chromatographic trace of a normal fresh blood sample.

2.4. Data analysis

Observed values from each laboratory and method were corrected by two-point calibration with assigned values of calibrators by JDS and the values were statistically calibrated and compared with that from KO40.

The results were assessed in respect of compatibility, comparability and precision. Five indices based upon R.S.D. or R.E. (relative error) were used for the assessment. For the compatibility and equivalency of obtained GHb values, the absolute R.E. was expressed by the normalized gap between respective results and the tentative reference

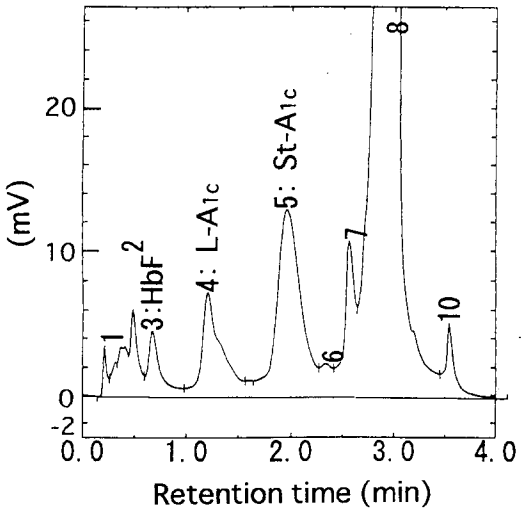

Fig. 1. Chromatogram by KO40 method.

value of KO40. For comparability and inter-assay differences of GHb measurement, the R.S.D. of variation of obtained values by every assays (inter-R.S.D.) was calculated together with the mutual difference of respective R.E. against average R.E. (mutual R.E.). For precision and intra-assay differences of GHb measurement, the R.S.D. of repeated measurements of single samples (intra-R.S.D.) was determined and the fluctuation of R.E. was expressed by its standard deviation (S.D.-R.E.).

Statistical analyses were performed with the use of Microsoft Excel for Windows V. 7.0. The results were separately analyzed whether laboratory or method, whether lyophilized sample or blood sample and whether eliminated labile components (group-E), non-eliminated components (group-N) or overall components (group-O). The permitted range $\leq 3\%$ and the sub-permitted range of $3\% < \sim \leq 6\%$ were used as the criteria of assessment for the indices.

3. Results

3.1. Intra- and inter-laboratory difference

Results were obtained from 32 out of 41 laboratories; results of master calibrators and five calibrators from 28, results of blood samples from 32, results of both calibrator and blood from 23, and results from 23 laboratories were correctable. The 23 laboratories included 12 of group-E which determined GHb eliminating labile components, 10 of group-N without elimination and the remaining one with no comment. The 32 laboratories measured GHb by immuno-method in 1 and by the HPLC method in 31 laboratories with the instrument of KY in 16, of TO in 13, of JO in 1, and with no comment in 1 laboratory.

In the measurement of seven calibrators six times, intra-R.S.D.s of the observed value in 28 laboratories except one were within the permitted range. However, all inter-R.S.D.s in the observed values of every seven calibrators among 28 laboratories were outside the permitted range and were distributed from 4.5–6.9%; the differences between the maximum and minimum of seven calibrators were 1.8–3.7% (Fig. 2(a)). After correction, the inter-R.S.D.s were reduced nearly into the permitted range of 0.9–3.0% and the differences were 0.9–1.5% (Fig. 2(b)). For the determination of calibrators, corrections were effective and the corrected results became comparable among the different laboratories. It can be said that in the determination of lyophilized sample the laboratories in the group were maintaining fairly good precision and comparability for the measurement of GHb with calibrators.

Overall R.E.s observed values of GHb in blood samples were 1.8–65.2% as plotted in Fig. 3(a) and the average R.E. were 12.2–41.7%. After the two-point calibration with master calibrators, the overall R.E.s were reduced to 0.02–42.0% and the average R.E.s for 23 laboratories to 1.9–20.9% (1.9–5.5% for group-E, 3.5–20.9% for group-N). From the average R.E.s of observed values for each of 32 laboratoies, none of the laboratories was within the sub-permitted range before correction. After correction, average R.E.s for each of 23 laboratories were reduced whereas 9/23 (39%) were still outside the sub-permitted range and only 6/23 (26%) were within the permitted range (Fig. 3(b)). Even after the correction, about three quarters of laboratories were found to be not comparable in the GHb measurement of blood samples. For the 12 laboratories of group-E, 6/12 (50%) were within the permitted range and 6/12 (50%) were within the sub-permitted range whereas in the 10 of group-N none was within the permitted range, 2/10 (20%) were within the sub-permitted range and 8/10 (80%) were outside this range. These data show that measuring only GHb and eliminating labile components increases compatibility of the results among different laboratories. JDS-correction is more useful in increasing compatibility of the results than the method of correction without eliminating liable components in the measurement of blood samples.

Averages of mutual-R.E.s by 32 laboratories calculated for the evaluation of the comparability of results were 0.3–17.2% before correction and were reduced to 1.2–13.0% after correction. The reduction was not so remarkable, although the average was reduced to 0.38–2.11% in group-E and 1.13–15.23% in group-N. After correction,

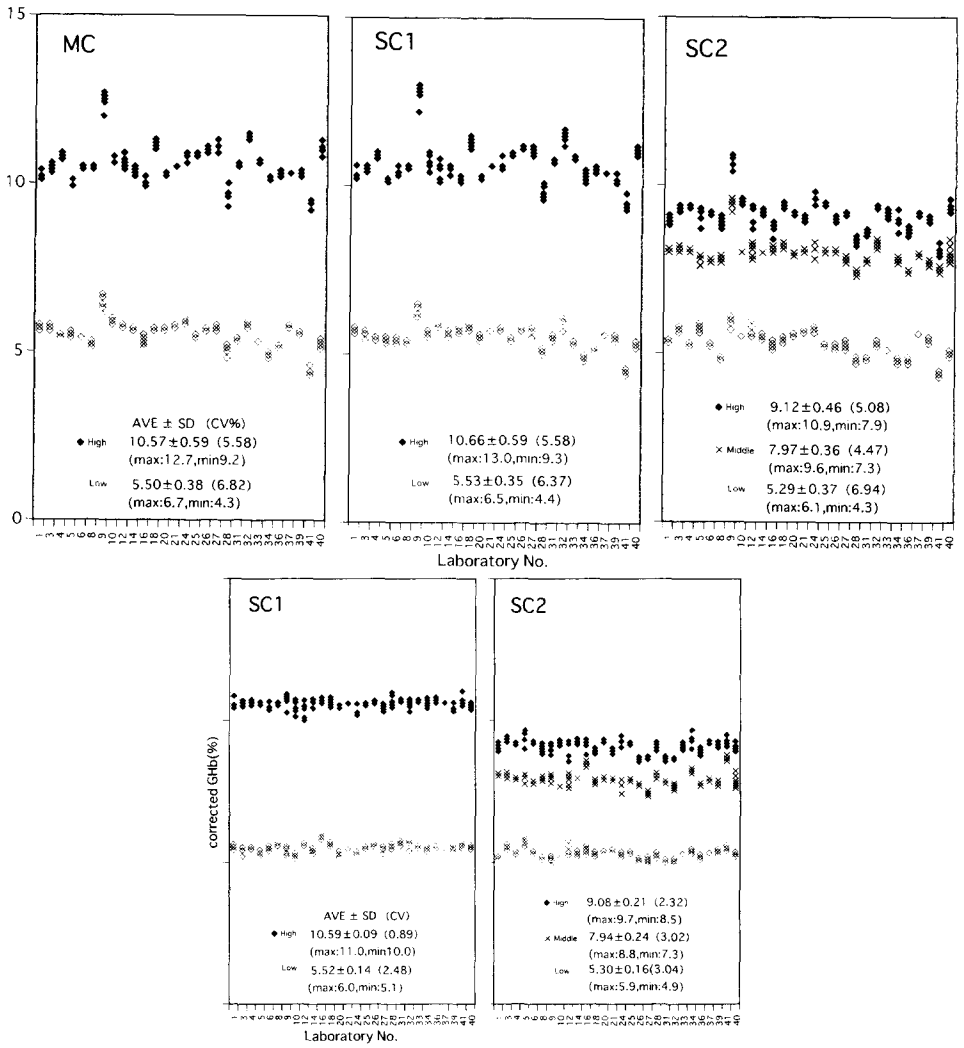

Fig. 2. (a) Differences of observed GHb values between laboratories/calibrators. (b) Distribution of corrected values by master calibrators/subcalibrators.

12/12 laboratories of group-E were within the permitted range but only 3/10 laboratories of group-N were within that range.

Averages of S.D.-R.E.s for 32 laboratories, calculated to estimate intra-assay precision of GHb measurement in blood samples, were 2.7–15.6% and were reduced to 1.0–13.3% after correction; reduction was to 1.0–4.2% in group-E and 3.0–13.3% in group-N, respectively. Then 11/12 of group-E and 1/12 laboratories of group-N were within the permitted range.

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From these findings it may be considered that JDS-correction is very effective in decreasing the bias against the postulated reference value and the

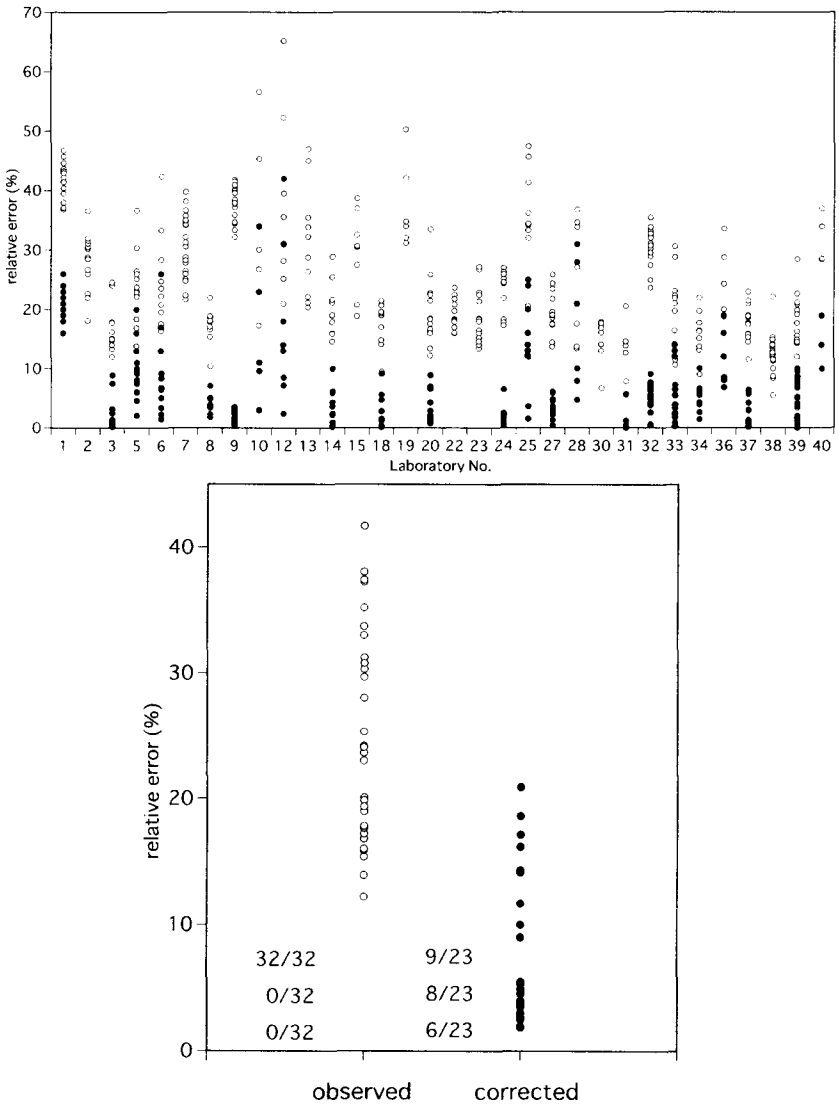

Fig. 3. (a) Relative errors of observed and corrected GHb values/fresh blood samples. (b) Reduction of mean relative errors to permitted range by correction/fresh blood samples.

dispersion of the results and in increasing the comparability of the results among 12 laboratories, while it is not enough.

3.2. Inter-method differences

All results from eleven assay methods were collected and calculated in the tentative reference laboratory. Results were collected partially for some methods. The collected results of six-measurements by each of nine calibrators from eleven provided methods are plotted in Fig. 4(a). As shown in this figure, values of single samples were different and variously dispersed among methods. In the GHb measurement of nine calibrators by eleven assay methods the overall intra-R.S.D.s were less than 6% and in 9 out of 11 assays the R.S.D.s were within the permitted range (Table

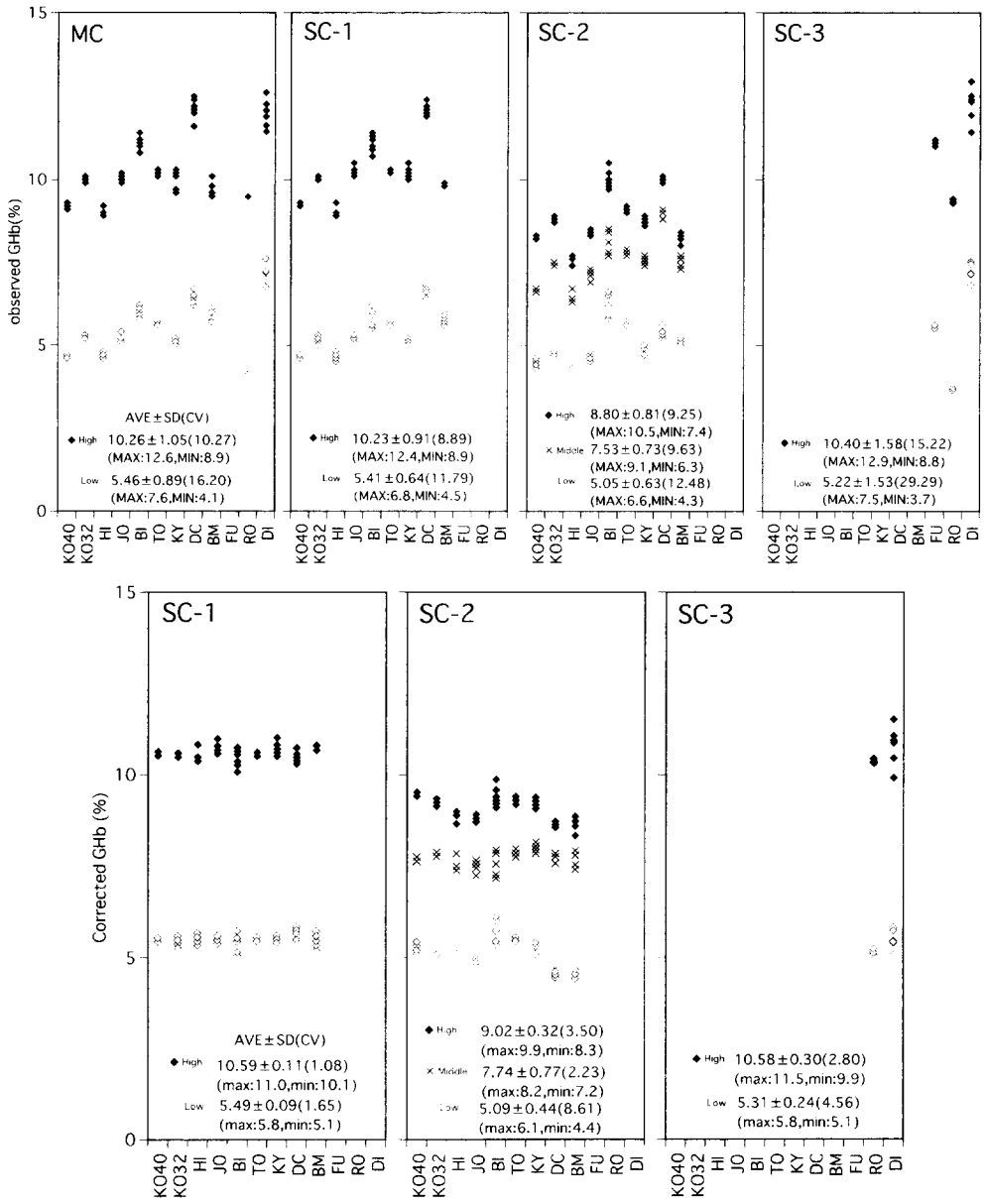

Fig. 4. (a) Differences of observed GHb values beteen different assay systems/calibrators. (b) Distribution of corrected values by master calibrator/subcalibrators.

1). Observed values of single calibrators by different eleven methods varied greatly depending on the methods and samples. The inter-R.S.D.s by 11 methods were 8.9–29.3% and the differences between the maxima and minima were 2.3–4.1% (Fig. 4(a)). After correction, the inter-R.S.D.s were reduced to 1.1–8.6% and the differences between maxima and minima were 0.7–1.7%. These were still great and not acceptable for comparison of the results from the different methods (Fig. 4(b)).

Table 1
R.S.D.s of GHb measurement by different assay systems/calibrators

		MC		SC1		SC2			SC3	
		L	H	L	H	L	M	H	L	H
Tentative reference method	KO40	*1.11*	*0.82*	*1.11*	*0.44*	*2.33*	*0.61*	*0.66*	*0.48*	*0.11*
HPLC method	KO32	*0.78*	*0.75*	*2.04*	*0.51*	*1.08*	*0.69*	*0.86*	N.D.	N.D.
	HI	*1.80*	*1.09*	*2.23*	*2.05*	*0.00*	*2.79*	*1.44*	N.D.	N.D.
	JO	*1.88*	*1.03*	*1.22*	*1.34*	*2.61*	*1.87*	*1.17*	N.D.	N.D.
	BI	*2.74*	*2.10*	**4.42**	*2.38*	**5.68**	**3.90**	*2.92*	N.D.	N.D.
	TO	*0.97*	*0.62*	*0.97*	*0.40*	*0.91*	*0.81*	*0.89*	N.D.	N.D.
	KY	*1.24*	*2.83*	*1.75*	*1.91*	*2.38*	*1.60*	*1.38*	N.D.	N.D.
Immuno-method	DC	*2.68*	*2.64*	*2.06*	*1.61*	*2.55*	*1.37*	*0.75*	N.D.	N.D.
	BM	*3.00*	*2.51*	*2.55*	*0.53*	*1.48*	*2.00*	*1.62*	N.D.	N.D.
	FU	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	*0.93*	*0.75*
	RO	N.C.	N.C.	N.D.	N.D.	N.D.	N.D.	N.D.	*2.20*	*0.58*
Affinity-method	DI	**4.62**	**3.57**	N.D.	N.D.	N.D.	N.D.	N.D.	**3.54**	**4.26**

Numbers in italic represent: permitted range $\leq 3\%$.
Numbers in bold represent: sub-permitted range $3\% < \sim \leq 6\%$.
N.D.: not done.
N.C.: not calibrated.

The averages of R.E.s by each of single calibrators from every 11 assays were 2.9–52.3% and 10/11 (91%) assays were outside the permitted range before correction. The averages of R.E.s in the corrected values were reduced to 1.9–5.9%; 4/10 (40%) assays including KO32, HI, TO and KY were within the permitted range (Fig. 5, left). From examination of average of the R.E., the inter-assay differences of GHb measurement were too great with or without correction even in the measurement of calibrators. Inter-assay variations were too large in both observed and corrected results to get comparable results among the assay methods.

In the determination of 20–425 samples of fresh blood by the 11 provided assay methods, averages of R.E.s in observed GHb value were 2.1–33.4% and ten out of eleven assays were out of these-permitted range. With correction by master calibrators, the averages of R.E.s by 11 assays were 1.6–15.9%; for six assay methods (KO32, HI, JO, TO, KY and RO), the R.E.s were reduced to within the permitted range, whereas five assay methods (BI, DC, MB, Fu and DI) were still outside the sub-permitted range. Even with the correction, 5/11 (45.5%) of assay methods still remain out of the sub-permitted range (Fig. 5, right).

These results suggest that about the half of the assay methods available are not comparable. This can be explained by the fact that the regression lines of the assay methods provided on KO40 were different in respect of their regression coefficients and intercepts between fresh blood samples and calibrators.

4. Discussion

In 1993, the Diabetes Control and Complications Trial (DCCT) reported the effectiveness of strict glyceamic control in patients with diabetes mellitus for the prevention of complications [28]. Similar results were reported by Reichard [29] and Yoshioka [30]. Since then several yardstick values of GHb have been proposed for the diagnosis of the diabetic state. It has become a consensus in Japan that a 7% of GHb level is an acceptable yardstick to prevent diabetic complications. The Ministry of Public Health recommended diagnos-

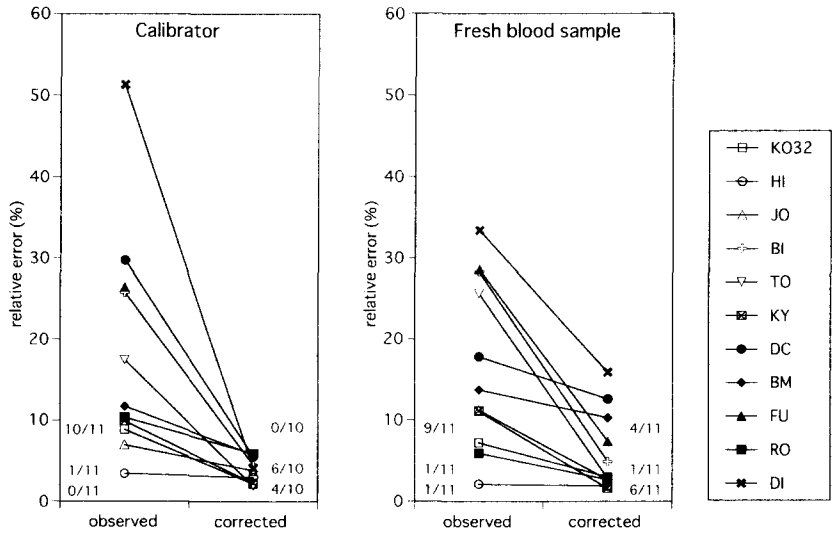

Fig. 5. Reduction of mean relative errors to permitted range by correction.

tic criteria of GHb values in the medical examination for diabetes: the therapeutic criterion was $\geq 6\%$ and the warning criterion was $\geq 5.6\%$ [31].

Not only to make efficient and possible use of these yardsticks but also to standardize glyceamic control in hospitals, it is necessary to reduce markedly the inter-laboratory differences of GHb measurement. Intra- and inter-laboratory differences should be kept less than 3% in indices which include R.S.D. or R.E. values.

The working groups on HbA1c/glycohaemoglobin standardization have been actively working for the standardization of GHb measurement: the International Federation of Clinical Chemistry (IFCC WG); the subcommittee on glycohaemoglobin standardization of The American Association of Clinical Chemistry (AACC SC); the HbA1c standardization committee of The Japan Diabetes Society (JDS SC); and the working group of the committee on diabetes diagnosis markers of the Japan Society of Clinical Chemistry (JSCC WG).

The IFCC WG had a joint meeting with the AACC SC in Columbia and proposed a candidate reference method of GHb measurement. They reported at the International Congress of Clinical Chemistry in London that they decided to develop a scientifically based reference system for the international standardization of HbA1c/Glycohaemoglobin determinations. The committee of JSCC WG is working for the establishment of the reference method and the reference material. The JDS WSC had made two surveys and recommended to measure only the stable HbA1c component and to correct the observed value by two-point calibration with assigned values [24]. However, the results of the third external quality survey of JDS to evaluate the inter-laboratory variation of GHb values measured by various methods in 1424 institutes did not meet expectations. The reason why the results were poor must be the lack of thorough use of the standardized procedure, i.e., the JDS recommendation. In the authors experience as shown in the results, correction according to the JDS recommendation is capable of providing comparable results in the GHb determination by HPLC methods. In the strictly conducted GHb measurement in The University of Tokushima and the backup laboratory of DCCT, there was excellent comparability between results in the two laboratories [25].

In respect of accuracy of the DCCTs results, the values are 1.5–2.5% higher than the true value which is expected from the present experiments. In a medical and biological sense, the true (or ultimately close to the true) value St-HbA1c

should be measured for the standardization of the GHb measurement; it is necessary to establish a system to measure a comparable and commutable value of GHb for the first step.

In this survey of GHb measurement in 41 laboratories there was a great inter-laboratory difference among them. In these laboratories, most measurements were performed by each HPLC method with or without elimination of labile components. In the case of analyzing the data separately for the methods with or without elimination, the averages of R.E.s of fresh blood samples by the method with elimination were 1.9–5.5% and without elimination were 3.5–20.90%; the averages of mutual-R.E. were 0.38–2.11% with elimination and 1.13–15.23% without elimination, respectively. This showed very high comparability. The recommendation of JDS SC was found effective; however it cannot be used as a compatible correcting method for three assay methods (DC, BM, DI) which measured GHb with elimination of labile components. Values from the three assay methods corrected by the JDS calibrators according to the directions of JDS recommendation were outside the correctable range and, from consideration of the averages of R.E.s and mutual-R.E.s, were not comparable.

GHb values of single samples measured by nine methods were very different even after correction and strongly depended not only on the principle of measurement but on the instruments used for the measurement, the sample lot and the sample itself (Fig. 4(a,b)). In the measurement of blood samples by the eleven assay methods, averages of R.E.s were not reduced so effectively as in the case of calibrators (Fig. 5). The regression lines of provided methods on the tentative reference method differed in their regression coefficients and intercepts between fresh blood samples and calibrators.

These differences, which must depend on the diversity of composition of blood samples and calibrators and on the poor performance of assay methods, cause much difficulty in performing comparable and compatible measurement of GHb by the diverse assay method. There are no compatible calibrators for every assay method and not reference material for the determination of GHb. It is necessary to develop a calibrator that behaves in the same way as blood samples in measurement of GHb.

These observations on imprecision and inaccuracy have been occasionally explained by reference to the matrix effects whereas the truth must be that the characteristics of measurement theory limits the method and the instruments used. It must be said that the performance of the methods or the instruments used is only poor at distinguishing the stable glycated haemoglobin itself. The authors are apprehensive that the introduction of diverse assay methods of GHb measurement might magnify the inter-laboratory differences if they were provided for routine analysis in clinical laboratories without strict checking.

5. Conclusions

The actual state of inter-laboratory differences of observed values was too great directly to compare results among laboratories. The recommendation of JDS to measure only the stable HbA1c component and to correct the observed percentage by two-point calibration with assigned values was effective but not sufficient for application to various assay methods. Inter-method differences among the eleven assay methods available were also great even after correction. The authors are concerned that the introduction of diverse assay methods of GHb measurement might magnify the inter-laboratory differences.

An urgent and worldwide problem to be solved is the removal of inter-laboratory differences in the measurement of GHb and the development of an assay system with a reference material and a reference method. Users in clinical practice must recognize this situation and the providers should check their analytical method and keep records that are readily traceable.

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