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IFCC Reference System for Measurement of Hemoglobin A_{1c} in Human Blood and the National Standardization Schemes in the United States, Japan, and Sweden: A Method-Comparison Study

Wieland Hoelzel,[1] Cas Weykamp,[2] Jan-Olof Jeppsson,[3] Kor Miedema,[4*] John R. Barr,[5] Ian Goodall,[6] Tadao Hoshino,[7] W. Garry John,[8] Uwe Kobold,[1] Randie Little,[9] Andrea Mosca,[10] Pierluigi Mauri,[11] Rita Paroni,[12] Fransiscus Susanto,[13] Izumu Takei,[14] Linda Thienpont,[15] Masao Umemoto,[16] and Hsiao-Mei Wiedmeyer,[9] on behalf of the IFCC Working Group on HbA_{1c} Standardization

Background: **The national programs for the harmonization of hemoglobin (Hb)A_{1c} measurements in the US [National Glycohemoglobin Standardization Program (NGSP)], Japan [Japanese Diabetes Society (JDS)/Japanese Society of Clinical Chemistry (JSCC)], and Sweden are based on different designated comparison methods (DCMs). The future basis for international standardization will be the reference system developed by the IFCC Working Group on HbA_{1c} Standardization. The aim of the present study was to determine the relationships between the IFCC Reference Method (RM) and the DCMs.**

Methods: **Four method-comparison studies were performed in 2001–2003. In each study five to eight pooled blood samples were measured by 11 reference laboratories of the IFCC Network of Reference Laboratories, 9 Secondary Reference Laboratories of the NGSP, 3 reference laboratories of the JDS/JSCC program, and a Swedish reference laboratory. Regression equations were determined for the relationship between the IFCC RM and each of the DCMs.**

Results: **Significant differences were observed between the HbA_{1c} results of the IFCC RM and those of the DCMs. Significant differences were also demonstrated between the three DCMs. However, in all cases the relationship of the DCMs with the RM were linear. There were no statistically significant differences between the regression equations calculated for each of the four studies; therefore, the results could be combined. The relationship is described by the following regression equations: NGSP-HbA_{1c} = 0.915(IFCC-HbA_{1c}) + 2.15% (r^2 = 0.998); JDS/JSCC-HbA_{1c} = 0.927(IFCC-HbA_{1c}) + 1.73% (r^2 = 0.997); Swedish-HbA_{1c} = 0.989(IFCC-HbA_{1c}) + 0.88% (r^2 = 0.996).**

Conclusion: **There is a firm and reproducible link between the IFCC RM and DCM HbA_{1c} values.**

[1] Roche Diagnostics GmbH, Penzberg, Germany.
[2] SKZL Queen Beatrix Hospital, Winterswijk, The Netherlands.
[3] Department of Clinical Chemistry, Malmo University Hospital, Lund University, Malmo, Sweden.
[4] Department of Clinical Chemistry, Isala Klinieken, NA 8000 GM Zwolle, The Netherlands.
[5] Centers for Disease Control and Prevention, Atlanta, GA.
[6] Department of Laboratory Medicine. Austin & Repatriation Medical Centre, Melbourne, Australia.
[7] Institute of Biopathological Medicine, Ono, Japan.
[8] Department of Clinical Biochemistry, Norfolk and Norwich University Hospital, Norwich, Norfolk, United Kingdom.
[9] University of Missouri School of Medicine, Columbia, MO.
[10] Department of Science and Biomedical Technology, University of Milan, Milan, Italy.
[11] Institute of Biomedical Technology, Consiglio Nazionale Delle Ricerche, Milan, Italy.
[12] Istituto di Ricovero e Cura a Carattere Scientifico, Hospital San Raffaele, Milan, Italy.
[13] German Diabetes Research Institute, Duesseldorf, Germany.
[14] Department of Laboratory Medicine, School of Medicine, Keio University, Tokyo, Japan.
[15] Laboratory of Analytical Chemistry, Faculty of Pharmaceutical Sciences, University of Gent, Ghent, Belgium.
[16] Standard Reference Center, Kawasaki, Japan.
*Author for correspondence. Fax 31-38-4242676; e-mail k.miedema@isala.nl.
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The measurement of hemoglobin A_{1c} (HbA_{1c})[17] in human blood is the most important marker for long-term assessment of the glycemic state in patients with diabetes, and goals for therapy are set at specific HbA_{1c} target values *(1–4)*. There are many commercial methods available for the routine measurement of HbA_{1c}. These methods are based on different analytical principles, such as immunoassays, ion-exchange chromatography, and affinity chromatography. Several surveys *(5–8)* have demonstrated that different results are produced by these different method principles. However, therapeutic targets for patients with diabetes require method-independent values for HbA_{1c}. Furthermore, if in the future HbA_{1c} is to be used for the diagnosis of diabetes or impaired glucose tolerance, it is essential that the different HbA_{1c} methods used routinely in medical laboratories provide comparable results.

Studies have shown that harmonization of test results obtained by different HbA_{1c} assays is feasible if all of the methods are calibrated with the same set of calibrators *(9, 10)* and/or are adjusted to a Designated Comparison Method (DCM) *(11)*. These principles have been used in national initiatives for the harmonization of HbA_{1c} results. In the US, the National Glycohemoglobin Standardization Program (NGSP) has been established *(12)*. The Japanese Diabetes Society (JDS) developed a set of national calibrators with the recommendation to adjust the calibration of all routine HbA_{1c} methods to these calibrators *(13)*. In Sweden, the Mono S method, a high-resolution ion-exchange HPLC method, has been chosen as the DCM for the harmonization of HbA_{1c} results *(14)*. All of these national initiatives were important steps toward improvement of the comparability of HbA_{1c} test results, but national standardization programs based on different DCMs cannot replace uniform worldwide standardization anchored on a metrologically sound international reference measurement system *(15, 16)* comprising (*a*) a clear definition of the analyte based on its molecular structure, (*b*) a primary reference material containing the analyte in a pure form, (*c*) a validated reference method that specifically measures the analyte in human samples, and (*d*) a global network of reference laboratories that guarantees that the reference method is performed with the necessary analytical quality and is capable of assigning reliable values to matrix-based secondary reference materials and calibrators.

The IFCC Working Group on HbA_{1c} Standardization has developed such a reference system for HbA_{1c} *(17)*. HbA_{1c} is defined as the stable adduct of glucose to the N-terminal valine of the β-chain of Hb. Primary reference materials of pure HbA_{1c} and HbA_0 have been prepared *(18)*, and a reference method that specifically measures HbA_{1c} has been developed *(19, 20)*. The reference method has been approved by all member national societies of the IFCC, and a global network of reference laboratories has been established *(20, 21)*.

When the IFCC Reference Method for the calibration of HbA_{1c} routine methods is used, laboratorians must consider that the current clinical interpretation of HbA_{1c} results is based on data from tests that were calibrated to DCMs that were less specific than the IFCC Reference Method and therefore generated HbA_{1c} values that are higher than those that will be obtained if the calibration is traced back to the IFCC Reference Method. To ensure the proper clinical use of the tests, it is important to understand the numeric relationship between the IFCC Reference Method and the DCMs used in the national standardization schemes. This study was designed to investigate this relationship.

Materials and Methods

IFCC REFERENCE METHOD

The reference method was developed as candidate reference method by Kobold et al. *(19)* on behalf of the IFCC Working Group on HbA_{1c} Standardization, and was thereafter thoroughly evaluated and optimized by the IFCC network of reference laboratories. In 2001, the final method was unanimously accepted in a ballot by the national member societies of the IFCC as the "Approved IFCC Reference Method for the Measurement of HbA_{1c} in Human Blood". The method was published in 2002 in the IFCC section of the journal *Clinical Chemistry and Laboratory Medicine (20)*. A PDF version of the method can be downloaded free of charge from the IFCC section of *Clinical Chemistry and Laboratory Medicine* (www.degruyter.de/journals/cclm/pdf/401_78.pdf).

The IFCC Reference Method has three steps. In the first step, Hb from washed and lysed erythrocytes is cleaved into peptides by the proteolytic enzyme endoproteinase Glu-C. The resulting glycated and nonglycated N-terminal hexapeptides of the β-chain are then separated from the crude peptide mixture by reversed-phase HPLC. In the third and final step, the glycated and nonglycated hexapeptides are quantified by mass spectrometry or by capillary electrophoresis with ultraviolet detection. The percentage of HbA_{1c} is determined by the ratio of glycated to nonglycated β-N-terminal hexapeptides of Hb.

The measurements in this study were performed by the reference laboratories of the IFCC Network of Reference Laboratories (IFCC-NRL), which are listed in the *Appendix*. The network comprises laboratories from Europe, Japan, and the US that have successfully established the reference method. The network is coordinated by the Network Coordinator, who is responsible for the organization of meetings, updating the Standard Operating Procedure of the reference method, and organizing regu-

[17] Nonstandard abbreviations: HbA_{1c}, hemoglobin A_{1c}; DCM, Designated Comparison Method; NGSP, National Glycohemoglobin Standardization Program; JDS, Japanese Diabetes Society; NRL, Network of Reference Measurement Laboratories; DCCT, Diabetes Control and Complications Trial; CPRL, Central Primary Reference Laboratory; PRL, Primary Reference Laboratory; SRL, Secondary Reference Laboratory; and JSCC, Japanese Society of Clinical Chemistry.

lar quality-control surveys within the network. The network works on behalf of, and is supervised by, the IFCC Working Group on HbA_{1c} Standardization *(21)*.

NGSP standardization scheme

The NGSP system was established after the Diabetes Control and Complications Trial (DCCT) study showed the relationship between HbA_{1c} and risks for development and/or progression of diabetes complications. Implementation of treatment goals based on the results of the DCCT in clinical settings made it necessary to harmonize HbA_{1c} results *(22)*. The anchor for the NGSP laboratory network is a DCM, which is ion-exchange HPLC using Bio-Rex 70 resin (Bio-Rad Laboratories) *(23)*. The method is performed in the Central Primary Reference Laboratory (CPRL) and backup Primary Reference Laboratories (PRLs) *(12)*. Secondary Reference Laboratories (SRLs) have been established to assist manufacturers with calibration to the DCCT as well as serving as the comparison methods for NGSP certification. These laboratories use HbA_{1c} assay methods of various method types (ion-exchange HPLC, affinity HPLC, and immunoassay) that are convenient and robust, provide excellent analytical performance, and are calibrated to the CPRL method. The CPRL, PRLs, and SRLs work closely together in a network of reference laboratories (NGSP-NRL). The network laboratories are monitored monthly by sample exchanges with the CPRL. The "NGSP-HbA_{1c} values" in this study were measured by the SRLs located in the US and Europe.

JDS/JSCC standardization scheme

The basis of the JDS/JSCC standardization scheme are national calibrators. In 1995, the JDS developed a first set of national calibrators, called JDS Calibrator Lot 1, which was recommended to be used for the calibration of all routine HbA_{1c} assays in Japan. The calibrator values were assigned with the HPLC ion-exchange chromatography methods of TOSOH and Kyoto Daiichi. These two methods were chosen because at the time when the calibrators were established, most of the Japanese medical laboratories used one of these HPLC methods. In recent years the Japanese standardization scheme has evolved. The Japanese Society of Clinical Chemistry (JSCC) developed a high-resolution ion-exchange HPLC method, named KO500 *(24)*, and a second set of national calibrators (deep-frozen blood), called JDS/JSCC Calibrator Lot 2. The KO500 method was used to assign target values to the Lot 2 calibrators and to samples for national proficiency testing. To keep consistency in the HbA_{1c} values, the calibration of the KO500 method was adjusted to the first lot of JDS calibrators. For the measurements in this study, the KO500 HPLC method was calibrated with JDS Calibrator Lot 2. The measurements were performed by the three Japanese IFCC reference laboratories, which are also reference laboratories of the JDS/JSCC standardization scheme.

Swedish standardization scheme

The Swedish standardization scheme uses the Mono S method (strong methylsulfonate cation exchanger on monobeads; Amersham Biosciences) as DCM for the harmonization of HbA_{1c} measurements *(25)*. The measurements in this study were performed by the Swedish IFCC reference laboratory at the Malmo University Hospital. The laboratory is a part of the EQAS organization, External Quality Assurance in Laboratory Medicine in Sweden (EQUALIS), located in Uppsala. Split samples of fresh EDTA blood are distributed once a month to 40 hospitals using different HPLC methods. Five of them are contracted to run the Mono S system in a national network. These laboratories are used for calibration of all hospital and point-of-care instruments in Sweden every second year.

Design and logistics

The study was designed by the IFCC Working Group on HbA_{1c} Standardization and organized logistically by the IFCC reference laboratory in Winterswijk (The Netherlands), which currently holds the function of the IFCC Network Coordinator, and the reference laboratory in Zwolle (The Netherlands), which was responsible for the blood collection. The design was adjusted to the aim of the study, which was to generate equations that describe reliably the relationship between the various reference systems. Therefore, low uncertainty of the resulting equations as well as an acceptable workload for the reference laboratories performing the measurements had to be considered. The uncertainty of the equations depends on many factors, such as the analytical performance of the measurements, the number of measurements per sample, systematic differences between the laboratories within the networks, the number of participating laboratories per system, the number of samples analyzed, the biological variation of samples, and the number of measurement campaigns (covering shifts attributable to changes in calibration, reagents, and instruments). To evaluate all factors that contribute to the uncertainty and to reduce the overall uncertainty, blood pools were used instead of single samples (explanation see below). The results among networks and not among laboratories were compared, each specimen was measured four times by each laboratory, and the experiments were repeated in four independent studies. The studies were performed 2001–2003 and were called Marrakech (2001), Chicago (2001), Kyoto I (2002), and Kyoto II (2003).

Samples

When evaluating the quantitative relationship between the reference systems, the biological variation in the samples used for the method comparison is a major confounding factor. The biological variation results from the fact that the composition of blood in each individual is slightly different. There are Hb derivatives such as carbamylated Hb and other adducts, and Hb forms that may

interfere with the NGSP-SRL methods in particular; these are commercial methods with a broad spectrum of method principles (ion-exchange chromatography, immunoassays, affinity chromatography) *(26)*. Theoretically, the influence of biological variation should be substantially reduced if the samples used are mixtures of blood from various donors rather than samples derived from single donors. This hypothesis was checked and confirmed to be true in a separate study in which 36 donations were used to prepare 36 single specimens as well as 6 pools (each pool being a mixture of 6 individual donations), and HbA_{1c} was measured by the network laboratories. The outcome was that the HbA_{1c} values of the pools were not significantly different from the mean of the HbA_{1c} values of the respective single donations (mean of singles, 6.84%; mean of mixtures, 6.85%; $P > 0.999$) and that the intralaboratory CV were the same for pools and singles donations (combined CV for singles 1.1%; for mixtures 1.1%; $P > 0.999$), but the scatter of the HbA_{1c} values around the regression line characterized by $S_{y|x}$ was significantly lower for pools than for single donations (e.g., for the relationship of the IFCC-NRL and the NGSP-NRL, $S_{y|x}$ was 0.17 for single donations and 0.08 for pools, respectively). This means that use of blood pools could substantially reduce the uncertainty of the resulting regression equations. Therefore, the blood pools used in this study were as follows: in the Marrakech study, eight samples in the range 3.04–9.65%; in the Chicago study, eight samples in the range 3.30–9.00%; in the Kyoto I study, five samples in the range 3.09–11.25%; and in the Kyoto II study, five samples in the range 3.48–8.65% HbA_{1c} (HbA_{1c} percentages determined by the IFCC Reference Method). Each sample was prepared from 10 donations.

Before mixing, each donation was checked for (*a*) hepatitis B surface antigen, anti-HIV, and anti-hepatitis C antibodies (Abbott Laboratories; criterion, samples must be negative); (*b*) abnormal Hb variants such as S, C, E, and F (Menarini 8140 method; A.Menarini; criterion, HbF <1% and other variants absent); and (*c*) abnormal amounts of urea to exclude increased concentrations of carbamylated Hb. The HbA_{1c} value was used to make pools with appropriate HbA_{1c} concentrations. The blood samples (60–90 donations for each campaign) were drawn and checked within a time frame of 32 h. Donations were stored in the refrigerator (2–8 °C) until pools were prepared. The pools were the starting material to supply both IFCC network laboratories and DCMs with specimens (described below).

specimens for the ifcc reference method

Both whole blood and hemolysates are suitable materials for the IFCC Reference Method and provide the same numerical HbA_{1c} results. This was demonstrated during the development of the reference method: a panel of whole-blood samples and hemolysates from the same blood were measured in parallel, and identical results were obtained [regression equation: $y = 1.006x - 0.035\%$ HbA_{1c}; $r^2 = 0.999$; slope and the intercept did not deviate significantly from 1 ($P > 0.999$) and 0 ($P > 0.99$), respectively]. However, whole blood is stable for ~1 week, whereas hemolysates stored at −70 °C are stable for many years (e.g., a hemolysate manufactured in 1999 and measured by the IFCC-NRL in 1999, 2001, and 2002 had HbA_{1c} values of 9.39%, 9.37%, and 9.32%, respectively). Because the IFCC Reference Method is time-consuming and the reference laboratories are spread globally, there was a need to have a time buffer for shipment and performing the measurements. Therefore, frozen hemolysates were used in this study. The hemolysates were prepared from the blood pools according to the Standard Operating Procedure of the IFCC Reference Method *(20)* within 32 h after blood drawing and stored at −70 °C until shipment. The samples were shipped on dry ice (−79 °C; amount sufficient for 5 days) by courier to the IFCC-NRL laboratories. The reference laboratories checked for the presence of dry ice, which was still present when the samples arrived at all laboratories during all studies, and stored the samples at −70 °C until analysis. The measurements were performed within 9 weeks of hemolysate preparation.

specimens for the DCMs

All of the DCMs work with hemolysates, but the methods use different hemolyzing agents and different dilutions. Therefore, whole-blood specimens were sent to the DCM laboratories, which were prepared locally for the measurement according to the specific protocol of the DCM used. Blood from the pools was dispensed in 1-mL aliquots, packed in special isolating boxes on cool packs (2–8 °C; capacity 4 days) within 8 h after preparation of the pools, and immediately shipped by courier. On arrival, the DCM laboratories checked the temperature and stored the specimens until the measurement. All measurements were performed within 5 days after blood drawing.

quality control

A precondition for the inclusion of the results of the IFCC network laboratories was that they had successfully passed the regular quality-control surveys of the IFCC-NRL. The IFCC-NRL control surveys were organized twice a year. Six hemolysates with HbA_{1c} concentrations covering the clinically relevant concentration range (3–13% HbA_{1c}) were distributed to the network laboratories, and each sample was measured four times by the reference laboratories. The criteria for passing were that the mean intralaboratory CV was ≤2.5% and the mean deviation from the overall mean of all NRL laboratories was ≤2.5%. The mean intralaboratory CV of the NRL laboratories in the surveys performed during this study was 1.0–1.2%, the intralaboratory CV varied from 0.50% to 2.2%, and the interlaboratory CV were in the range of 1.4–1.9%. In two studies, one reference laboratory did not

meet the criteria; its results were therefore not included when calculating the overall IFCC-NRL HbA_{1c} values.

The laboratories of the NGSP-NRL participated in a monthly monitoring program with specific precision and bias limits *(12)*. All laboratories met the requirements of the program during the time of the method-comparison studies (mean difference between CPRL and SRL HbA_{1c} values <0.35%; SD of difference replicates <0.23%).

The JDS/JSCC-NRL laboratories twice a year exchanged a set of samples (deep-frozen blood) with five HbA_{1c} concentrations (4.05–12.63%) and participated in the IFCC/DCM trials. The interlaboratory CV in these studies were <1.0%.

The Swedish reference laboratory participated in the national network of Mono S reference laboratories, which performed regular intercomparison studies. Split samples of fresh EDTA blood were distributed once a month. The interlaboratory CV in this Mono S network intercomparison studies were <1.5%.

STATISTICAL EVALUATION

The mean values of the four measurements per sample and the SDs and CV for these measurements were calculated for each individual laboratory. The mean values of the laboratories were used to calculate the "overall mean values " of the IFCC-NRL, the NGSP-NRL, and the JDS/JSCC-NRL. The overall mean values were used as "IFCC-HbA_{1c} values", "NGSP-HbA_{1c} values", "JDS/JSCC-HbA_{1c} values", and "Swedish-HbA_{1c} values" for the calculation of the relationship between the IFCC reference system and the DCM-based systems. Each of the four method-comparison studies was evaluated separately. Whether the correlation between the IFCC Reference Method and the DCMs fits a linear regression model was checked by visual inspection. Because this could be confirmed for all comparisons, linear regression analysis was used for calculating slopes, intercepts, and r^2, with use of SAS software, Ver. 8.2 (SAS Institute). The SAS software was also used to apply the Kruskal–Wallis test to check whether there were statistically significant differences in the outcomes of the four studies. Finally, the results of the four studies were combined, and the overall regression equations were calculated for the correlation between the DCM-based systems and the IFCC reference system. The presence of statistically significant differences between the system values was checked, as indicated by the slope deviating statistically significantly from 1 and/or the intercept from 0, respectively.

Results

The mean intralaboratory CV in the four studies was 1.1% (range, 0.38–2.1%) for the 11 laboratories of the IFCC-NRL; 1.0% (range, 0.47–2.4%) for the 9 SRLs of the NGSP; 0.54% (0.27–0.87%) for the three Japanese reference laboratories, and 0.57% (0.30–0.58%) for the Swedish reference laboratory. The mean interlaboratory CV were 1.9% for the IFCC-NRL, 1.9% for the NGSP-NRL, and 0.66% for the JDS/JSCC-NRL. The slopes and intercepts of the regression equations and the r^2 for the relationship between the IFCC-HbA_{1c} values and the DCM-HbA_{1c} values of the four studies are listed in Table 1. The Kruskal–Wallis test demonstrated that there were no statistically significant differences in the outcomes of the four studies [IFCC-NRL, $\chi^2 = 0.70$ ($P = 0.87$); NGSP-NRL, $\chi^2 = 0.83$ ($P = 0.84$); JDS/JSCC-NRL, $\chi^2 = 0.90$ ($P = 0.82$); Swedish-RL, $\chi^2 = 0.88$ ($P = 0.83$)] so that the results of the four studies could be combined to calculate the overall regression equations (Table 2). The slope and the intercept of the equation for the IFCC vs NGSP methods deviated significantly from 1 and 0, respectively (P <0.001). However, the relationship between the two systems was linear, and

Table 1. Comparison of IFCC HbA_{1c} Reference Method with the national DCMs: individual results for the four method-comparison studies.

	Study[a]			
Reference system	Marrakech (n = 8)	Chicago (n = 8)	Kyoto I (n = 5)	Kyoto II (n = 5)
NGSP-NRL (9 laboratories)				
Slope	0.926	0.926	0.906	0.912
Intercept, %	2.14	2.05	2.21	2.17
r^2	0.999	0.999	0.999	0.999
JDS/JSCC-NRL (3 laboratories)				
Slope	0.934	0.926	0.920	0.943
Intercept, %	1.76	1.67	1.78	1.68
r^2	0.996	0.999	0.999	0.999
Swedish-RL (1 laboratory)				
Slope	1.008	0.941	1.002	0.968
Intercept, %	0.90	1.09	0.78	1.08
r^2	0.999	0.999	0.999	0.999

[a] n, number of samples.

Table 2. Comparison of the IFCC HbA_{1c} Reference Method with the national DCMs: combined results for the four method-comparison studies.

	Reference system		
	NGSP-NRL	JDS/JSCC-NRL	Swedish-RL
No. of samples	26	26	26
Intercept, %	2.152[a]	1.724[a]	0.884[a]
SE, %	0.050	0.065	0.080
95% confidence interval, %	2.049–2.256	1.590–1.859	0.718–1.049
Slope	0.9148[a]	0.9274[a]	0.9890
SE	0.0075	0.0098	0.012
95% confidence interval	0.899–0.930	0.907–0.948	0.964–1.014
RMSE[b]	0.084	0.109	0.134
r^2	0.998	0.997	0.997

[a] Significant deviation of slope from 1 or intercept from 0 (P <0.001).
[b] RMSE, root mean square error.

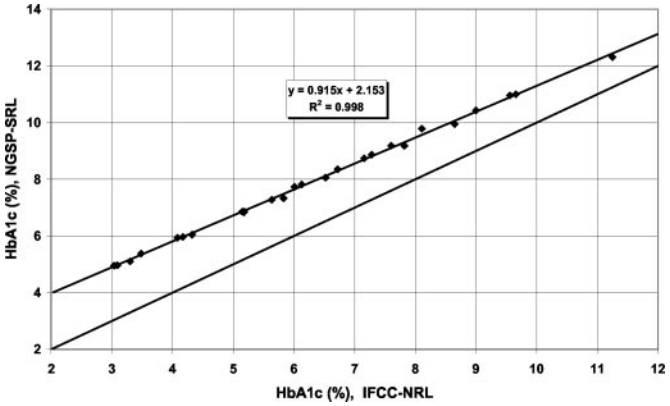

Fig. 1. HbA_{1c} values measured in 26 pooled blood samples by the IFCC-NRL, applying the IFCC Reference Method (mean value of 11 network laboratories), and by the NGSP-NRL, applying various methods adjusted to the NGSP DCM Bio-Rex 70 HPLC (mean values of 9 network laboratories).

The *lines* are the regression line and the $y = x$ line, respectively.

there was very little dispersion of the measured values around the regression line ($r^2 = 0.998$; see Fig. 1). There were also statistically significant differences between the IFCC-HbA_{1c} values and the JDS/JSCC-HbA_{1c} values. However, as with the NGSP, there was a strong linear correlation between both systems ($r^2 = 0.997$; see Fig. 2). There was a slightly higher variation in the outcome of the four method-comparisons studies of the IFCC-NRL vs the Swedish-RL, but the differences were not statistically significant. The higher random variation was attributable to the HbA_{1c} values from the Swedish standardization scheme not being the mean values of a network of reference laboratories but rather the results from one laboratory. In contrast to the two other systems, the overall slope of 0.989 did not deviate significantly from 1 ($P > 0.95$; see Fig. 3), whereas the intercept was significantly different from 0 ($P < 0.001$).

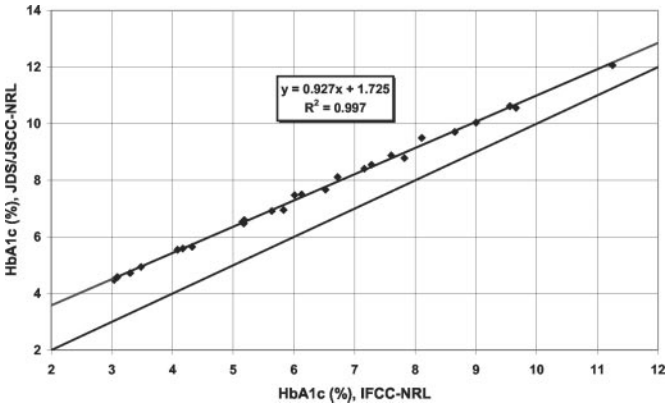

Fig. 2. HbA_{1c} values measured in 26 pooled blood samples by the IFCC-NRL, applying the IFCC Reference Method (mean value of 11 network laboratories), and by the JDS/JSCC-NRL, applying the JDS/JSCC DCM KO 500 calibrated with JDS calibrators (mean values of 3 network laboratories).

The *lines* are the regression line and the $y = x$ line, respectively.

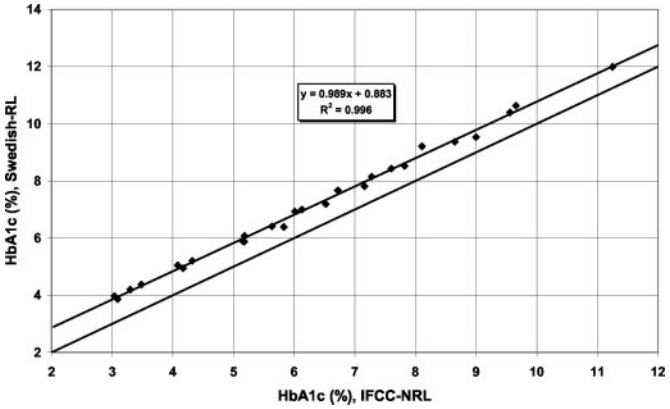

Fig. 3. HbA_{1c} values measured in 26 pooled blood samples by the IFCC-NRL, applying the IFCC Reference Method (mean value of 11 network laboratories), and by the Swedish-RL, applying the DCM Mono S HPLC.

The *lines* are the regression line and the $y = x$ line, respectively.

There were also statistically significant differences in the slopes as well in the intercepts between the three DCM-based systems (Table 3).

Discussion

To ensure optimum quality of patient care and a valid interpretation of clinical trials, good interlaboratory agreement of results in laboratory medicine is essential. The most effective approach for achieving universal comparability of test results is the ability to trace the calibration of the routine assays back to an international metrologically sound reference measurement system. Therefore, an essential requirement of the IVD Directive of the European Union is that manufacturers have to trace back the calibration of their tests to reference methods or reference materials of higher metrologic order if available *(27)*.

With the development of the IFCC reference system, a

Table 3. Comparison of the national DCMs: combined results for the four method-comparison studies.

	DCM		
	NGSP-NRL	JDS/JSCC-NRL	Swedish-RL
NGSP-NRL (9 laboratories)			
Slope		0.985[a]	0.923[b]
Intercept		0.459[b]	1.345[b]
r^2		0.999	0.998
JDS/JSCC-NRL (3 laboratories)			
Slope	1.014[a]		0.936[b]
Intercept	−0.459[b]		0.907[b]
r^2	0.999		0.997
Swedish-RL (1 laboratory)			
Slope	1.081[b]	1.065[b]	
Intercept	−1.442[b]	−0.947[b]	
r^2	0.998	0.997	

[a,b] Significant deviation of slope from 1 or intercept from 0: [a] $P < 0.05$; [b] $P < 0.001$.

reference method and reference materials of higher metrologic order are now available for the measurement of HbA_{1c}. In the IFCC reference system, HbA_{1c} is defined on its molecular structure and specifically measured with a reference method, whereas in the existing DCM-based national standardization schemes HbA_{1c} is defined and measured as the "HbA_{1c}" peak of the chromatogram of the chosen DCM. These HbA_{1c} peaks contain not only HbA_{1c} but also, depending on the resolution of the resin used, to some extent substances that are not HbA_{1c} *(28–32)*. To minimize the confounding effect of biological variation, blood pools were used as samples in this study, and blood with abnormal Hb variants such as S, C, E, and F and abnormal urea concentrations (carbamylated Hb) were excluded.

Considering the lack of specificity of the DCMs, it is not surprising that all three DCMs generate significantly higher results than the IFCC Reference Method and that there are significant differences among the results of the three DCMs as well (see Table 3). The NGSP-SRL generates the highest HbA_{1c} values because the HbA_{1c} peak of the Bio-Rex 70 method, used as anchor of the NGSP, contains a high proportion of non-HbA_{1c} substances, such as HbF, minor Hb forms, and carbamylated Hb, and the peak is not clearly separated from the neighboring non-HbA_{1c} peaks *(23)*. The KO500 used in the Japanese scheme is a high-resolution HPLC method, but because this method is based on calibrators with values that were assigned with the older HPLC methods from TOSOH and Kyoto Daiichi, the JDS/JSCC-NRL values reflect the low specificities of these methods. The results of the JDS/JSCC-NRL are slightly lower than those of the NGSP-NRL. The Swedish DCM generates the lowest DCM HbA_{1c} values. The Mono S system, developed in 1983, shows an almost homogeneous HbA_{1c} peak in the chromatogram, but it contains carbamylated Hb as well as free α-globulin chains *(25, 31, 32)*, so that the values are higher than those measured with the IFCC Reference Method. In contrast to the DCM methods, new dedicated HPLC systems are today eliminating many of these interfering adducts such as carbamylated Hb by use of more modern chromatographic material and improved gradients *(33)*.

There is, however, a strong correlation between the IFCC values and the DCM values, which can be described by a linear regression model. The relationships were identical in all four studies, and the dispersion of the values around the regression line was small. It is therefore possible to establish reliable numerical links between the results of the IFCC Reference Method and the DCMs described by linear regression equations. When calibrators with IFCC values are used, the HbA_{1c} values of the routine methods will be lower than those generated with the previous calibration. There are significant numerical differences between HbA_{1c} values based on the IFCC Reference Method calibration and HbA_{1c} values based on the DCM calibrations (see Table 4). These changes are similar to the change in glucose values four decades ago when the nonspecific glucose routine methods, based on the measurement of the reduction caused by glucose and other substances in blood, were replaced by methods that specifically measured glucose *(34, 35)*. Because of the close correlation between the IFCC Reference Method and the DCMs, it is possible to derive IFCC values from the existing scales of numbers by use of linear regression equations. Because the Bio-Rex 70 method was used as reference for harmonization of the HbA_{1c} test results in the DCCT study *(1)*, it is also possible to "translate" the risk curves generated in this landmark study and the HbA_{1c} data of the important UKPDS study *(2)*, which were also adjusted to the Bio-Rex 70 calibration. When regression equations are used for transforming IFCC HbA_{1c} values into DCM HbA_{1c} values and vice versa, the additional uncertainty introduced by the uncertainty of the regression equations must be considered. This uncertainty must be added to the uncertainty of the analytical measurement. The uncertainty values given in Table 4 were calculated according to the Eurochem/Citac Guide for Quantifying Uncertainty in Analytical Measurement *(36)*, considering the IFCC value as independent variable. The standard uncertainty is the standard error of the predicted DCM value.

Table 4. Examples of the numeric relationship between IFCC HbA_{1c} values and DCM-based values.

IFCC	NGSP HbA_{1c}		JDS/JSCC HbA_{1c}		Swedish HbA_{1c}	
HbA_{1c}, %	HbA_{1c}, %	u_{trans},[a] %	HbA_{1c}, %	u_{trans}, %	HbA_{1c}, %	u_{trans}, %
5.30	7.00[b]	0.018	6.64	0.024	6.13	0.029
7.00	8.56	0.017	8.22	0.022	7.80	0.028

[a] u_{trans} is the standard uncertainty, which is generated if the equations described in this report are used to transform IFCC HbA_{1c} values into DCM-based values.

[b] HbA_{1c} target value for NGSP.

Changing medical decision criteria is not just a matter for laboratory professionals but also for the healthcare providers and patients who use these criteria. Therefore, the IFCC Working Group on HbA_{1c} Standardization has contacted the international scientific societies of diabetologists to discuss appropriate ways of adopting the IFCC standardization for HbA_{1c} routine tests in clinical practice.

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Appendix

PARTICIPATING IFCC REFERENCE LABORATORIES

Department of Clinical Chemistry, Malmo University Hospital, Malmo, Sweden; Roche Diagnostics GmbH, Penzberg, Germany; Department of Clinical Chemistry, Isala Klinieken, Zwolle, The Netherlands; Department of Clinical Chemistry, Queen Beatrix Hospital, Winterswijk,

The Netherlands; Department of Science and Biomedical Technology, University of Milan, Milan, Italy; IRCCS Hospital San Raffaele, Milan, Italy; Institute of Biomedical Technology, Consiglio Nazionale Delle Richerche, Milan, Italy; Centers for Disease Control and Prevention, Atlanta, GA; Standardization Reference Center, Kawasaki, Japan; Institute of Biopathological Medicine, Ono, Japan; Laboratory of Analytical Chemistry, Faculty of Pharmaceutical Sciences, University of Gent, Ghent, Belgium.

PARTICIPATING NGSP SRLs

Methods are given in parentheses: Diabetes Diagnostic Laboratory, University of Missouri School of Medicine, Columbia, MO (Bio-Rad Diamat HPLC; Tosoh 2.2 Plus HPLC; Primus CLC330 HPLC); Collaborative Studies Clinical Laboratory, Fairview University Medical Center, Minneapolis, MN (Bio-Rad Diamat HPLC; Tosoh 2.2 Plus HPLC); Core Laboratory for Clinical Studies, Washington University School of Medicine, St. Louis, MO (Roche Tina-quant II on Hitachi 917); Queen Beatrix Hospital, Winterwijk, The Netherlands (Beckman CE; Bio-Rad Diamat; Menarini HA8160 HPLC); Isala Klinieken, Zwolle, The Netherlands (Roche Unimate-Modular Analytics; Primus CLC385 HPLC).

PARTICIPATING JDS REFERENCE LABORATORIES

Institute of Biopathological Medicine, Ono, Japan; Standard Reference Center, Kawasaki, Japan; Department of Laboratory Medicine, School of Medicine, Keio University, Tokyo, Japan.

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