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Evaluation of enzymatic BM Test HbA_{1c} on the JCA-BM6010/C and comparison with Bio-Rad Variant II Turbo, Tosoh HLC 723 G8, and AutoLab immunoturbidimetry assay

Abstract

Background: A novel enzymatic HbA_{1c} assay was introduced for use in an automated chemistry analyzer. With this unique method, HbA_{1c} and plasma glucose can be measured from the same EDTA tube. We evaluated the analytical performance of this enzymatic HbA_{1c} assay in a JCA-BM6010/C analyzer and compared the HbA_{1c} values with the results from other widely used methodological instruments.

Methods: The imprecision, linearity, carry-over and concordance rate of the enzymatic HbA_{1c} test (BM Test HbA_{1c}) using the JCA-BM6010/C analyzer were evaluated. Three hundred and seventy-seven specimens with HbA_{1c} concentrations from 16 to 133 mmol/mol were used for a comparison study with two high performance liquid chromatography methods: Variant II Turbo and Tosoh HLC 723 G8 and the AutoLab Hemoglobin A_{1c} immunoturbidimetry reagent using a Hitachi 7600-110. Forty specimens were used for the glucose method comparison.

Results: The HbA_{1c} coefficients of variation of the within-run imprecision for low and high levels were 0.6% and 0.4%, respectively. The linearity of the BM Test HbA_{1c} using the JCA-BM6010/C analyzer was excellent in the range between 31 mmol/mol and 143 mmol/mol. The carry-over rate was 0.2%. The relationships between the BM test and the other three methods were 0.916×Tosoh G8+3.644, r=0.986; 0.887×Bio-Rad Variant II+1.896, r=0.972; and 0.941×AutoLab+4.532, r=0.977. The concordance rates using a cut-off of 48 mmol/mol were 91.5% with Tosoh G8, 82.8% with Bio-Rad Variant II, and 91.0% with AutoLab. The simultaneously assayed plasma glucose with HbA_{1c} was 1.002×Routine plasma glucose+0.625, r=1.000

Conclusions: The enzymatic BM Test HbA_{1c} in the JCA-BM6010/C analyzer showed excellent precision and linearity, and a minimal carry-over rate. The simultaneously assayed plasma glucose analysis showed good performance.

Keywords: diabetes; enzymatic HbA_{1c} assay; evaluation; glucose; HbA_{1c}; multicenter study.

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Introduction

The maintenance of a stable blood glucose concentration is very important in patients with diabetes mellitus. The rate of formation of HbA_{1c} is directly proportional to the concentration of glucose in the blood; each 1% increase in HbA_{1c} is associated with an increase in mean glucose concentrations of approximately 1.9–2.2 mmol/L [1]. The HbA_{1c} concentration represents the integrated values for glucose over the preceding 8–12 weeks. Hemoglobin A (HbA) is the major component of human adult Hb, comprising approximately 97%, along with 2.5% HbA_2 and 0.5% HbF. HbA_{1c} is formed by the condensation of glucose with the N-terminal valine residue of each β-chain of HbA to form an unstable Schiff base. The Schiff base may dissociate or may undergo an Amadori rearrangement to form a stable ketoamine, HbA_{1c}. Labile intermediates (pre-HbA_{1c}, Schiff base) may be included in measurements of HbA_{1c}, especially in the common ion-exchange methods, and produce misleadingly high results [2]. An International Expert Committee advised that HbA_{1c} could be used for the diagnosis of diabetes. A HbA_{1c} value ≥48 mmol/mol was selected as the decision point, based on the prevalence of retinopathy [3]. This recommendation has been endorsed by the American Diabetes Association and World Health

Organization. HbA_{1c} concentrations of 39 mmol/mol to 46 mmol/mol indicate that subjects are at a high risk of developing diabetes. HbA_{1c} has also been recommended as an alternative to glucose in screening for diabetes [4].

The number of diabetic patients is continuously increasing worldwide. The prevalence of diabetes for all age groups worldwide was estimated to be 2.8% in 2000 and is anticipated to increase to 4.4% in 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 [5]. As a result, the number of HbA_{1c} determinations will continue to increase precipitously. Precise, economical, rapid, and convenient assay methods are required.

HbA_{1c} measurements rely mostly on high-performance liquid chromatography (HPLC) and immunoassays. HPLC is precise [6, 7], but a dedicated device and a long run time are required. The immunoassay can be adapted to an automated analyzer, but the reproducibility is not good. Both HPLC and the immunoassay approaches can be affected by hemoglobin variants in the whole blood sample [8]. An enzymatic HbA_{1c} assay has been adapted for use in the automated chemistry analyzer [9]. HbA_{1c} and plasma glucose can be measured from the same sample tube by the JCA-BM6010/C automatic analyzer (JEOL, Tokyo, Japan).

In this study, we evaluated the enzymatic determination of HbA_{1c} and the simultaneous plasma glucose test using the JCA-BM6010/C analyzer. We also compared the performance of the enzymatic HbA_{1c} assay with HPLC and immunoassay.

Materials and methods

Characteristics of HbA_{1c} and glucose test using the JCA-BM6010/C

In the HbA_{1c} assay, red blood cells in the sample are hemolyzed to generate methemoglobin from hemoglobin by the reaction of an oxidizing agent. Fructosyl dipeptides (fructosyl-VH) are generated from the N-terminus of the β-chain of hemoglobin by the reaction with protease. At the same time, azide methemoglobin is generated from the reaction of sodium azide, which is measured via the absorbance at the wavelength of 505/805 nm as a measurement of Hb. Hydroperoxide, which is generated by the reaction of fructosil dipeptides (fructosyl-VH) with fructosyl peptide oxidase (FPOX), allows the coloring agent to develop a color in the presence of peroxidase, which is measured via the absorbance at the wavelength of 658/805 nm as a measurement of HbA_{1c}. The JCM-BM6010 analyzer enables the simultaneous assay of blood cells (HbA_{1c}) and plasma (glucose and other chemical analytes) from one sample (Figure 1).

Study design

This enzymatic method is traceable to the Diabetes Control and Complications Trial (DCCT) reference method and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) reference measurement procedure [10]. The trueness of the method is not checked in this study. A JCA-BM6010/C analyzer was calibrated at the beginning of the study with the calibrators supplied by the manufacturer. Data from the enzymatic assay using the JCA-BM6010/C analyzer with the BM Test HbA_{1c} reagent were compared with the results using Variant II Turbo (Bio-Rad Laboratories, Hercules, CA, USA), Tosoh HLC 723 G8 (Tosoh Corporation, Tokyo, Japan), and AutoLab Hemoglobin A_{1c} (IVD Lab Co., Uiwang, Korea) immunoturbidimetry reagent on a Hitachi 7600-110 apparatus (Hitachi, Tokyo, Japan). All of these methods are certified by the IFCC and NGSP, except for AutoLab Hemoglobin A_{1c}, which is only NGSP certified.

The enzymatic HbA_{1c} test and plasma glucose test (BioMajesty glucose hexokinase FS*; DiaSys Diagnostic Systems GmbH, Holzheim Germany) using the JCA-BM6010/C analyzer can be evaluated simultaneously. Simultaneous plasma glucose testing with HbA_{1c} from the same sample tube was evaluated and compared with routine plasma glucose analysis using the JCA-BM6010/C analyzer.

Method description

The study was approved by the Institutional Review Boards of Seoul St. Mary's Hospital and St. Paul's Hospital. Analyses were performed using fresh whole blood samples collected in EDTA tubes from patients admitted to Seoul St. Mary's Hospital and St. Paul's Hospital for routine HbA_{1c} assay. The concentrations of the chosen samples included a broad measuring range. The specimens were sent to two

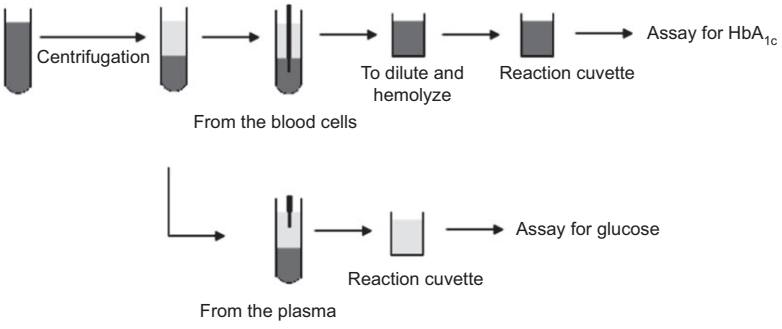


Figure 1 Schematic procedure for assays of HbA_{1c} and glucose, provided by manufacturer JEOL Ltd.

affiliated hospital laboratories and a commercial laboratory to be analyzed with other methods. All laboratories are located in Seoul. No additional samples were necessary for this study, and no samples were stored after completion of the study. All tests were finished within one working day. Samples were analyzed in duplicate with the enzymatic BM Test HbA_{1c} assay using the JCA-BM6010/C and Tosoh HLC 723 G8 analyzers.

The HbA_{1c} values were quantified in 377 samples from 16 mmol/mol to 133 mmol/mol. Forty specimens from 1.4 mmol/L to 26.2 mmol/L were used for glucose method comparisons using the JCA-BM6010/C analyzer. The evaluation was conducted over 3 months.

Precision

We investigated the precision using CLSI EP5-A2 [11]. Low and high controls were assayed in duplicate twice a day in an analytical run on 20 working days. We used controls from the manufacturer (BM Test HbA_{1c} Control). To evaluate the assay's precision, two levels of controls for HbA_{1c} (29–42 mmol/mol and 76–89 mmol/mol) and for glucose (4.4–6.0 mmol/L and 13.4–18.5 mmol/L) were used.

Linearity

We investigated the linearity using CLSI EP6-A [12]. Using samples with HbA_{1c} concentrations of 31 mmol/mol (low) and 143 mmol/mol (high), five concentration samples (low:high, 4:0, 3:1, 2:2, 1:3, 0:4) were made. The expected concentration was 31 mmol/mol, 59 mmol/mol, 87 mmol/mol, 115 mmol/mol, and 143 mmol/mol for each sample. Each concentration sample was assayed four times, and the means were used to examine the linearity. The linearity was evaluated with a linear regression analysis and Spearman's correlation coefficient (r).

Carry-over

We investigated the carry-over using EP10-A3 [13]. Three concentrations of HbA_{1c} [27 mmol/mol (Low), 63 mmol/mol (Mid), 99 mmol/mol (High)] were measured with a sequence as follows: Mid, High, Low, Mid, Mid, Low, Low, High, High, and Mid. The sequence was specifically designed to allow for the nearly uncorrelated estimation of the effects of non-linearity, sample carry-over, and linear drift.

Method comparison

We investigated method comparison using EP9-A2 [14]. A total of 377 specimens with HbA_{1c} concentrations from 16 mmol/mol to 133 mmol/mol were used for the HbA_{1c} comparison study. Each whole blood sample was analyzed using the enzymatic BM Test HbA_{1c} assay on the JCM-BM6010/C, Tosoh HLC 728 G8, Variant II Turbo, and immunoturbidimetric AutoLab HbA_{1c} methods.

Statistics

The relationships between the BM test and the other three methods were analyzed with a linear regression analysis, and the confidence limits of the expected value and the prediction limits of the predicted value at 48 mmol/mol HbA_{1c} were calculated. The concordance rates using a cut-off of 48 mmol/mol were defined as the proportions of total observed agreements both at ≥48 mmol/mol and <48 mmol/mol. The statistical evaluation was performed with Analyse-it for Microsoft Excel v.2.30 (Analyse-it Software, Leeds, UK) and Statistics Pro (www.praetersoftware.com).

Results

Validation of the BM Test HbA_{1c} using the JCA-BM6010/C analyzer

The within-run imprecision for the low and high levels of HbA_{1c} were 0.6% and 0.4%, respectively. The between-run and between-day imprecisions fell between 0.0% and 0.9%, and the total CVs of the low and high HbA_{1c} were 1.2% and 0.7%, respectively (Table 1). The linearity of the BM Test HbA_{1c} using the JCA-BM6010/C analyzer was excellent in the range between 31 mmol/mol and 143 mmol/mol, which is also shown in the equation of the linear regression analysis plotted against the theoretical HbA_{1c} concentration ($y=0.99x-0.06$; $r=0.99$; $p<0.0001$). The linearity, carry-over and linear drift according to CLSI EP-10 protocol were not statistically significant at a 5% significance level (Table 2). The linear regression with Passing-Bablok fit and Bland-Altman plots of comparison studies are shown in Figure 2. The analysis took 11 min for the first sample, 4–6 s for all the subsequent samples, and 20 min for 100 tests.

Table 1 Imprecision of the BM Test HbA_{1c} using the JCA-BM6010/C analyzer.

	HbA_{1c}	
	Low	**High**
Within-run CV (%)	0.6	0.4
Between-run CV (%)	0.9	0.6
Between-day CV (%)	0.6	0.0
Total CV (%)	1.2	0.7

Table 2 Linearity, carry-over and linear drift of the BM Test HbA_{1c} using the JCA-BM6010/C analyzer.

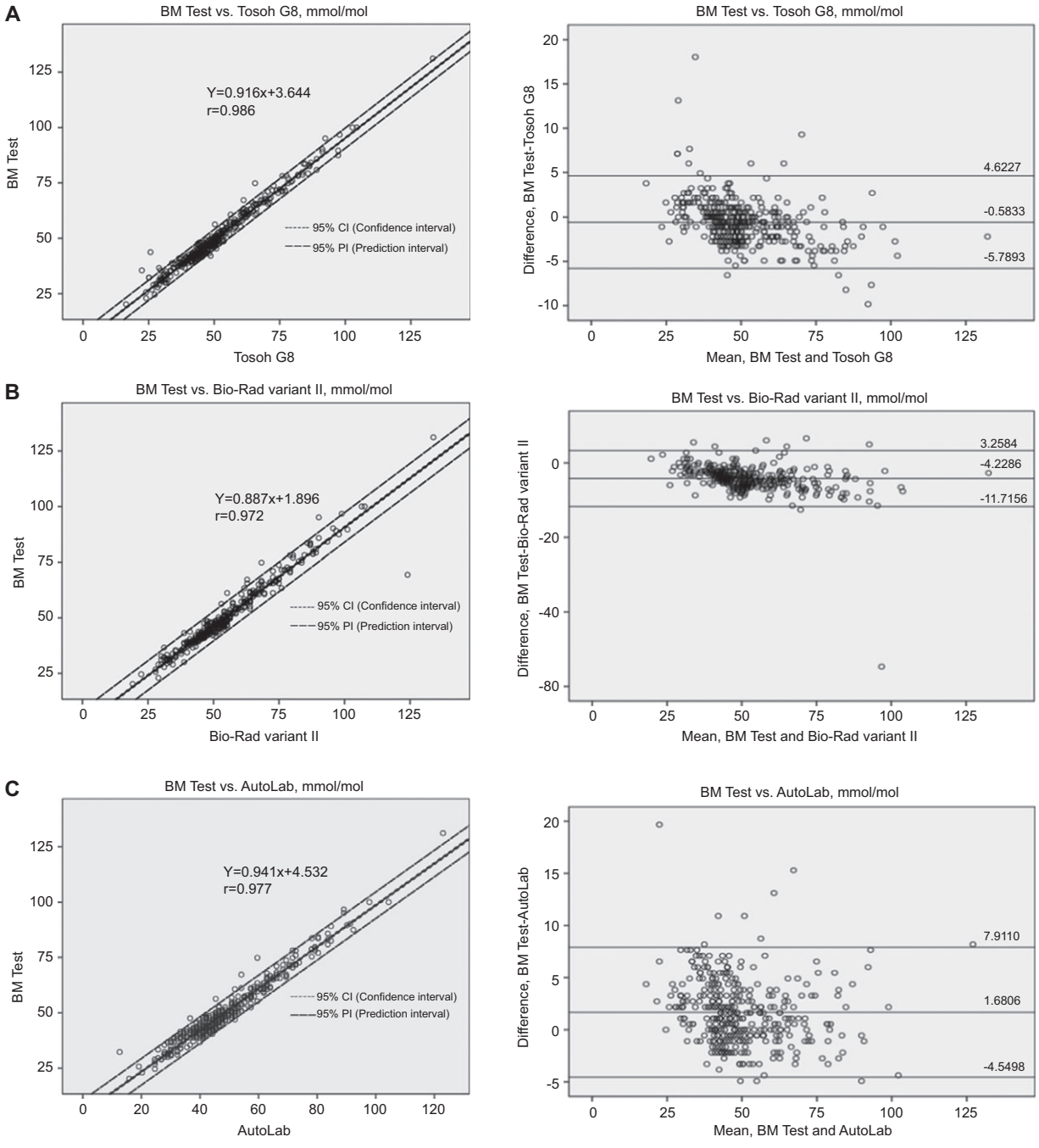
Parameter	Outcome	Sign statistic	p-Value
Intercept	0.020	5	0.0625
Slope	1.000	2	1.0000
% Carry-over	0.2	3	1.0000
Non-linearity	−0.0054	0	0.0625
Drift	0.0018	2	1.0000

Validation of simultaneously assayed glucose with the BM Test HbA_{1c} using the JCA-BM6010/C analyzer

The total CVs of the low and high simultaneous measurements of plasma glucose were 1.0% and 2.0%, respectively, and they showed a remarkable correlation with the routine plasma glucose assay (linear regression, y=1.002x+0.625; r=1.00; p<0.0001).

Comparison between the BM Test HbA_{1c} using the JCA-BM6010/C and Tosoh HLC G8

The comparisons were performed using 377 samples. The relationship between the BM Test HbA_{1c} using the JCA-BM6010/C and Tosoh HLC G8 analyzers was BM=0.916×Tosoh G8+3.644, r=0.986 (p<0.001), and the concordance rate using a cut-off of 48 mmol/mol was 91.5% (95% CI 88.6%–94.5%) (Table 3).



(**Figure 2** Continued)

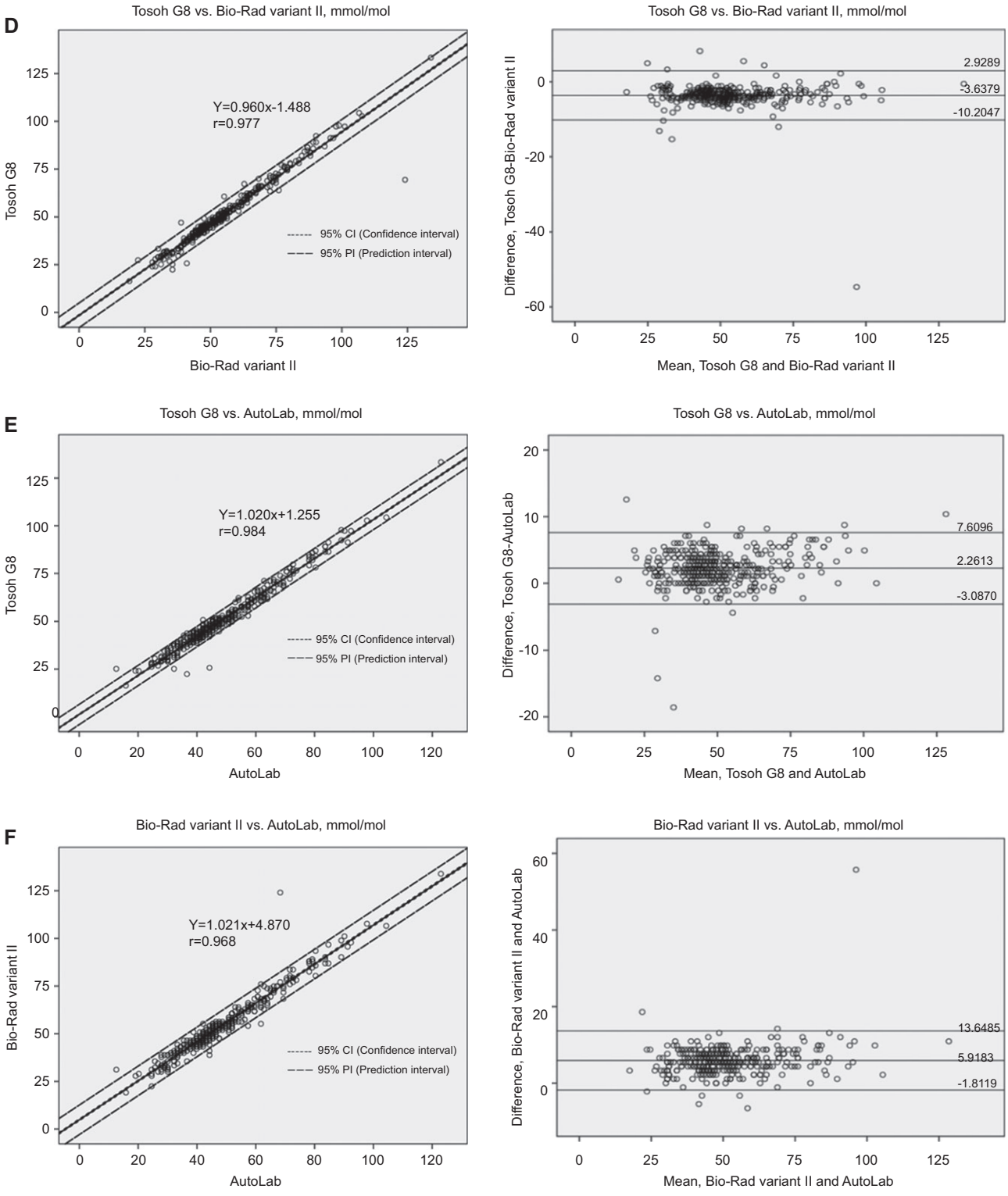


Figure 2 Linear regression with Passing-Bablok fit and Bland-Altman plot of the comparison study for HbA_{1c}; between the BM Test HbA_{1c} using JCA-BM6010/C and Tosoh HLC G8 (A), Bio-Rad Variant II Turbo (B), and AutoLab HbA_{1c} (C), between Tosoh HLC G8 and Bio-Rad Variant II Turbo (D), and AutoLab HbA_{1c} (E), between Bio-Rad Variant II Turbo and AutoLab HbA_{1c} (F).
Dotted lines in the Bland-Altman plots show the mean difference and mean difference±1.96 SD, respectively.

Table 3 Concordance rates using a cut-off of 48 mmol/mol HbA_{1c} concentration.

	HbA_{1c} at HbA_{1c} of 48 mmol/mol	Difference	95% CL	95% PL	Concordance rate (%) (95% CI)
BM Test with Tosoh G8	47.6	−0.4	(47.4, 47.9)	(43.0, 52.2)	91.5 (88.6–94.5)
BM Test with Variant II	44.5	−3.5	(44.1, 44.8)	(37.8, 51.1)	82.8 (78.6–87.0)
BM Test with AutoLab	49.7	1.7	(49.4, 50.0)	(43.7, 55.7)	91.0 (88.0–94.0)
Tosoh G8 with Variant II	44.6	−3.4	(44.2, 45.0)	(38.1, 51.1)	87.5 (84.0–91.1)
Tosoh G8 with AutoLab	50.2	2.2	(49.9, 50.5)	(44.9, 55.5)	89.9 (86.7–93.1)
Variant II with AutoLab	53.9	5.9	(53.5, 54.3)	(46.1, 61.6)	78.0 (73.3–82.7)

CL, confidence limits of the expected value at 48 mmol/mol; PL, prediction limits of the predicted value at 48 mmol/mol.

Comparison between the BM Test HbA_{1c} using the JCA-BM6010/C and Bio-Rad Variant II Turbo

The comparisons were performed using 377 samples. The relationship between the BM Test HbA_{1c} using the JCA-BM6010/C and Bio-Rad Variant II Turbo analyzers was BM=0.887×Bio-Rad Variant II+1.896, r=0.972 (p<0.001), and the concordance rate using a cut-off of 48 mmol/mol was 82.8% (95% CI 78.6%–87.0%).

Comparison between the BM Test HbA_{1c} using the JCA-BM6010/C and AutoLab HbA_{1c}

The comparisons were performed using 377 samples. The relationship between the BM Test HbA_{1c} using the JCA-BM6010/C and AutoLab HbA_{1c} was BM=0.941×AutoLab+4.532, r=0.977 (p<0.001), and the concordance rate using a cut-off of 48 mmol/mol was 91.0% (95% CI 88.0%–94.0%).

Comparison between Tosoh HLC G8 and Bio-Rad Variant II Turbo

The comparisons were performed using 377 samples. The relationship between the Tosoh HLC G8 and Bio-Rad Variant II Turbo analyzers was TosohG8=0.960×Variant II–1.488, r=0.977 (p<0.001), and the concordance rate using a cut-off of 48 mmol/mol was 87.5% (95% CI 84.0%–91.1%).

Comparison between Tosoh HLC G8 and AutoLab HbA_{1c}

The comparisons were performed using 377 samples. The relationship between the Tosoh HLC G8 and AutoLab HbA_{1c} analyzers was TosohG8=1.020×AutoLab+1.255, r=0.984 (p<0.001), and the concordance rate using a cut-off of 48 mmol/mol was 89.9% (95% CI 86.7%–93.1%).

Comparison between Bio-Rad Variant II Turbo and AutoLab HbA_{1c}

The comparisons were performed using 377 samples. The relationship between the Bio-Rad Variant II Turbo and AutoLab HbA_{1c} was Variant II=1.021×AutoLab+4.870, r=0.968 (p<0.001), and the concordance rate using a cut-off of 48 mmol/mol was 78.0% (95% CI 73.3%–82.7%).

Interferences

We investigated four clinically important potential interferences. Interference of hematocrit was performed by adding or removing plasma in order to obtain hematocrit between 10% and 67%. Interference of glucose, triglycerides and bilirubin was assessed by overloading a patient sample in order to obtain a final concentration of 33.3 mmol/L for glucose, 4.75 mmol/L for triglycerides and 414.8 µmol/L for bilirubin. No interferences were evident.

Discussion

Currently, the methods used for HbA_{1c} are certified traceability of manufacturers to the IFCC reference measurement procedure by IFCC and to the DCCT reference method by NGSP [10, 15]. The enzymatic assay relies on the specific cleavage of the hemoglobin, digested with a specific protease to generate fructosyl amino acid, in a whole blood sample matrix. Several types of fructosyl amino acid oxidase enzymes have been reported that are potentially useful for determining glycated hemoglobin [16, 17]. The enzymatic method is not interfered with by hemoglobin

variants (HbD, HbE, HbC, HbS, HbF), hematocrit, sodium cyanate, acetylsalicylic acid, acetaldehyde, glucose, bilirubin, albumin, ascorbic acid, uric acid, chyle, and intralipos according to other reports [9, 18]. We did not investigate the interference from hemoglobin variants.

The novel enzymatic HbA_{1c} assay analyzes the HbA_{1c} in whole blood and simultaneously the plasma chemistry analytes, including glucose in one sample, which is a unique feature of the JCA-BM6010/C automatic analyzer. We can use the EDTA sample for HbA_{1c}, glucose and other chemistry tests when the turnaround time is quick enough for the glucose test without a significant decrease.

The performance characteristics of the new enzymatic HbA_{1c} assay were precise (total CVs for low and high levels of controls were 1.2% and 0.7%, respectively). This enzymatic HbA_{1c} study satisfied the recommendation of an intralaboratory CV <2% [19]. Simultaneously assayed plasma glucose analysis correlated closely with the routine plasma glucose test method using the JCA-BM6010/C analyzer. In the HbA_{1c} comparison study, there were differences in the HbA_{1c} concentration of 48 mmol/mol among the four methods. There was a 3.5 mmol/mol difference at the 48 mmol/mol between the BM Test HbA_{1c} and Bio-Rad Variant II Turbo and a 3.4 mmol/mol difference between the Bio-Rad Variant II Turbo and Tosoh G8, although those methods had good precision (total CVs <2%). A difference of 5.9 mmol/mol between the Bio-Rad Variant II Turbo and AutoLab HbA_{1c} is unacceptable for one method to replace the other. There are several factors that interfere with HbA_{1c} assays. Genetic variants (e.g., HbS trait, HbC trait), elevated fetal hemoglobin (HbF) and chemically modified derivatives of hemoglobin (e.g., carbamylated Hb in patients with renal failure) can affect the accuracy of HbA_{1c} measurements. The effects vary depending on the specific Hb variant or derivative and the specific HbA_{1c} method [20]. If we could exclude the specimens with possible interferences, the concordance rates between each method would show a higher correlation.

In conclusion, the new enzymatic BM Test HbA_{1c} using the JCA-BM6010/C analyzer, which has good performance characteristics, has the advantages of rapid reporting and the simultaneous measurement of plasma glucose from the same sample tube is convenient for monitoring the glycemic status of diabetes patients.

Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article. Research funding played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.
Research funding: This study was supported by a grant from Alere Healthcare Inc., Seoul, Korea.
Employment or leadership: None declared.
Honorarium: None declared.

Received March 30, 2013; accepted July 2, 2013

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