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SHORT COMMUNICATION

Performance Evaluation of the Bio-Rad D-100 System for Hemoglobin A1c Assay

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SUMMARY

Background: **Recently, the Bio-Rad D-100 system (Bio-Rad Laboratories) for hemoglobin A1c (HbA1c) assay was introduced.**
Methods: **We evaluated the precision, linearity, and method comparison of the D-100 system. The results of HbA1c from the D-100 were compared with that of the Variant II Turbo 2.0 (Bio-Rad) and G8 variant mode (Tosoh Bioscience). An additional 17 variant hemoglobin samples were compared to the immunoassay (Integra 800) results.**
Results: **The within-laboratory imprecision coefficient of variation was 1.28 - 1.58% for the control materials and 1.01 - 1.03% for the patient samples. The linearity was confirmed ranging from 3.6 to 19.2% in NGSP units. The mean difference (NGSP units) was as follows: D-100 vs. G8, -0.02%; D-100 vs. Variant II Turbo 2.0, 0.07%. There were no clinically significant differences in the HbA1c results in the 17 hemoglobin variants between the D-100 and immunoassay.**
Conclusions: **The analytical performance of the Bio-Rad D-100 system for HbA1c assay is clinically acceptable.**
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INTRODUCTION

The hemoglobin A1c (HbA1c) is a representative test used for the diagnosis and monitoring of diabetic patients [1-3]. An accurate and precise HbA1c assay is essential for evidence-based medical decision making in clinical practice. Efforts to standardize HbA1c tests have reduced bias and inter-laboratory variability, and technological advances in instruments and reagents have led to accurate and precise HbA1c assays by routine methods [4-7]. Most clinical laboratories use automated high-performance liquid chromatography (HPLC) or immunoassay as routine methods for the measurement of HbA1c levels due to convenience and turnaround time.
Recently, the Bio-Rad D-100 system (Bio-Rad Laboratories, USA) for hemoglobin A1c assay was introduced.

Several studies have addressed the performance of the D-100 system [8-13]. They evaluated the analytical performance of the D-100 system and have reported the clinical impact of rare hemoglobin variants. However, the comparison data between D-100 system and Variant II Turbo 2.0 (Bio-Rad Laboratories) or Tosoh G8 variant mode (Tosoh Bioscience, Japan) was limited. Also, there was little data available about the flag sign of hemoglobin variants on the routine HbA1c analyzer.
In this study, we evaluated the analytical performance of the D-100 system for HbA1c assay, and we investigated the clinical impact of the hemoglobin variants on routine HPLC HbA1c analyzers.

MATERIALS AND METHODS

The institutional review board of Ewha Womans University Mokdong Hospital approved the study (EUMC 2016-04-032).

Precision

The precision of the D-100 system was evaluated according to the CLSI document EP15-A3 [14]. Two levels of control solutions (Lyphochek diabetes control, Bio-Rad Laboratories) and patient venous blood samples anticoagulated with ethylenediaminetetraacetic acid (EDTA) were used. Fresh venous whole blood samples were aliquoted and stored at -70°C before measurement. The imprecision estimates were analyzed in both National Glycohemoglobin Standardization Program (NGSP) units (%) and International Federation of Clinical Chemistry (IFCC) units (mmol/mol). In this study, the acceptable criterion for the within-laboratory imprecision coefficient of variation (% CV) was 2.0% in NGSP units and 3.0% in IFCC units [15].

Linearity

The Lyphochek Hemoglobin A1c Linearity Set (Bio-Rad Laboratories) and patient venous blood samples anticoagulated with EDTA were used to evaluate the linearity of the D-100 according to the CLSI document EP06-A [16]. The six concentrations of the Linearity Set were measured twice per run, and the five levels of patient samples were measured four times per run. We used polynomial regression analysis for linearity evaluation. The allowable bias for nonlinearity was within ± 6.0% of each HbA1c level.

Method comparison

The method comparison of the D-100 system was evaluated according to the CLSI document EP09-A3 [17] using patient samples. The HbA1c concentration of 40 samples ranged from 4.7% - 16.0% in NGSP units (< 6%, n = 19; 6.0 - 7.9%, n = 33; 8.0 - 9.9%, n = 16; and ≥ 10%, n = 12). All venous blood samples anticoagulated with EDTA were analyzed immediately after blood draw. The duplicated results from the D-100 system were compared with those of the Variant II Turbo 2.0 and Tosoh G8 variant mode.

Hemoglobin variants

Seventeen venous blood samples anticoagulated with EDTA that revealed a flag sign on the Variant II Turbo 2.0 or G8 analyzer were collected. All samples were stored below -70°C before measurement. The variant hemoglobin samples were simultaneously measured by three HPLC methods (D-100, Variant II Turbo 2.0, and G8 variant mode) and one immunoassay (Cobas Integra 800, Roche Diagnostics, Switzerland). In addition, the type of variant hemoglobin was determined by capillary zone electrophoresis (Capillarys 2 Flex-Piercing, Sebia, France). Immunoassay measurements without interfering factors such as Hb S, C, E, and D variant hemoglobin were used for comparison [18]. The bias (HPLC-immunoassay) was calculated and analyzed according to capillary zone electrophoresis findings and the pattern of chromatogram on the HPLC instrument.

RESULTS

Precision

The repeatability (% CV) of the control material was 1.18% - 1.56% and that of patient sample was 1.01% - 1.03% (Table 1). The within-laboratory imprecision was 1.28% - 1.58% for the control materials and 1.01% - 1.03% for the patient samples (Table 1). Between-day imprecision was zero for patient samples. The precision estimates in IFCC units are shown in Table 1.

Linearity

In the polynomial regression, the best fit line was linear within the range of 3.6% - 19.2% for control solutions and 3.1% - 16.1% for patient samples in NGSP units. In the control solution, the linear regression equation of the corresponding interval was $y = 1.021 x +0.0232$, and the coefficient of determination (R^2) was 1,000. In patient samples, the linear regression equation was $y = 0.9993 x +0.0071$, and the R^2 was 0.9993.

Method comparison

The linear equation between D-100 and G8 was $y = 1.0167 x -0.1233$ ($r = 0.997$), and the mean bias was -0.02% in NGSP units (Figure 1A and 1B). Similarly, the linear equation between D-100 and Variant II Turbo 2.0 was $y = 0.9946 x +0.1162$ ($r = 0.997$) and the mean bias was 0.07% in NGSP units (Figure 1C and 1D).

Variant hemoglobin

Capillary zone electrophoresis of 17 variant Hb specimens revealed 15 HbD traits, 1 HbE trait, and 1 HbS trait. The instrument flag sign was reported in all specimens on the D-100, while only for 15 of 17 specimens on both the Variant II Turbo 2.0 and G8. The HbA1c results of each variant Hb specimen, the equipment flag sign type, and the Hb EP results are described in detail in Supplementary Table S1. The mean ± SD of bias

Table 1. Precision of the D-100 HbA1c assay.

Level	Control solutions					Patient samples				
	Mean	Repeatability		Within-laboratory imprecision		Mean	Repeatability		Within-laboratory imprecision	
		SD	% CV	SD	% CV		SD	% CV	SD	% CV
NGSP units (%)										
1	5.34	0.06	1.18	0.07	1.28	5.60	0.06	1.01	0.06	1.01
2	10.01	0.16	1.56	0.16	1.58	10.61	0.11	1.03	0.11	1.03
IFCC units (mmol/mol)										
1	34.87	0.70	1.99	0.75	2.15	37.65	0.62	1.65	0.62	1.65
2	85.92	1.70	1.99	1.72	2.01	92.51	1.20	1.30	1.20	1.30

Abbreviations: CV - coefficient of variation, IFCC - International Federation of Clinical Chemistry and Laboratory Medicine, NGSP - National Glycohemoglobin Standardization Program, SD - standard deviation.

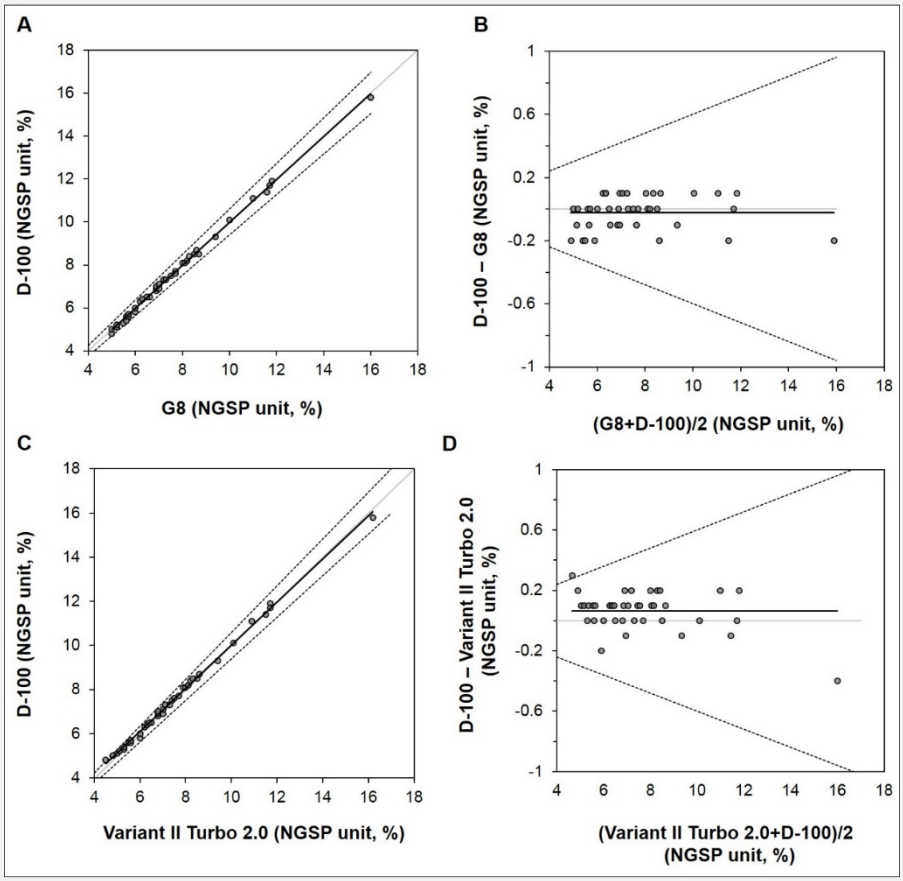

Figure 1. Comparison between Bio-Rad D-100, Variant II turbo 2.0, and Tosoh G8 hemoglobin A1c analyzers.

(A) Scatter and (B) Bland-Altman difference plots between D-100 and G8. (C) Scatter and (D) Bland-Altman difference plots between D-100 and Variant II Turbo 2.0. In the scatter plot, the solid black line represents the best fit linear line, and the dotted line depicts the allowable difference of ± 6.0%. In the Bland-Altman difference plot, the solid black line represents the mean difference, and the dotted line depicts the allowable difference of ± 6.0%.

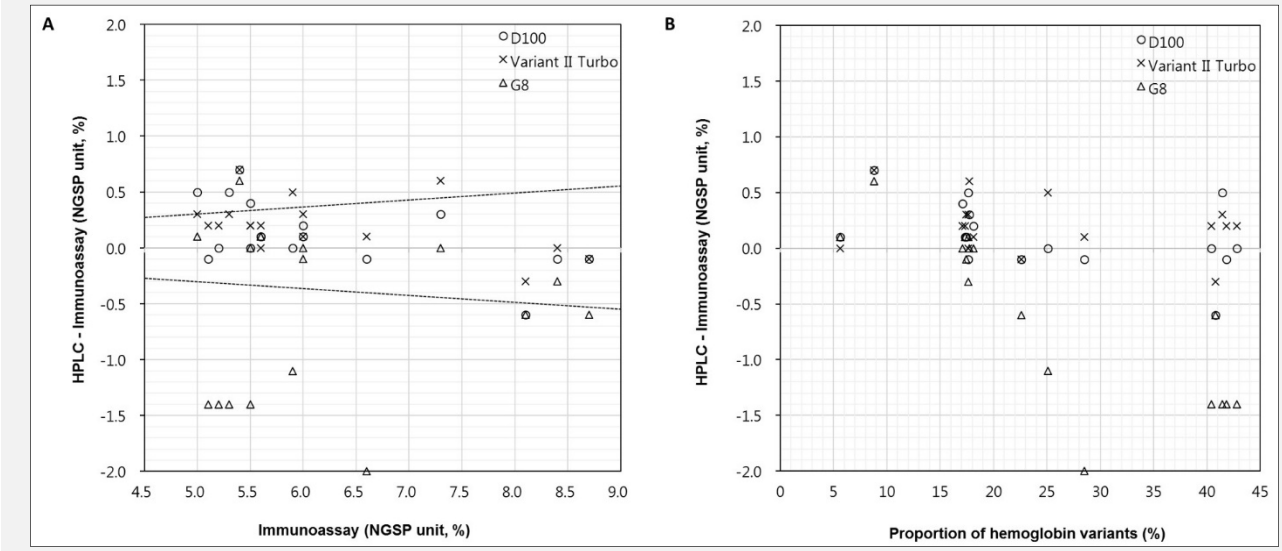

Figure 2. Differences between the immunoassay and each HPLC method for hemoglobin A1c assay in variant hemoglobin samples.

Differences depending on (A) immunoassay results and (B) the percentage of variant hemoglobin by capillary electrophoresis analysis. The dotted line represents the allowable difference of ± 6.0%. The proportion of hemoglobin variants represents 100 x variant hemoglobin/total hemoglobin (%).

(HPLC-Immunoassay; NGSP unit) of 15 samples showing the HbD trait on capillary zone electrophoresis was 0.12% ± 0.33% for D-100, 0.20% ± 0.24% for the Variant II Turbo 2.0, and 0.56% ± 0.77% for the G8. Five samples on the D-100, 4 samples on the Variant II Turbo, and 8 samples on the G8 had a percentage bias of ± 6.0% or more between the immunoassay and the three HPLC methods (Figure 2). The mean bias (NGSP units) analyzed by dividing two groups based on the proportion of variant Hb (100 x variant Hb/Total Hb; < 25% vs. ≥ 25%) in the capillary zone electrophoresis findings were: D-100, 0.22 vs. -0.04, $p = 0.08$; Variant II Turbo 2.0, -0.23 vs. -0.17, $p = 0.644$; and G8, 0.01 vs. 1.33, $p < 0.001$. Example of chromatograms in variant hemoglobin samples depending on the type of HPLC analyzer are shown in Supplementary Figure S2. One example showed a variant peak on the chromatogram in all three methods, and the HbA1c value was 5.7% for the D-100, 5.8% for the Variant II Turbo 2.0, 5.7% for the G8, and 5.6% for the immunoassay (Figure S2A). On the other hand, one example showed a variant peak only on the D-100 and Variant II Turbo 2.0, but not on the G8, and the HbA1c value was 6.5% for the D-100, 6.7% for the Variant II Turbo 2.0, 4.5% for the G8, and 6.6% for the immunoassay (Figure S2B).

DISCUSSION

In this study, the within-laboratory imprecision (% CV) of the D-100 was similar to those reported in previous studies: 0.78% - 1.46% in NGSP units and 0.99% - 2.27% in IFCC units [8-10]. Our imprecision results satisfied the acceptance criteria for the HbA1c assay recommended by the American Society for Clinical Chemistry [19]. The linearity of the D-100 system was confirmed with a clinically significant concentration interval of HbA1c.

The D-100 results were 0.07% (NGSP units) higher than those of the Variant II Turbo 2.0, and the percentage difference between the two methods ranged from -3.3% to 6.7%; 79 of 80 samples were within ± 6.0%. The D-100 result was lower than that of the G8 by -0.02% (NGSP units), and all samples showed a percentage difference (range, -4.0% to 1.6%) within ± 6.0%, indicating that the difference in HbA1c between methods was clinically acceptable. Many clinicians have suggested that treatment regimens could be altered if successive HbA1c results change by more than 0.5% [6]. With our results, the differences in HbA1c level between the three HPLC devices were within ± 0.5% (NGSP unit) or 5 mmol/mol (IFCC units).

Although an immunoassay is not a reference measurement procedure for measuring the HbA1c concentra-

tion, the reagent used in the present study was the Tinaquant Hemoglobin A1c Gen. 2 (Roche Diagnostics), which is not significantly affected by variant hemoglobin including HbS, C, E, or D [18]. As the variant hemoglobin samples used in this study were identified as HbS, E, and D by capillary electrophoresis, we thought that the influence of variant hemoglobin on the immunoassay was negligible.

In this study, the difference between HPLC and immunoassay was significantly increased depending on the percentage of variant hemoglobin only in the G8 analyzer. Six samples, mostly in the G8, had a falsely decreased HbA1c of more than 1.0% (NGSP units) from the immunoassay. It is well documented that the same variant hemoglobin can show a falsely increased or decreased value depending on the method [20]. Unlike the D-100 and the Variant II Turbo 2.0, the fraction of variant hemoglobin in these 6 samples was not separated from the HbA0 peak in the G8; therefore, the concentration of HbA1c was underestimated. These 6 samples showed specific warning flags in both the D-100 and Variant II Turbo 2.0 instruments, while 2 samples showed no flags, and 4 specimens revealed a flag code of '07', indicating a 'number of theoretical plates' on the G8. The flag code of '07' on the G8 was generated when the shape of chromatogram was poor. Although the number of evaluated samples was small, the HbA1c results with the specific flag code '07' on the G8 might have a significant impact on HbA1c assay. Therefore, these samples should be confirmed with another instrument or other assay principles.

CONCLUSION

The performance of the Bio-Rad D-100 system for HbA1c assay is clinically acceptable. Variant hemoglobin is rare in clinical practice, but care should be taken to determine HbA1c values from some variant hemoglobin samples by routine HPLC-based HbA1c assay.

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None declared.

Declaration of Interest:
No competing financial interests exist.

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Supplementary Table and Figures.

Table S1. Hemoglobin A1c results and the corresponding flag signs of 17 variant hemoglobin samples.

Sample ID	G8 (variant mode)		Variant II Turbo 2.0		D-100		Immunoassay	Hb EP		Absolute difference (immunoassay-HPLC)		
	Flag	HbA1c (%)	Flag	HbA1c (%)	Flag	HbA1c (%)	HbA1c (%)	Results	Variant (%)	D-100	Variant II	G8
16113867811	H-V0 (variant D)	5.7	None	5.6	S-win-dow	5.7	5.6	Hb S trait	5.6	0.1	0.0	-0.1
16123839013	No flag	4.8	Variant win-dow	6.4	E-win-dow	5.9	5.9	Hb E trait	25.1	0.0	-0.5	1.1
15382077313	H-V1 (variant S)	6.0	No flag	6.1	S-win-dow	6.1	5.4	Hb D trait	8.8	0.7	-0.7	-0.6
15394873113	H-V1 (variant S)	5.5	Variant win-dow	5.7	S-win-dow	5.9	5.5	Hb D trait	17.1	0.4	-0.2	0
16119668113	H-V1 (variant S)	5.7	Variant win-dow	5.8	S-win-dow	5.7	5.6	Hb D trait	17.3	0.1	-0.2	-0.1
16106553111	H-V1 (variant S)	5.9	Variant win-dow	6.3	S-win-dow	6.1	6.0	Hb D trait	17.4	0.1	-0.3	0.1
16095584311	H-V1 (variant S)	8.1	Variant win-dow	8.4	S-win-dow	8.3	8.4	Hb D trait	17.6	-0.1	0.0	0.3
15373920113	H-V1 (variant S)	5.1	Variant win-dow	5.3	S-win-dow	5.5	5.0	Hb D trait	17.6	0.5	-0.3	-0.1
15346837613	H-V1 (variant S)	7.3	No flag	7.9	S-win-dow	7.6	7.3	Hb D trait	17.7	0.3	-0.6	0.0
16132563613	H-V1 (variant S)	6.0	Variant win-dow	6.1	S-win-dow	6.2	6.0	Hb D trait	18.1	0.2	-0.1	0.0
16122957211	H-V1 (variant S)	8.1	Variant win-dow	8.6	S-win-dow	8.6	8.7	Hb D trait	22.6	-0.1	0.1	0.6
15366445613	No flag	4.6	Variant win-dow	6.7	E-win-dow	6.5	6.6	Hb D trait	28.5	-0.1	-0.1	2.0
16107494313	7 (peak not good)	4.1	Variant win-dow	5.7	E-win-dow	5.5	5.5	Hb D trait	40.4	0.0	-0.2	1.4
16106053711	H-V2 (variant C)	7.5	Variant win-dow	7.8	Minor peak	7.5	8.1	Hb D trait	40.8	-0.6	0.3	0.6
16039380613	7 (peak not good)	3.9	Variant win-dow	5.6	E-win-dow	5.8	5.3	Hb D trait	41.4	0.5	-0.3	1.4
15382208413	7 (peak not good)	3.7	Variant win-dow	5.3	E-win-dow	5.0	5.1	Hb D trait	41.8	-0.1	-0.2	1.4
15358218713	7 (peak not good)	3.8	Variant win-dow	5.4	E-win-dow	5.2	5.2	Hb D trait	42.8	0.0	-0.2	1.4

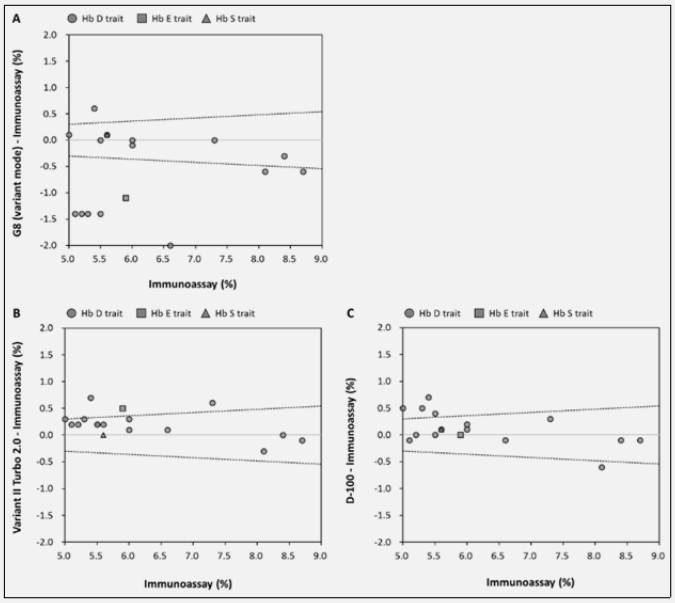

Figure S1. Differences between immunoassay and each HPLC method for hemoglobin A1c assay in variant hemoglobin samples.

The dotted line represents the allowable difference of ± 6.0%. Circle, hemoglobin D (Hb D) trait; square, hemoglobin E (Hb E) trait; triangle, hemoglobin S (Hb S) trait.

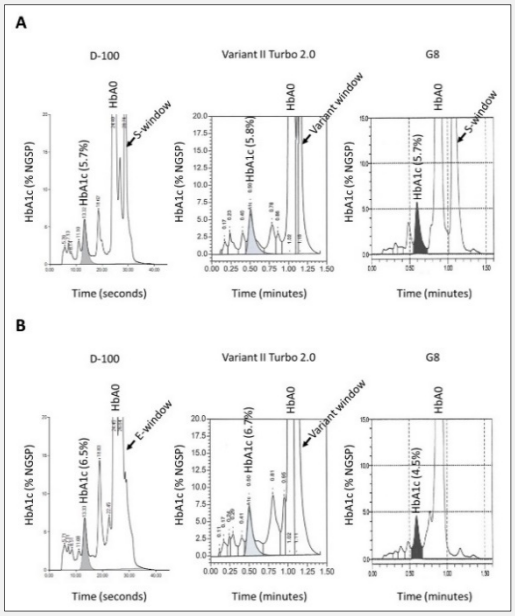

Figure S2. Sample chromatograms for the variant hemoglobin on the three HPLC methods.

(A) This sample shows a variant peak on the chromatogram in all three methods. HbA1c values were: D-100, 5.7%; Variant II Turbo 2.0, 5.8%; G8, 5.7%; and immunoassay, 5.6%. (B) This sample shows a variant peak in D-100 and Variant II Turbo 2.0, but not G8. HbA1c values were: D-100, 6.5%; Variant II Turbo 2.0, 6.7%; G8, 4.5%; and immunoassay, 6.6%.