Evaluation of the Bio-Rad D-100™ system for the measurement of glycated hemoglobin (HbA_{1c})

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ABSTRACT

Background: HbA_{1c}, the main form of glycated hemoglobin, is the gold standard for the monitoring of glycemic control in diabetic patients and has recently been recommended for the diagnosis of diabetes. HbA_{1c} levels also correlate with the development of long-term complications in diabetic patients. It is therefore essential that HbA_{1c} measurements be performed on robust and reliable methods. The aim of this study was to evaluate the D-100™ system (Bio-Rad Laboratories) for the accurate quantification of HbA_{1c}.
Methodology: Detection of HbA_{1c} in whole blood by the D-100 system is based on ion-exchange quantitative high performance liquid chromatography (HPLC) in a 45 second separation per sample. Precision was assessed for 24 days by measuring Bio-Rad quality control (QC) materials in addition to four patient samples, in duplicate, twice daily. Linearity and accuracy was assessed using proficiency testing (PT) material from the College of American Pathologists (CAP) or Institute for Quality Management in Healthcare (IQMH). Remnant samples after routine analysis were collected and utilized for comparative testing against the Bio-Rad VARIANT™ II Turbo. Interference from known hemoglobin variants (AC, n=55; AD, n=41; AE, n=43; AS, n=37) was assessed by comparing results to those obtained by the Trinity Biotech™ ultra² boronate affinity high performance liquid chromatography (HPLC) at a National Glycohemoglobin Standardization Program (NGSP) reference laboratory. An overall test of coincidence of least-squares regression lines was used to test for statistically significant differences compared to AA samples; clinical significance was defined as a relative difference exceeding ±7% versus AA samples at HbA_{1c} levels of 6 and 9 $\%HbA_{1c}$ based on Deming regression analysis.
Validation: The Bio-Rad Lyphochek and Liquicheck QC showed within run and total coefficient of variation (CV) of 0.8-1.0% and 0.9-1.1%, respectively. HbA_{1c} levels in patient samples ranging from 4.8 $\%HbA_{1c}$ to 12.1 $\%HbA_{1c}$ showed total CVs of 0.7–0.8%. Linearity over a measuring range of 5.10-11.17 % HbA_{1c} was acceptable with a slope of 0.947 and intercept of -0.06. PT sample results met CAP and IQMH criteria (allowable error of 6% and 7%, respectively). For method comparison, samples were selected to maximally cover the measuring range of the assay, 3.5 % HbA_{1c} to 20.0 % HbA_{1c}. One hundred samples were run in duplicate on the D-100 analyzer and compared to routine measurements on the Bio-Rad Variant II Turbo analyzer. Deming regression analysis showed R=0.9983, slope of 0.944 (0.937-0.952), y-intercept of 0.08 (0.03-0.14); the standard error of the estimate was 0.09 $\%HbA_{1c}$. Bias plots showed a mean difference of -0.3 % HbA_{1c} (95% CI: -0.5 – 0.0 % HbA_{1c}). The variant interference evaluation showed no clinically significant interferences for the four variants tested, although there were statistically significant differences for AE and AS ($p<0.05$). In addition, the D-100 Advisor software correctly provided the presumptive identification of the 176 known AS, AC, AD, and AE variants according to defined chromatographic time windows.
Conclusions: The Bio-Rad D-100 system is a robust, high-throughput method for the routine determination of HbA_{1c} in clinical laboratories.

BACKGROUND

What is HbA_{1c}?

- HbA_{1c}, the main form of glycated hemoglobin, is the gold standard for monitoring glycemic control in diabetic patients. Recently, it has been recommend for use as a diagnostic marker for type 2 diabetes mellitus.
- Tightly controlled blood glucose levels predict better outcome for patients and it is essential to have an accurate and reliable method for determining HbA_{1c} levels.
- HbA_{1c} results from the non-enzymatic, irreversible, binding of glucose to the N-terminal valine of the β-chain of hemoglobin.
- Methods used to measure HbA_{1c} include assays based on separation techniques (ion-exchange HPLC, boronate affinity HPLC or capillary electrophoresis) as well as enzymatic and immunoassays.

Bio-Rad D-100™ System

- Fully-integrated stand-alone workstation
- Separation using cation exchange HPLC
- Samples are pre-filtered prior to chromatography column (up to 10,000 tests)
- Minimum blood volume of 1 mL (lower volume requires manual dilution using 5 µL)
- Automated two-point calibration upon the installation of a new cartridge, which is stable on-board the D-100 for the lifetime of the cartridge (10,000 injections or 90 days).
- Once reconstituted by the system, Calibrator Pack is stable for 24 hours after initial use when stored at 2-8 °C
- Separates Hb fractions within 45 seconds; first result after 2 minutes 15 seconds with a throughput of 80 samples per hour
- HbA_{1c} is eluted just after a peak containing the labile fraction of HbA_{1c} (LA1c) and carbamylated Hb (cHb) and before HbA_0. HbA_{1c} result = $HbA_{1c}/(HbA_0 + HbA_{1c})$
- Results may be expressed in IFCC units (mmol/mol) and/or in NGSP units (%)

OBJECTIVE

- The aim of this study was to assess the analytical performance of the D-100 system for the routine determination of HbA_{1c} in a clinical laboratory setting.

MATERIALS AND METHODS

Precision - Coefficient of variation (CV) was assessed from mean HbA_{1c} values obtained from the D-100 system from replicate measurements of Bio-Rad Lyphochek Diabetes Control (Lot 33920), Bio-Rad Liquichek Diabetes Control (Lot 38540) and four patient samples for 24 days, in duplicate, twice daily.

Linearity - Assessment was completed with six levels from the CAP LN15-B 2017 CVL Survey measured in quadruplicate. Assigned values were 5.44 $\%HbA_{1c}$, 6.75 $\%HbA_{1c}$, 8.07 $\%HbA_{1c}$, 9.36 $\%HbA_{1c}$, 10.72 $\%HbA_{1c}$, and 12.07 $\%HbA_{1c}$. Accuracy was assessed using proficiency testing (PT) material from CAP GH5-C 2016 and IQMH CHEM 1609 and 1701 HB surveys measured in duplicate.

Method Comparison - Performed by selecting patient samples to maximally cover the measuring range of the assay, 3.5 $\%HbA_{1c}$ to 20.0 $\%HbA_{1c}$. One hundred samples were run in duplicate on the D-100 analyzer and compared to routine measurements on the Bio-Rad Variant II Turbo analyzer in the UHN Core Laboratory by Deming regression analysis and bias plots.

Interferences - Known hemoglobin variants AC, AD, AE, and AS, were assessed by comparing results to those obtained by the Trinity Biotech ultra² boronate affinity HPLC at a NGSP reference laboratory. An overall test of coincidence of least-squares regression lines was used to test for statistically significant differences compared to AA samples; clinical significance was defined as a relative difference exceeding ±7% versus AA samples at HbA_{1c} levels of 6 $\%HbA_{1c}$ and 9 $\%HbA_{1c}$ based on Deming regression.

RESULTS

Precision

	Level 1					Level 2				
Control Material	N	Mean ($\%HbA_{1c}$)		SD	CV (%)	n	Mean ($\%HbA_{1c}$)		SD	CV (%)
Lyphochek	96	5.39	*Within Run*	0.05	0.9	96	9.55	*Within Run*	0.08	0.8
			Between Day	0.02	0.5			*Between Day*	0.02	0.2
			Total Imprecision	0.05	1.0			*Total Imprecision*	0.08	0.9
Liquichek	92	5.38	*Within Run*	0.05	1.0	92	9.39	*Within Run*	0.08	0.8
			Between Day	0.02	0.4			*Between Day*	0.02	0.2
			Total Imprecision	0.06	1.1			*Total Imprecision*	0.08	0.9

Table 1. Precision for Bio-Rad Quality Control Material was run in duplicate, twice per day for 24 days (Lyphochek Diabetes QC) and 23 days (Liquichek Diabetes QC).

Patient Sample Mean HbA_{1c}	4.8 $\%HbA_{1c}$		6.6 $\%HbA_{1c}$		9.5 $\%HbA_{1c}$		12.1 $\%HbA_{1c}$	
Imprecision	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**
Within Run	0.03	0.5	0.05	0.8	0.07	0.7	0.09	0.7
Between Day	0.01	0.2	0.01	0.2	0.03	0.3	0.03	0.2
Total Imprecision	0.03	0.7	0.05	0.8	0.07	0.8	0.09	0.8

Table 2. Precision for Patient Samples run in duplicate, twice per day for 22 days (n=88).

Linearity and Calibration Verification

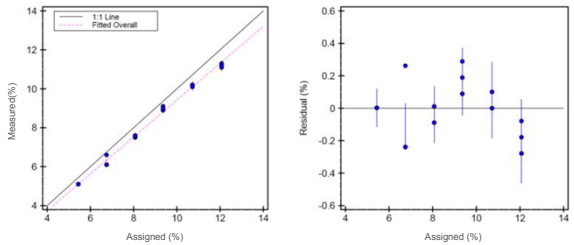

Figure 1. Linearity and Calibration Verification assessment of HbA_{1c} performed on the D-100 analyzer using CAP LN15-B 2016 survey material. The measuring range was 5.44% to 12.07 $\%HbA_{1c}$ as assigned by NGSP. Total allowable error was 6%. Linearity verification revealed slope = 0.947, y-intercept = -0.06 and maximum error of 1.8%. Each level was measured in four replicates.

RESULTS

Accuracy

Source	Survey	Vial	Assigned Value ($\%HbA_{1c}$)	D-100 Run 1	D-100 Run 2	Average % Difference
IQMH	CHEM 1609 HB	1	5.8	6.0	6.0	3.45
IQMH	CHEM 1609 HB	2	5.2	5.1	5.1	-1.92
IQMH	CHEM 1701 HB	2	5.2	5.3	5.2	0.96
CAP	GH5-C 2016	11	9.11	8.7	8.7	-4.50
CAP	GH5-C 2016	12	6.01	6.0	6.0	-0.17
CAP	GH5-C 2016	13	11.71	10.9	10.9	-6.92
CAP	GH5-C 2016	14	5.02	5.0	5.0	-0.40
CAP	GH5-C 2016	15	7.58	7.3	7.3	-3.69

Table 3. Accuracy assessment of HbA_{1c} measurement on the D-100 using eight vials from CAP and IQMH surveys. The measuring range was 5.0 to 11.7 $\%HbA_{1c}$. All samples met the IQMH TE_a criteria of 7%. All samples with the exception of one high sample (>10 $\%HbA_{1c}$) met the CAP TE_a criteria of 6%.

Method Comparison

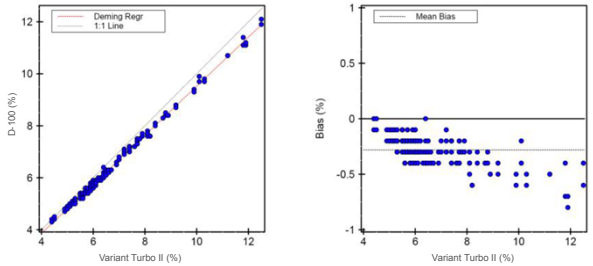

Figure 2. Method comparison of D-100 vs. Bio-Rad Variant II Turbo using 100 patient samples measured in duplicate. Scatter plot (left) shows the 1:1 (dashed line) and Deming regression (red line). Bias plot (right) shows mean bias (dotted line). Statistics include: slope = 0.944; y-intercept = 0.08; correlation coefficient = 0.983; mean bias = -0.3 $\%HbA_{1c}$.

Variant Interference Evaluation

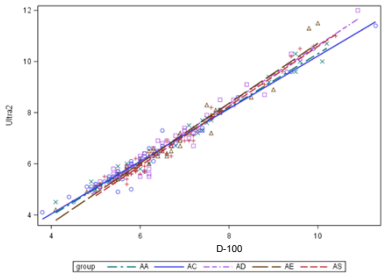

Figure 3. Evaluation of interference from 176 known hemoglobin variants measured on the Trinity Biotech Ultra² boronate affinity HPLC vs. the D-100 system. There were no clinically significant interferences for the four variants tested (AC, n=55; AD, n=41; AE, n=43; AS, n=37), although there were statistically significant differences for AE and AS ($p<0.05$). In addition, the D-100 Advisor software correctly provided the presumptive identification of all 176 known AS, AC, AD, and AE variants according to defined chromatographic time windows.

CONCLUSIONS

- The Bio-Rad D-100 system performed well and met the evaluation criteria for the measurement of HbA_{1c} using both quality control materials (Bio-Rad Lyphochek and Liquichek) and patient samples. The D-100 met linearity and accuracy assessments using survey materials from IQMH and CAP.
- Method comparison showed a mean negative bias of 0.3 $\%HbA_{1c}$ when compared to the Core Lab Bio-Rad Variant II Turbo. The variant interference evaluation showed no clinically significant interferences for the four variants tested (AC, AD, AE and AS).
- The Bio-Rad D-100 system is a robust, high-throughput method for the routine determination of HbA_{1c} in clinical laboratories.