Evaluation of the Bio-Rad D-100 Hemoglobin Testing System

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Background

The D-100 is a high throughput automated test system used to quantify hemoglobin A1c (A1c) in whole blood samples. The D-100 uses High Performance Liquid Chromatography (HPLC) to separate hemoglobin fractions and obtain an HbA1c result that is expressed in NGSP or IFCC units. The D-100 also utilizes a bi-directional LIS interface and offers the capability of automatically reviewing (flagging or releasing) the HbA1c results on the system.

Objective

The purpose of this study is to verify and validate the manufacturer's claims of 10,000 tests (injections) per analytical cartridge with prefilter change every 2,000 tests on a single calibration. This testing included precision and accuracy checks, sample throughput, hemoglobin variant detection with no interference from heterozygous hemoglobins S, C, D, E and F up to 30%, and workflow studies.

Methods

Beginning January 4, 2017 a new analytical cartridge and prefilter were installed on a D-100. Calibration and QC were run per guidelines. During the 10,000 test sample run study, 4 patient EDTA whole blood samples at ~ 5, 6.5, 8 and 12% HbA1c were selected and run as precision samples in duplicates, twice a day for 10 days. Additionally, 40 samples whose NGSP values were pre-assigned by SRL (Secondary Research Laboratory) were run in duplicate along with a linearity kit and 2 levels of diabetes controls. The HbA1c values from D-100 were compared to the NGSP values. Workflow timing was evaluated on 2 separate daily runs from start to finish. Hemoglobin variants were compared to gel electrophoresis.

Results

The analytical cartridge performed flawlessly over 30 days for 10,000 tests without recalibration, loss of precision or abnormal chromatography. Total precision calculations for the 4 precision samples were 0.86, 0.73, 0.87, and 0.86%. The sample throughput was confirmed to be 45 seconds per sample. The "time to first result" was confirmed to be 2 minutes 15 seconds, where the 45 second assay of the first sample in the run follows a 1 minute 30 second system flush. Each successive sample of the run is 45 seconds.

Conclusion

The performance of the Bio-Rad D-100 met all of the manufacturer's stated claims. With the Onboard Advisor built-in result review criteria and the bi-directional LIS interface, >90% of our patient samples are run and verified with no technician intervention.

BIO-RAD

Introduction

With growing HbA1c testing needs in our laboratory we wanted a platform that would address the HbA1c testing volumes that are expected to grow over the next few years but at the same time not make more work for our already over-extended staff. The D-100 Hemoglobin Testing System seemed to be an ideal solution for our laboratory because of faster throughput. We believe that HPLC is the gold standard when it comes to HbA1c testing. We needed a platform that provides an HbA1c value without compromising the variant hemoglobin information.

With growing diversity in the testing population we believe that it is important to know if there is a variant hemoglobin in the sample. Ion-exchange HPLC provides that information. With the global trend of using HbA1c to aid in the diagnosis of diabetes mellitus, it is more important than ever to use assays that include the detection of hemoglobin variants. In the absence of hemoglobin variant detection some HbA1c methods might lead to misdiagnosis and unnecessary or delayed patient treatment. We evaluated the D-100 to see if it meets our needs.

Materials and Methods

Materials

D-100 Hemoglobin Testing System, analytical cartridge – 1, Prefilters – 5, Buffers, Calibrators and Bio-Rad HbA1c Quality Control.

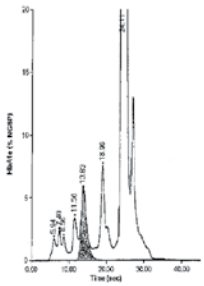
Method

The D-100 was installed with a fresh analytical cartridge and a new prefilter. Calibration and QC were run per guidelines. During the 10,000 test sample run study, 4 patient EDTA whole blood samples at ~ 5, 6.5, 8 and 12% HbA1c were selected and run as precision samples in duplicate, twice a day for 10 days. Additionally, 40 samples whose NGSP values were pre-assigned by SRL (Secondary Research Laboratory) were run in duplicate along with a linearity kit and 2 levels of diabetes controls. The HbA1c values from D-100 were compared to the NGSP values. Workflow timing was evaluated on 2 separate daily runs from start to finish. Hemoglobin variants were compared to gel electrophoresis.

Results

The analytical cartridge performed flawlessly over 30 days for 10,000 tests without recalibration, loss of precision or abnormal chromatography. Figure 1a shows the chromatography at the start of the cartridge and Figure 1b shows the chromatography at the end of the same cartridge at 10,000 injections. The chromatography remained uncompromised through the lifetime of the cartridge.

Fig. 1a. D-100 Chromatogram at the Start of the Cartridge

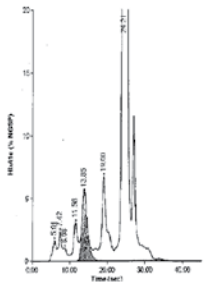


Peak Name	RT	Area	Area %	Concentration (% NGSP)
A1a	5.94	2319.74	0.91	–
A1b	7.40	2388.85	0.94	–
F	8.56	2299.35	0.90	–
LA1c	11.56	5305.23	2.08	–
HbA1c	13.83	10767.87	–	5.7
P3	18.96	14496.18	5.68	–
A0	24.11	217723.48	85.28	–

Total Area: 255301

Status: **Released**

Fig. 1b. D-100 Chromatogram at the End of the Cartridge



Peak Name	RT	Area	Area %	Concentration (% NGSP)
A1a	5.91	1731.18	0.82	–
A1b	7.42	2169.10	1.03	–
F	8.68	1065.31	0.50	–
LA1c	11.56	3962.42	1.87	–
HbA1c	13.85	8573.25	–	5.5
P3	19.00	11007.84	5.20	–
A0	24.21	183095.49	86.53	–

Total Area: 211605

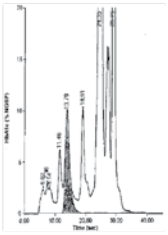
Status: **Released**

Figures 2a, 2b, 2c, 2d and 2e show the chromatograms of patient samples with the 4 most commonly encountered variant hemoglobin traits HbS, C, D, E, and HbF. Each variant hemoglobin has a dedicated window in the table adjacent to the chromatograms. The variant concentrations on the chromatograms were compared with electrophoresis results for the variants (Figure 2). We performed the screening with an alkaline gel and confirmed with acid gels on the Helena SPIFE Touch system. Electrophoresis results validate the variants seen on the chromatogram.

Fig. 2 Electrophoresis results for the variant hemoglobins

HbS	Hemoglobin A = 60.6% Hemoglobin S = 36.6% Hemoglobin A2 = 2.8%	**HbC**	Hemoglobin A = 63.9% Hemoglobin C = 36.1%	**HbD**	Hemoglobin A = 61.6% Hemoglobin D = 36.6% Hemoglobin A2 = 1.8%	**HbE**	Hemoglobin A = 70.2% Hemoglobin E = 29.8%

Fig. 2a. D-100 Chromatogram showing HbA1c results in presence of HbS trait

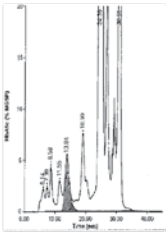


Peak Name	RT	Area	Area %	Concentration (% NGSP)
Unknown	6.02	1422.88	1.07	–
A1b	7.36	1105.20	0.83	–
F	8.14	1299.90	0.98	–
LA1c	11.46	3424.41	2.58	–
HbA1c	13.79	6798.59	–	9.7
P3	18.91	6476.59	4.87	–
A0	24.35	65931.47	49.58	–
S-Window	28.75	46524.59	34.99	–

Total Area: 132984

Status: **Released**

Fig. 2b. D-100 Chromatogram showing HbA1c results in presence of HbC trait

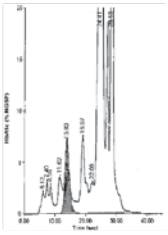


Peak Name	RT	Area	Area %	Concentration (% NGSP)
Unknown	6.14	1189.35	0.88	–
A1b	7.39	841.78	0.62	–
F	8.58	1894.50	1.40	–
LA1c	11.55	1743.43	1.29	–
HbA1c	13.84	3694.17	–	5.3
P3	18.99	5523.63	4.09	–
A0	24.39	80963.11	59.91	–
C-Window	30.61	39288.46	29.07	–

Total Area: 135138

Status: **Released**

Fig. 2c. D-100 Chromatogram showing HbA1c results in presence of HbD trait

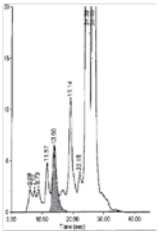


Peak Name	RT	Area	Area %	Concentration (% NGSP)
Unknown	6.12	1130.41	0.74	–
A1b	7.40	1246.76	0.81	–
F	8.56	1427.78	0.93	–
LA1c	11.62	2287.01	1.49	–
HbA1c	13.83	5327.70	–	6.9
P3	18.97	5605.54	3.65	–
A0	24.41	81811.21	53.22	–
D-Window	28.13	53040.06	34.51	–

Total Area: 153712

Status: **Released**

Fig. 2d. D-100 Chromatogram showing HbA1c results in presence of HbE trait



Peak Name	RT	Area	Area %	Concentration (% NGSP)
Unknown	6.08	1683.48	0.94	–
A1b	7.51	1096.64	0.61	–
F	8.73	1966.11	1.10	–
LA1c	11.57	3544.74	1.98	–
HbA1c	13.90	5920.48	–	6.2
P3	19.14	11310.29	6.32	–
A0	24.39	97779.11	54.66	–
E-Window	26.40	52786.55	29.51	–

Total Area: 178872

Status: **Released**

Figure 2e shows the chromatogram obtained from a patient sample with HbF concentrations of 17.4%. The D-100 claims reportability of HbA1c in presence of HbF as high as 30%.

Fig. 2e. D-100 Chromatogram showing HbA1c results in presence of HbF

Peak Name	RT	Area	Area %	Concentration (% NGSP)
Unknown	6.22	8776.03	4.75	–
A1b	7.38	2010.55	1.09	–
F	8.60	32208.61	17.44	–
LA1c	11.34	6351.84	3.44	–
HbA1c	13.80	8527.85	–	6.8
P3	19.01	9693.90	5.25	–
A0	24.25	117157.18	63.42	–

Total Area: 184726

Status: **Released**

Table 1 shows the total precision. The samples were selected over a clinically broad range of HbA1c values. The precision for the 4 samples were 0.86, 0.73, 0.87, and 0.86%.

Table 1. Precision

Variation Source	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4
Concentration HbA1c (NGSP%)	**5.38%**	**9.71%**	**5.02%**	**6.51%**	**8.03%**	**12.48%**
Repeatability	**0.78**	**0.80**	**0.77**	**0.64**	**0.86**	**0.54**
Betwen-Run	**0.29**	**0.65**	**0.00**	**0.49**	**0.44**	**0.46**
Between-Day	**0.34**	**0.20**	**0.37**	**0.00**	**0.46**	**0.49**
Total Precision	**0.90**	**1.05**	**.086**	**0.73**	**0.87**	**0.86**

A set of samples were run and the HbA1c values were compared with the NGSP values assigned to the samples. Table 2 shows the %Bias when compared to value assignments ranged from -0.01% to -0.38%. The %CV for all samples were within 1% which is an excellent CV.

Table 2. Total Error

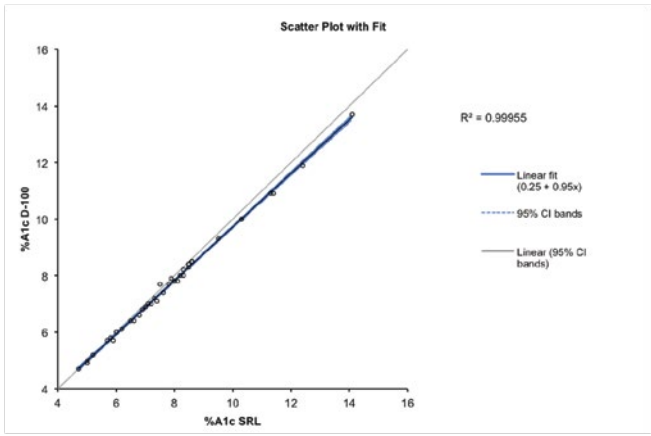
Total Allowable Error (Linear Fit)

Sample Range, % HbA1c	% HbA1c Bias to NGSP	% Bias to NGSP	% CV (Precision)	% TE
5.0	-0.01	-0.19	0.86	1.9
6.5	-0.09	-1.36	0.73	2.8
8.0	-0.17	-2.10	0.87	3.8
12.0	-0.38	-3.15	0.86	4.8

Total Allowable Error (Deming)

Sample Range, % HbA1c	% HbA1c Bias to NGSP	% Bias to NGSP	% CV (Precision)	% TE
5.0	-0.01	-0.26	0.86	1.9
6.5	-0.09	-1.39	0.73	2.8
8.0	-0.17	-2.10	0.87	3.8
12.0	-0.38	-3.15	0.86	4.8

Figure 3: Correlation of HbA1c values from D-100 with the values from Secondary Reference Laboratory (SRL).



The sample throughput was confirmed to be 45 seconds per sample. The “time to first result” was confirmed to be 2 minutes 15 seconds, where the 45 second assay of the first sample in the run follows a 1 minute 30 second system flush. Each successive sample of the run is 45 seconds.

The D-100 satisfies the workflow needs of our laboratory. Barcoded samples are loaded on the sample racks. When the “RUN” button is pushed, the samples advance through the sampling system. Up to 100 samples may be loaded at one time.

The D-100 queries the host LIS for patient demographics and they are downloaded to the D-100. After an initial 2 minute 15 second warm-up, the D-100 reports another result every 45 seconds.

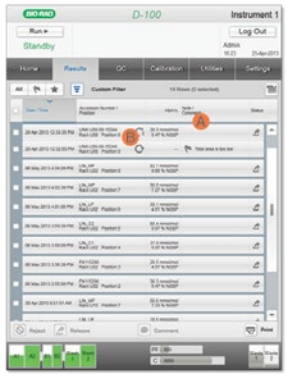
Using the Onboard Advisor and customizing the review criteria for holds, we were able to cut down our manual chromatogram technician review time by approximately 80%. We now only need to review samples that are held in the instrument. More than 90% of our HbA1c samples are now run, reported and autoverified without tech intervention. Figures 4a, b and c show screenshots of the user interface on the D-100. The user interface is easy to use.

Figure 4a

This is the home screen which shows you where you press run to start a run. It also shows the reagent status.

A Press Run
B Reagent Status

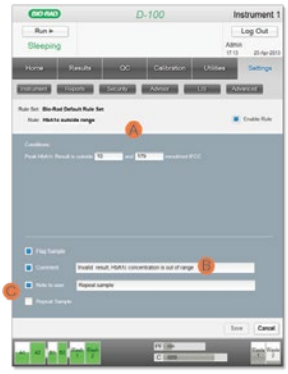
Figure 4b



On-Board Advisor
Simplifies result review while ensuring quality

A Flags results in real-time
B Automatic sample repeat

Figure 4c



On-Board Advisor

A Customizable rules
B Apply comments, notes, and flags
C Turn on automatic resampling

Conclusion

The performance of the Bio-Rad D-100 met all of the manufacturers stated claims. The D-100 Hemoglobin Testing System produces an HbA1c in just 45 seconds without interference from common hemoglobin variants and HbF up to 30%. With an 80-test hourly throughput and a 10,000 sample analytical column, the system meets the needs of high volume laboratories. The D-100 also maximizes workflow efficiency. Onboard Advisor software rules simplify chromatogram interpretation. We found that it provides a consistent and thorough review of every result. Normal results may be released automatically to the LIS, while flagged results are held for further review. This ensures nothing is missed while dramatically reducing hands-on time. With the built-in result review criteria and the bi-directional LIS interface, >90% of our patient samples are run and verified with no tech intervention. The consumables use RFID technology and buffer can be changed while the system is running.

Thank you to the MIHS Immunochemistry staff for their help performing this study.

BIO-RAD

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