How the D-100 System is Making World Class Diabetes Testing as Easy as A1c

An evaluation of the analytical performance and the user-friendliness of the Bio-Rad D-100 system
Performance Comparison – HbA1c Testing – D-100

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Introduction

For efficient diagnosis and monitoring of diabetes, clinical chemistry labs require the gold standard accuracy of HPLC in a reliable and high throughput format. This comparison study was initiated to evaluate the analytical performance and the user accessibility of Bio-Rad's D-100 system against a number of reference systems and procedures for HbA1c testing. This comparison uncovers the outstanding speed, accuracy and precision of the D-100 that lead to its "world class" analytical performance, whilst also highlighting its no compromise approach to ease of use, traceability and reliability.

Methods

The CLSI EP-5, EP-6, EP-9 protocols and the IFCC monitoring samples were used to investigate assay imprecision, linearity and accuracy. The IFCC monitoring samples consist of 24 frozen whole blood samples (12 samples in duplicate). Values were assigned with the whole IFCC network. Samples were analyzed on one day in one run.

Aliquots were made from two patient samples for the imprecision study (EP-5) and stored at minus 80 °C degrees until analysis (duplicate measurements twice per day for 20 days). CVs were also calculated on the basis of the duplicates of the fresh patient samples in the EP-9 protocol. The EP-9 protocol was performed with 40 frozen samples and the data were used to investigate the bias between the D-100 and the 6 Secondary Reference Measurement Procedures (SRMPs) (n=40, 8 samples per day for 5 days, duplicate measurements). The data were also used to calculate the NGSP certification criteria. Beginning in January 2014, 37 of 40 results need to be within 6% (relative) of an individual NGSP SRMP to pass certification.

HbA1c value determination of the patient samples was performed with 6 certified SRMPs:

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- Roche Tina-quant Gen.2 HbA1c on Integra 800, immunoassay, IFCC and NGSP certified (Roche Diagnostics);
- Premier Hb9210, affinity chromatography HPLC, IFCC and NGSP certified (Trinity Biotech)
- Tosoh G8, cation-exchange HPLC, IFCC certified (Tosoh Bioscience).

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- Premier Hb9210, affinity chromatography HPLC, IFCC certified (Trinity Biotech)
- Menarini HA8180V, cation-exchange HPLC, IFCC and NGSP certified (Menarini Diagnostics)
- Sebia Capillarys 2 Flex Piercing, IFCC and NGSP certified (Sebia).

To check overall calibration and bias independently of the chosen SRMP, the results of the D-100 instruments in the EP-9 procedure were compared with the mean of the 6 SRMPs, and medical decision point (MDP) analysis was performed at an HbA1c value of 48 mmol/mol (6.5% Diabetes Control and Complications Trial (DCCT) units) and 75 mmol/mol (9.0% DCCT units). When the 2 methods are statistically identical, the 95% CI for each y MDP includes the corresponding x MDP.

Beside an EP-9 protocol with frozen samples also a method comparison was performed with 100 fresh patient samples compared to the Tosoh G8.

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The linearity of the D-100 was tested with two different sample types. With the first linearity test we used 2 fresh patient samples with a low and a high HbA1c value. Dilutions were made with both samples. With the second linearity test we used the Bio-Rad Lyphochek Hemoglobin A1c Linearity Set Level 1 to 6.

Interference from common Hb variants HbAS, HbAC, HbAD, HbAE, HbAJ, increased A2 (ß-thalassemia), and HbF was investigated by the D-100. Five samples of each variant with different HbA1c values were analyzed in 1 day.

Results

Table 1: Results of the EP-5 protocol and the CVs on the basis of the duplicate samples analyzed in EP-9.

	CV (%) SI units	CV (%) DCCT units
EP-5	0.8 (46.8 mmol/mol) 1.0 (72.9 mmol/mol)	0.6 (6.43%) 0.7 (8.82%)
EP-9	0.7	0.5

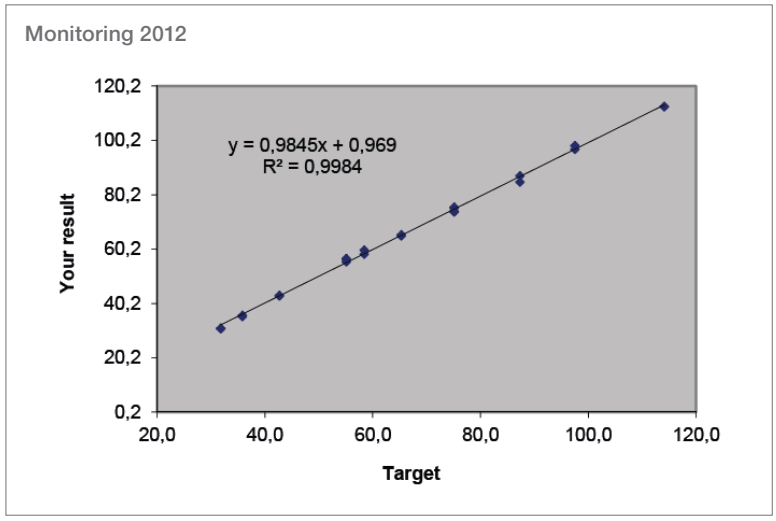
Table 2: NGSP certification pass/fail criteria with respect to the results of the EP-9 protocol performed with frozen patient samples.

Deming regression lines	Lot number 020268	Bias	SEE	Out ± 6% SRM	NGSP criteria*
D-100 (Y) vs Premier Isala (X)	Y=0.97X+0.16	-0.09	0.16	0	Pass
vs Tina-quant Isala (X)	Y=0.98X+0.02	-0.09	0.20	3	Pass
vs Tosoh G8 Isala (X)	Y=0.98X+0.04	-0.09	0.06	0	Pass
vs Premier SKB (X)	Y=0.98X+0.08	-0.06	0.15	0	Pass
vs Menarini HA8180 SKB (X)	Y=0.97X+0.13	-0.07	0.09	0	Pass
vs Sebia SKB (X)	Y=0.98X+0.13	-0.02	0.14	0	Pass

* 37 of 40 results need to be within 6% (relative) of an individual NGSP SRMP to pass certification.

Figure 1 Overall results of the IFCC monitoring samples.

D-100			
ID	Target	Actual	DEV
01	111,4	112,7	-1,3
02	55,1	56,6	1,5
03	75,1	74,2	-0,9
04	55,1	56,7	1,6
05	97,5	98,3	0,8
06	58,4	59,8	1,4
07	31,8	30,9	-0,9
08	75,1	73,9	-1,2
09	35,8	35,4	-0,4
10	65,3	65,1	-0,2
11	42,7	43,2	0,5
12	87,3	84,9	-2,4
13	42,7	42,9	0,2
14	65,3	65,5	0,2
15	97,5	96,9	-0,6
16	75,1	75,7	0,6
17	55,1	56,6	1,5
18	31,8	31,1	-0,7
19	58,4	58,4	0,0
20	114,1	112,6	-1,5
21	35,8	35,8	0,0
22	55,1	55,5	0,4
23	87,3	87,2	-0,1
24	75,1	75,3	0,2



Issue	Deviation	Reproducibility	Linearity
Excellent	**0.0 – 1.9**	**<2%**	**>0.9950**
Good	2.0 – 3.9	2.00 – 3.49%	0.9901 – 0.9950
Acceptable	4.0 – 6.9	3.50 – 4.99%	0.9851 – 0.9900
Poor	7.0 – 9.9	5.00 – 6.99%	.9801 – .9850
Unacceptable	>9.9	>6.99%	<0.9801

IFCC mmol/mol	D-100	dev from IFCC	Mean
30	30,5	0,5	0.04
60	60,0	0,0	
90	89,6	-0,4	

Correlation coefficient = 0,9992

Results

Table 3: Results of the medical decision point analysis of 48 mmol/mol and 75 mmol/mol compared to the mean of the 6 SRMP and the individual SRMP.

Medical Decision Point Analysis		
System	**HbA1c (mmol/mol)**	**HbA1c (mmol/mol)**
Mean SRMP	**47.3 (47.1 – 47.6)**	**73.7 (73.3 – 74.1)**
Tosoh G8 Isala	47.1 (47.0 – 47.3)	73.7 (73.4 – 73.9)
Roche Tina-quant Integra 800 Isala	47.1 (46.5 – 47.6)	73.6 (72.8 – 74.4)
Premier Hb9210 Isala	47.2 (46.8 – 47.6)	73.2 (72.6 – 73.9)
Premier Hb9210 SKB	47.4 (47.0 – 47.8)	73.9 (73.2 – 74.5)
Menarini HA8180v SKB	47.4 (47.2 – 47.6)	73.6 (73.3 – 74.0)
Sebia Capillarys SKB	47.9 (47.5 – 48.2)	74.2 (73.6 – 74.7)

Figure 2: EP-9 results of the D-100 measured and off-line calibrated with IFCC secondary reference material compared to the mean of the 6 SRMP. The mean bias compared to the mean of the 6 SRMP is -0.8 mmol/mol and off-line cali-brated with IFCC secondary reference material -0.6 mmol/mol.

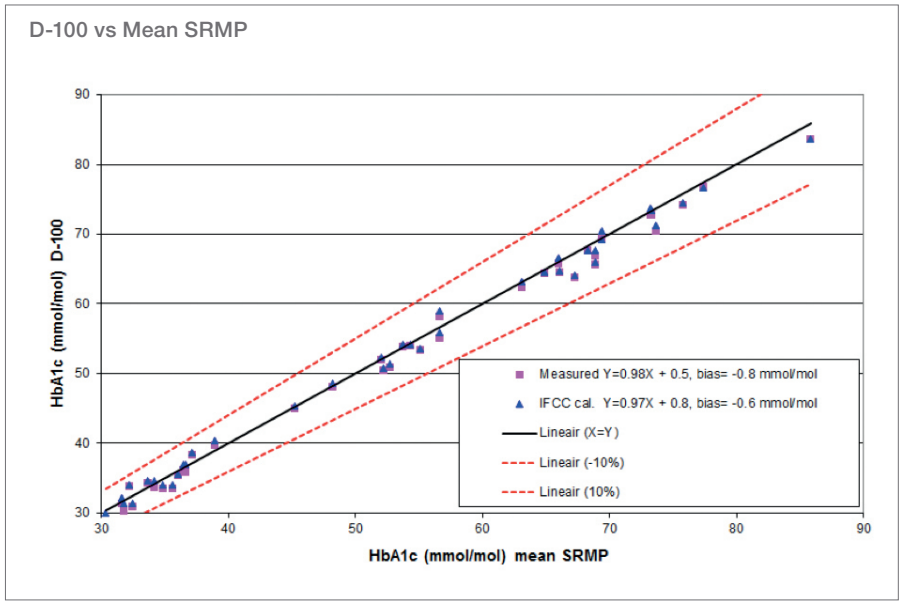


Figure 3: Analytical performance of the D-100 in sigma metrics,

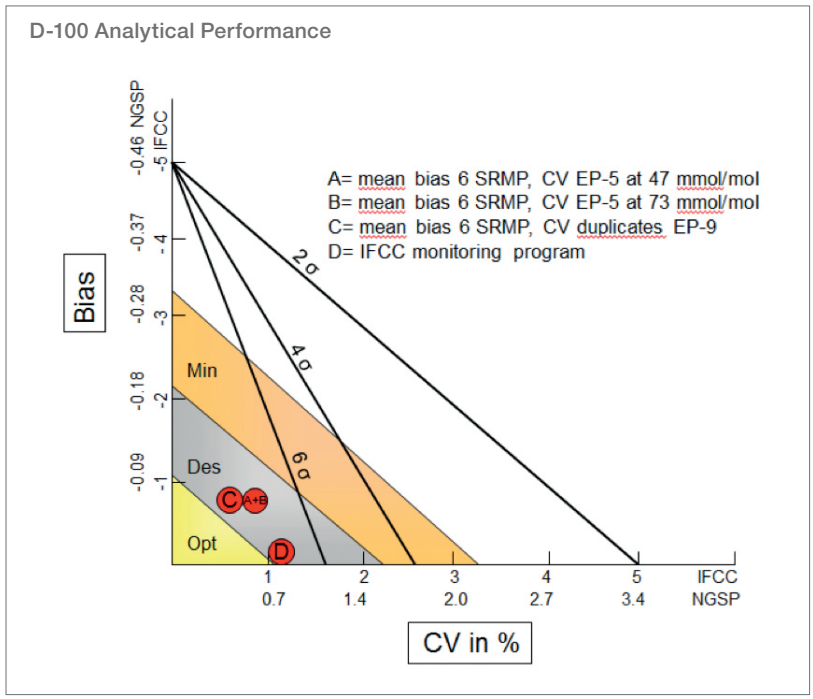


Figure 4: Results of the method comparison with fresh patient samples between Tosoh G8 and D-100. The Deming regression line between the measured samples on the D-100 and the samples off-line calibrated with IFCC secondary reference material was Y=1.00X (95% CI: 0.995 to 1.10) – 0.37 (95% CI: -0.79 to 0.05). Bias was -0.23 mmol/mol. So, statistically, there was no difference,

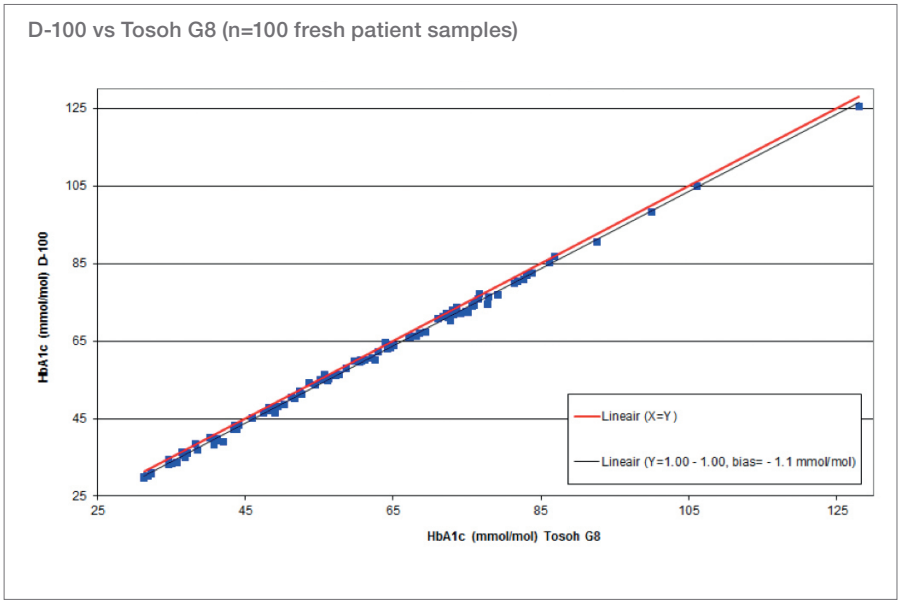


Figure 5: Results of 40 frozen normal non-variant samples and frozen Hb-variants samples (n=5 per Hb-variant). The mean relative difference of the Hb-variants compared to the assigned value was for HbAS -0.7%, HbAC -6.6%, HbAD –3.9%, HbAE 1.7%, HbAJ -42.9% elevated A2 5.3% and HbF -3.0%. The percentage HbF in the samples was 3.2%, 4.6%, 8.6%, 15% and 18%. These results have been corrected for the bias with the Premier Hb9210 with frozen normal non-variant samples (bias was – 1.0 mmol/mol).

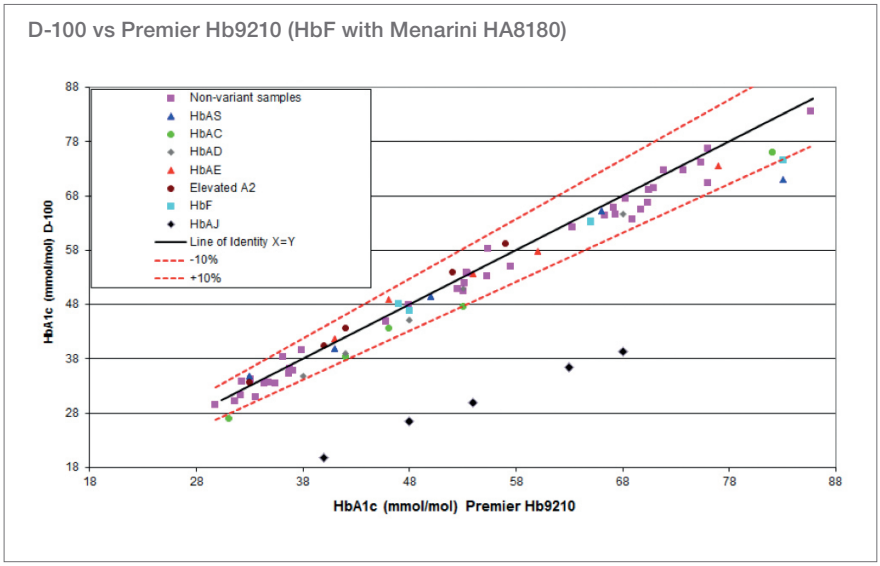


Figure 6a: the linearity of the D-100 with the use of Bio-Rad Lyphochek.

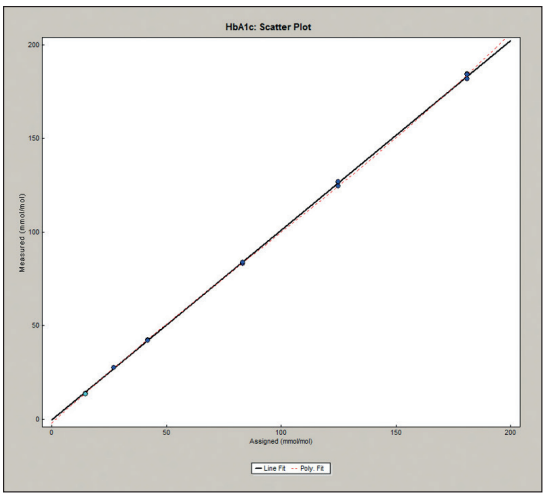
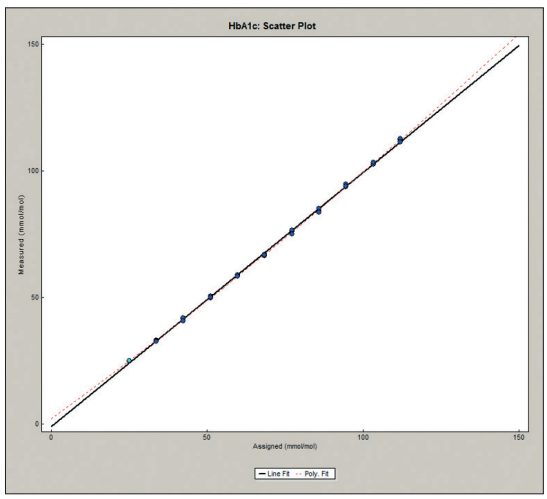


Figure 6b: The linearity of the D-100 with the use of a low and a high patient sample.



Evaluation of User Friendliness

In general we can say that we have never worked with an instrument which was as easy to use as the D-100 is. Bio-Rad has really listened to the wishes of the users with regard to ease of use of the instrument. For example, changing a column, a frit or reagent is so easy that it is almost impossible to make a mistake. Also having two positions for one buffer avoids delay in analysis time and avoids air in the HPLC system which is a common problem with HPLC's. Due to the chips in the reagents and columns, the traceability of lot numbers and all relevant data like expiry date and start of use date of the reagent etc. is documented very well within the instrument. This is a big step forward. The evaluation of the results is very easy to do because of the flags within the system. What we have seen so far is that these flags are sufficient to evaluate the results in a correct way.

However, we were not always patient enough with the touch screen. We think it reacts a little bit slow. We also saw a few times that, even when there is no sample left in a rack with magnets for manual diluted samples, the D-100 thought there was a sample present. This needs more investigation.

Discussion

The analytical performance of the D-100 in the IFCC monitoring program with respect to deviation from the target value (values assigned with the whole IFCC network), reproducibility and linearity, all fell in the category "excellent". The CVs in the EP-5 protocol and calculated from the duplicates in the EP-9 were also excellent. All were <1.0% in DCCT and even <1.0% in SI units.

The calibration of the D-100 is sufficient which was confirmed by passing the NGSP criteria compared with 6 different SRMPs. Also the off-line IFCC calibrated samples were not statistically and clinically different from the measured samples. The mean bias compared with the mean of the 6 SRMPs was well within the acceptable criteria of < ± 2 mmol/mol. Medical decision point analysis at an HbA1c value of 48 mmol/mol and 75 mmol/mol showed a significant statistical difference compared with the mean of the SRMP and the individual SRMPs, mainly due to the fact that the 95% CI was very small. Clinically seen, the differences were not significant. The analytical performance of the D-100 in sigma metrics was classified as "world class" performance as sigma was >6. There was no issue with linearity, even though the patient samples showed a little bit more deviation from the calculated values than the lyphocheck samples. This can be explained by the fact that it is not easy to dilute whole blood samples very accurately because of the viscosity of whole blood. There was no interference of the common Hb-variants HbAS, HbAC, HbAD, HbAE, elevated A2 and HbF. However, samples with HbAS /HbAC and a high HbA1c value (> 80 mmol/mol), had more deviation from the target value than samples with a lower HbA1c value and HbAS/HbAC. I recommend further investigating this. The D-100 cannot analyze samples with HbAJ correctly.

Conclusion

The analytical performance of the Bio-Rad D-100 is excellent.

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