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Laboratory Medicine and Clinical Practice:
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DETERMINATION OF GLYCATED HAEMOGLOBIN: ASSESSMENT OF THE BIO-RAD D-100 INSTRUMENT

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INTRODUCTION

Glycated haemoglobin (HbA1c) is a form of haemoglobin produced in a non-enzymatic reaction as a result of the exposure of normal haemoglobin to high plasma glucose concentrations. The measurement of HbA1c is mainly used to identify the average plasma concentration of glucose over a long period of time: which is very important in the monitoring of diabetic patients.

PURPOSE

- ✓ Assess the analytical performance of the instrument in terms of precision, accuracy and carry-over.
- ✓ Compare the HbA1c results obtained from samples received at the U.O.C. Department of Laboratory Medicine with those obtained with the Adams HA8160V and Adams HA8180V instruments (A. Menarini Diagnostics). This included samples that also contained haemoglobin variants.

INSTRUMENTS

MATERIALS AND METHODS

1. Methods

BIO-RAD D-100	
Parameters measured	HbA1c with separation and detection of variants HbE, HbD, HbS, HbC, separation of other fractions, such as HbF, HbA0, LHbA1c, P3 (Hb acetylated/glycated), HbA1a, HbA1b)
Measuring principle	HPLC with cation exchange chromatography, photometric measurement of absorbance at 415 nm
Measuring range	Linearity: NGSP (%) 3.5-20.0; IFCC (mmol/mol) 15-195
Sample volume	5 µL (whole blood)
Sample loading capacity	The sampler with 100 spaces with continuous loading up to 2000 samples per session; 3 emergency positions (STAT)
Duration of the analysis	45 seconds/test
Analytical column	Latest generation cation-exchange column

MENARINI Adams HA8180V	
Parameters measured	HbA1c and HbF (HbS and HbC detected in Variant mode)
Measuring principle	Reverse phase cation exchange chromatography. Dual wavelength detection
Measuring range	Linearity: NGSP (%) 3-20%, IFCC (mmol/mol) 9-195
Sample volume	Approx. 14 µL (whole blood)
Sample loading capacity	100 samples
Duration of the analysis	Variant mode: 90 sec/test
Analytical column	Cation-exchange column, non-porous material, negative charge

MENARINI Adams HA8160V	
Parameters measured	HbA1c, HbA1, HbF, HbA2, detection of haemoglobin variants and fragmentation patterns
Measuring principle	Reverse phase cation exchange chromatography. Dual wavelength detection
Measuring range	Linearity: NGSP (%) 3-20%, IFCC (mmol/mol) 9-195
Sample volume	4 µL (whole blood)
Sample loading capacity	100 samples
Duration of the analysis	2.9 min/test
Analytical column	Cation-exchange column, non-porous material, negative charge

2. Assessment protocol

- **IMPRECISION** assessed according to protocol CLSI EP5-A2 using 4 pools of whole blood with different concentrations of HbA1c
- **ACCURACY** assessed using 11 samples from the external CRB quality assessment programme with target value assigned (IFCC Reference Procedure)
- **CARRY-OVER** assessed following a precise sequence of samples with a high (H) and low (L) concentration
- **COMPARISON BETWEEN INSTRUMENTS** The results obtained on 1000 samples of whole blood with request for HbA1c, without haemoglobin variants, analysed routinely with the Adams HA8180 V (A. Menarini Diagnostics) instrument and a further 36 samples that had different types of haemoglobin variants (HbS, HbD Punjab, Hb Hasharon, HbC, HbE, HbG, Hb Camperdown). These additional samples were analysed with the Adams HA8160 V instrument (A. Menarini Diagnostics). All of the results were compared with those from ~~of~~ the Bio-Rad D-100 instrument.

RESULTS

1. Imprecision

4 pools were prepared with different concentrations of HbA1c (30 - 40 - 48 - 75 mmol/mol)

	POOL 1	POOL 2	POOL 3	POOL 4
Mean, mmol/mol	35	40	47	75
DS, mmol/mol	0.92	0.96	0.73	1.38
CV %	**2.6**	2.4	1.5	1.9

2. Accuracy

	Ex. 1	Ex. 2	Ex. 3	Ex. 4	Ex. 5	Ex. 6	Ex. 7	Ex. 8	Ex. 9	Ex. 10	Ex. 11
Target value	56	84	69	41	54	45	80	36	59	71	63
Value obtained	54	84	69	43	54	44	79	36	59	70	62
Bias %	3.3	-0.2	0.3	**3.7**	0.6	-2.0	-1.8	-0.3	0.3	-0.8	-1.0

3. Carry-over

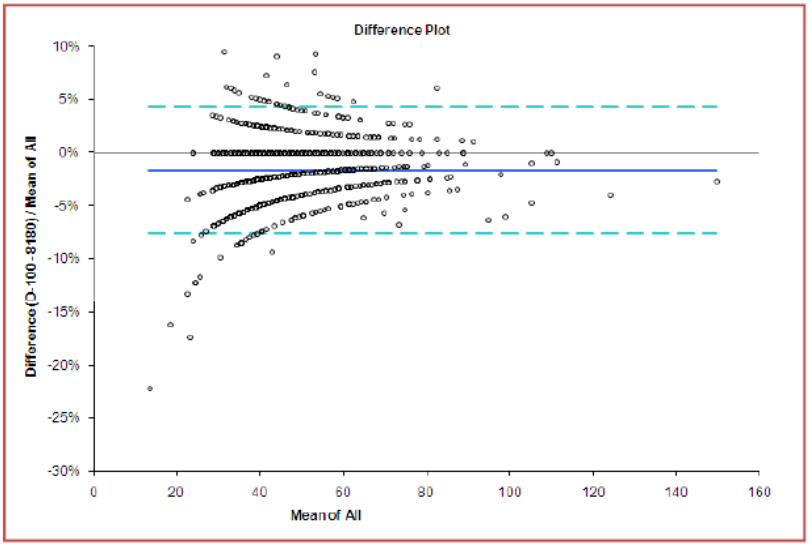
A sample with a high (H) HbA1c value is followed by a sample with low (L) HbA1c value in an established order. If there is no carry-over, the difference between the highest value and the lowest of series L will have an SD less than 3 of the concentrations observed in the sequence of low samples.

L	L	L	H	H	L	H	H	L	L	L	L	H	H	L	H	H	L	H	H	L
24	24	23	125	123	24	126	124	24	24	24	23	124	124	24	124	124	24	125	124	24

3ds L=3*0.4=1.2
HL–LL=1

NO Carry-over

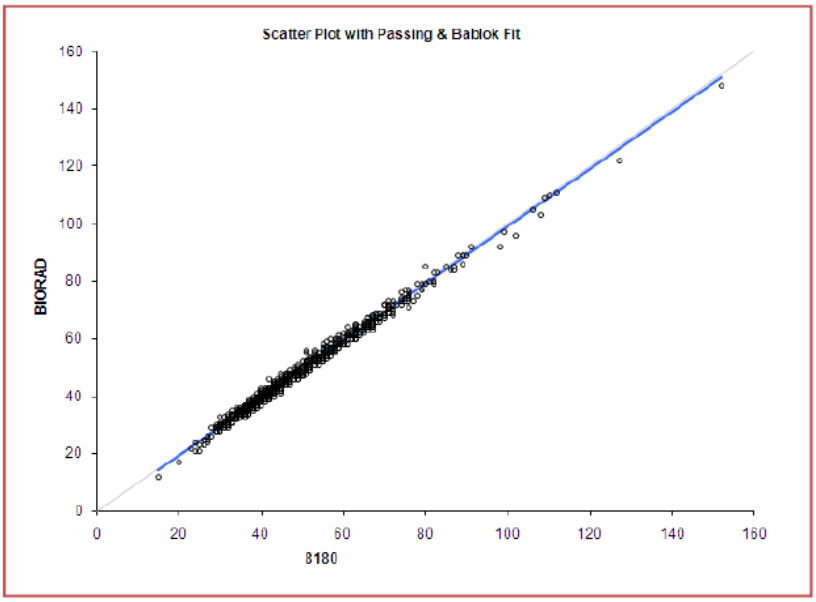
4. Comparison between instruments



1000 samples

Bias -1.6%
95% CI: -1.8 to -1.4

STATISTICALLY BUT NOT CLINICALLY SIGNIFICANT

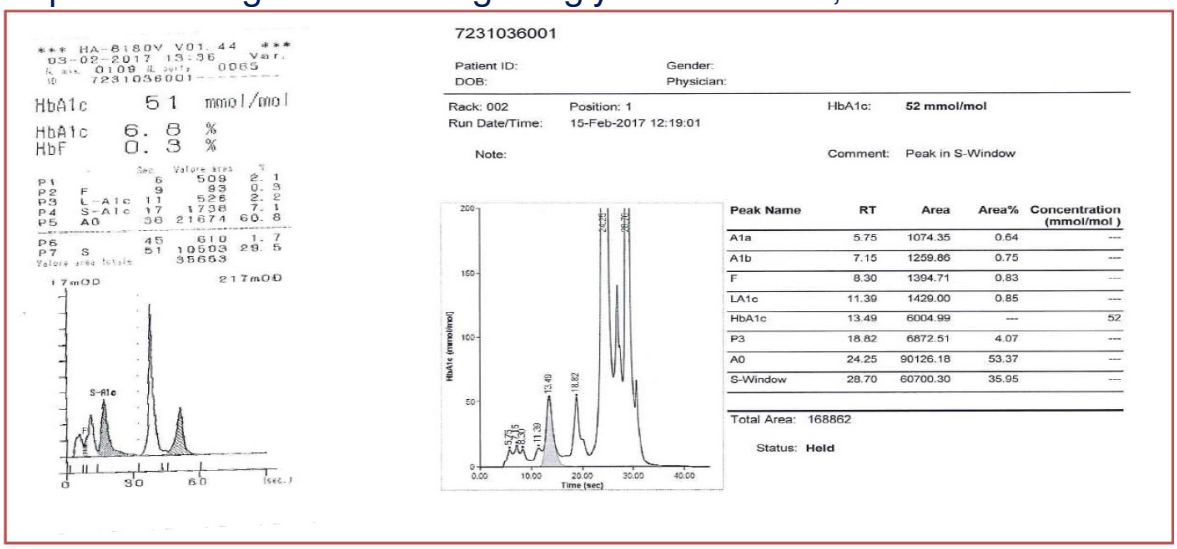


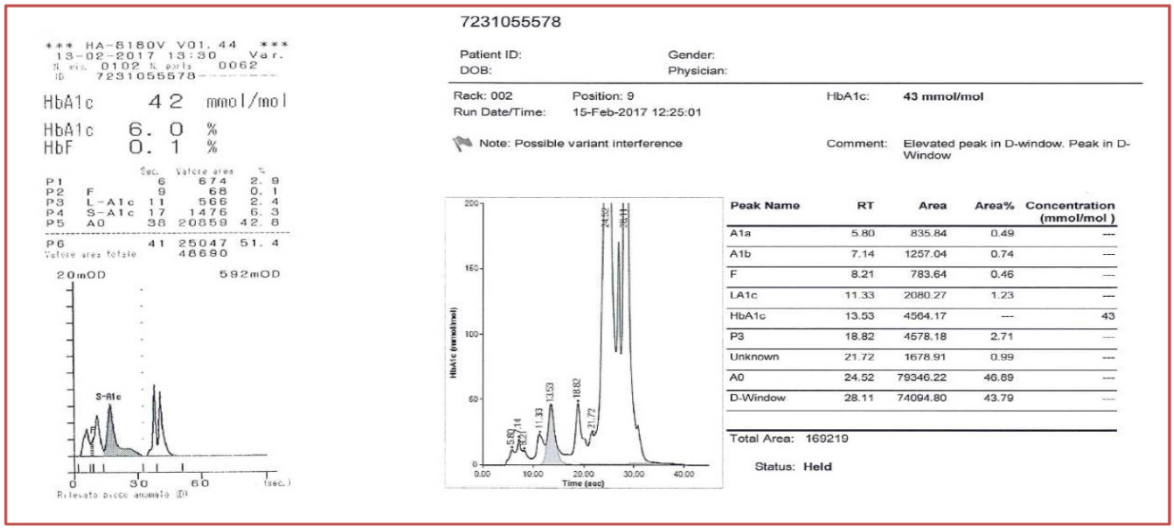
EXCELLENT CORRELATION

y =1.00x-1.00
R2= 0.99
P < 0.0001

SAMPLES WITH VARIANTS

36 samples were selected that had different types of haemoglobin variants: 18 HbS (characterised by sickling testing), 6 HbD Punjab, 4 Hb Hasharon, 3 HbC and 5 various ones (HbE, HbG, Hb Camperdown). The low number of units in the group of patients with haemoglobinopathies does not allow definitive statistical data to be obtained. Differences > 5 mmol/mol, which correspond to a significant change in glycemic control, were not observed.





CONCLUSIONS

The D-100 analytical performance, both in terms of imprecision, accuracy and correlation with the method in use, proved to be very satisfactory. The most common haemoglobin variants studied (HbS, HbC HbD, HbE) are automatically identified by the software and did not demonstrate analytical interference with HbA1c measurement. The ease of use, robustness and hourly productivity attest to the possible application in clinical laboratory diagnostics.