EVALUATION OF THE D-100 HPLC SYSTEM FOR THE DETERMINATION OF GLYCATED HEMOGLOBIN

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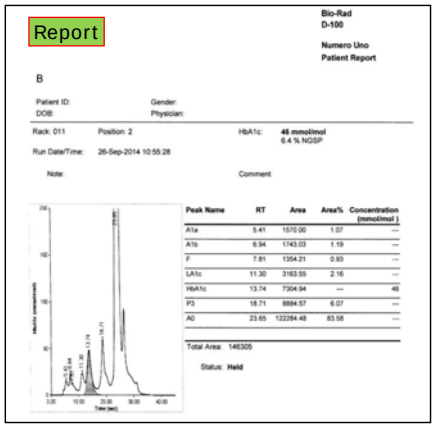
Background. The HbA_{1c} testing is set to assume a greater role in the next few years as a consequence of the introduction of HbA_{1c} for the diagnosis in addition to its conventional use for monitoring of diabetic patients, and of the global increasing frequency of diabetes. Accordingly, the methods used for HbA_{1c} determination need to provide excellent performance in terms of analytical quality as well as robustness, usability and throughput. The analytical performance of a new HPLC analyzer, the D-100 System from Bio-Rad Laboratories, has been evaluated

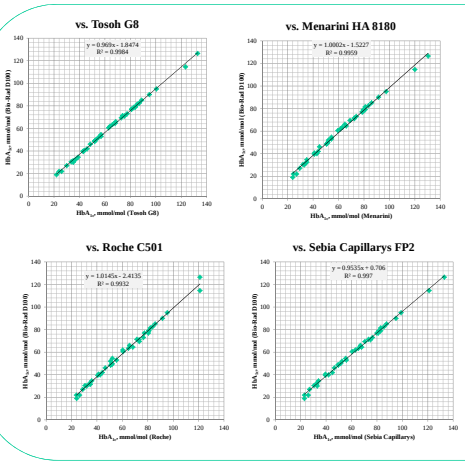
Methods. Precision was tested by using the CLSI-EP5 protocol and measured for 20 working days, 2 runs per day, run in duplicate, on aliquots of frozen blood samples at four different HbA_{1c} levels (30, 47, 62, and 108 mmol/mol). For method comparison, 40 blood samples with HbA_{1c} values well distributed over the measuring interval were analyzed in duplicate according to EP9-A2IR. The following comparison methods were used: Menarini HA 8180, Roche Cobas 501, Sebia Capillarys FP2, Tosoh G8. Trueness was evaluated by analyzing two IFCC value-assigned samples. The influence of two common hemoglobin variants (HbS and HbC) was also evaluated.

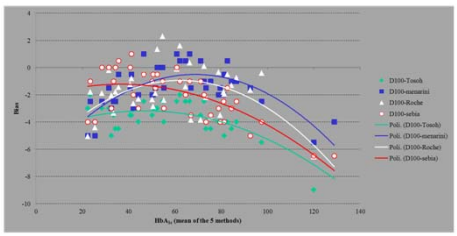
Results. Total reproducibility was found to be very good at any HbA_{1c} level tested, with CV values always <2 %, well below the recommended goal for imprecision of CV<3%. Method comparison study proved D-100 results were well correlated with those obtained with other methods and provided the following linear regression (least square) equations (D-100 was considered as y, other methods as x), y=1.000x-1.52, R^2=0.995 (D-100 vs Menarini); y=1.014x-2.41, R^2=0.993 (D-100 vs Roche); y=0.953x+0.71, R^2=0.997 (D-100 vs Sebia); y=0.969 x-1.85, R^2=0.998 (D-100 vs Tosoh). With regard to trueness, D-100 presented a bias of -1.2 mmol/mol at HbA_{1c} level of 31.7 mmol/mol and +1.5 mmol/mol at 78.0 mmol/mol respect to the IFCC target values. HbC and HbS were clearly eluted after HbA_0 and not integrated for the calculation of HbA_{1c}.

Report



Methods comparison





Precision

Lyophilized samples (Lyphocheck)

HbA_{1c} mmol/mol	Tosoh G8 35.4	Menarini HA 8180 33.6	Sebia Cap FP2 31.0	Roche C501 35.6	Bio-Rad D-100 33.1
Repeatability	0.9	0.7	4.3	2.6	1.9
Between run	0.9	0.7	0.0	0.0	0.6
Between day	0.5	1.1	1.5	1.3	1.2
Total	1.4	1.5	4.5	2.9	2.3

HbA_{1c} mmol/mol	Tosoh G8 84.1	Menarini HA 8180 79.5	Sebia Cap FP2 84.7	Roche C501 83.1	Bio-Rad D-100 81.8
Repeatability	0.4	0.4	1.1	1.4	1.2
Between run	0.0	0.3	0.2	0.8	0.0
Between day	0.2	0.8	1.3	0.8	0.6
Total	0.4	1.0	1.7	1.8	1.3

Fresh human blood

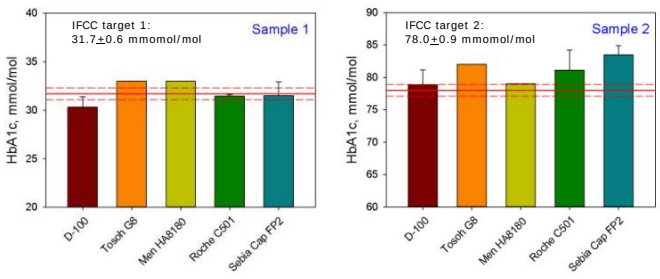
HbA_{1c} 30 mmol/mol	Tosoh G8	Menarini 8180	Sebia Cap FP2	Roche C501	Bio-Rad D-100
Repeatability	1.0	0.8	2.1	2.1	1.9
Between run	0.5	0.5	0.0	0.0	0.0
Between day	0.4	1.2	0.5	1.7	0.5
Total	1.2	1.5	2.2	2.7	1.9

HbA_{1c} 47 mmol/mol	Tosoh G8	Menarini 8180	Sebia Cap FP2	Roche C501	Bio-Rad D-100
Repeatability	0.4	0.6	1.9	1.8	1.8
Between run	0.4	0.8	0.0	0.7	0.0
Between day	0.2	0.5	1.2	1.0	0.7
Total	0.6	1.1	2.3	2.2	1.9

HbA_{1c} 62 mmol/mol	Tosoh G8	Menarini 8180	Sebia Cap FP2	Roche C501	Bio-Rad D-100
Repeatability	0.6	0.5	1.6	1.7	1.2
Between run	0.0	0.7	0.0	0.0	0.0
Between day	0.1	0.7	0.5	0.6	0.7
Total	0.6	1.2	1.6	1.8	1.4

HbA_{1c} 108 mmol/mol	Tosoh G8	Menarini 8180	Sebia Cap FP2	Roche C501	Bio-Rad D-100
Repeatability	0.4	0.5	1.1	1.7	1.1
Between run	0.2	0.3	0.0	0.0	0.6
Between day	0.4	0.7	0.6	1.6	0.6
Total	0.5	0.9	1.2	2.3	1.3

Trueness



Analytical interferences

Hb Variant (heterozygosis)	Sample	HbA_{1c}, mmol/mol Bio-Rad D 100	Menarini HA8180	Sebia Capillarys	Tosoh G8
Hb C	1	34	34	35	37
	2	39	39	46	41
	3	75	75	89	78
	4	79	79	98	83
Hb D	1	91	65	108	102
Hb S	1	41	38	39	42
	2	42	37	40	43
	3	55	50	55	54
	4	77	76	81	79
(Hb F)	1 (1.8%)	29	30	29	33
	2 (3.5%)	35	36	32	39
	3 (18%)	36	34	nd	36
	4 (22%)	41	36	nd	39
	5(26%)	40	35	nd	39

Method	HbA_{1c}, mmol/mol			
	Influence of triglycerides		Influence of bilirubin	
	Basal	Spiked	Basal	Spiked
Bio-Rad D100	40	40	42	43
Menarini HA8180	40	40	41	41
Sebia Capillarys 2FP	39	38	39	38
Tosoh G8	43	43	45	45

Conclusions. The Bio-Rad D-100 system is a fully automated, user-friendly, high throughput HPLC system giving accurate and reproducible results. The system showed a reduced hands on time, a simple calibration process, a very good workflow efficiency thanks to the intuitive interface, and an high speed HbA_{1c} assay in 45 sec. Bio-Rad D-100 therefore displays the appropriate characteristics to be used as a routine method in clinical laboratories.