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

Paleari R1, Saibene D2, Moia R3, Besozzi M3, Varini M4, Pezzo M4, Ceriotti F2, Mosca A1

1 Centro per la Riferibilità Metrologica in Medicina di Laboratorio (CIRME), Dipartimento di Fisiopatologia Medico-Chirurgica e dei Trapianti, Università degli Studi, Milano (MI)
2 Laboratorio di Standardizzazione, Servizio Medicina di Laboratorio, Ospedale San Raffaele, Milano (MI)
3 Laboratorio di Analisi Cliniche, IRCCS Istituto Auxologico Italiano, Milano (MI)
4 Laboratorio Analisi, Ospedale di Suzzara, Suzzara (MN)

Background: The HbA1c testing is set to assume a greater role in the next few years as a consequence of the introduction of HbA1c 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 HbA1c 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 HbA1c levels (30, 47, 62, and 108 mmol/mol). For method comparison, 40 blood samples with HbA1c 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 (HbsS and HbC) was also evaluated.

Results: Total reproducibility was found to be very good at any HbA1c 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); \( y = 0.969x - 1.85 \), \( R^2 = 0.998 \) (D-100 vs Tosoh). With regard to trueness, D-100 presented a bias of -1.2 mmol/mol at HbA1c 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 HbA0 and not integrated for the calculation of HbA1c.

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 HbA1c assay in 45 sec. Bio-Rad D-100 therefore displays the appropriate characteristics to be used as a routine method in clinical laboratories.