High Resolution HbA_{1C} Separation using the Sebia CAPILLARYS 3 TERA

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INTRODUCTION

HbA_{1C} is widely used for the management and diagnosis of diabetes mellitus (DM). Correlation between HbA_{1C} with clinical outcomes emphasises the necessity for accurate and precise measurement. Galway University Hospital has experienced an exponential rise in HbA_{1C} requesting since the WHO endorsed its use for diagnosing DM in 2011. Numbers of requests for HbA_{1c} testing have tripled in the past 6 years. The Sebia Capillarys 3 Tera **(CAPI3)** separates the different fractions of haemoglobin based on the technique of capillary electrophoresis **(CE).** HbA_{1C} quantitation is achieved using high resolution CE in combination with ultra violet (UV) detection.

AIM

The primary study aim was to evaluate CAPI3 with respect to the following assay performance characteristics: linearity, precision, bias and method comparison to High Performance Liquid Chromatography (HPLC) using the Menarini HA-8160V analyser. The secondary aim was to assess the stability of HbA_{1c} over a period of 15 days.

MATERIALS & METHODS

Samples: Blood samples collected in K_2–EDTA specimen tubes for routine HbA_{1C} analysis were used in this evaluation.

Instrument: The CAPI3 Capillary Electrophoresis analyser and the Sebia HbA_{1C} kit (Product Number: 2515). The CAPI3 incorporates 12 individual silica capillaries functioning in parallel, allowing 12 simultaneous analyses.

Assay performance characteristics were assessed in accord with Clinical Laboratory Standards Institute guidelines[1].

Assay precision was assessed using replicate analysis of each of two quality contol materials (Sebia, Product Number: 4768) across each of the 12 capillaries, 5 times a day for 5 consecutive days (n=600). Linearity was evaluated by serially diluting two patient samples with almost identical total haemoglobin concentrations (15g/dL) and HbA_{1C} values of 29 and 120 mmol/mol respectively. Five EQA samples with target values assigned by an IFCC reference method were employed to determine assay bias. Method comparison was performed using patient samples (n=145) with HbA_{1C} concentrations that ranged from 16-147 mmol/mol. Method agreement was evaluated using Bland-Altman difference plot and Passing-Bablok regression analysis. Stability of HbA_{1C} was assessed using two patient samples with HbA_{1C} concentrations of 73 and 36 mmol/mol stored at room temperature (RT:19°C±1°C) and 4°C±1°C respectively, with both samples being reanalysed daily for a total of 15 days. All data was recorded using Microsoft Excel 2010 and statistical analyses carried out using Excel and MethVal software.

RESULTS

Both within and between run analytical variation (CV_A%) at a mean HbA_{1C} concentration of 36 and 71 mmol/mol was <1.4% **(Table 1).** The method was linear up to a concentration of 71 mmol/mol. The use of EQA samples with target IFCC values demonstrated minimal method bias (Range:-2.0 to 1.0 mmol/mol **(Table 2).**

Passing-Bablok Regression analysis provided the equation y=1.018+0.1; R=0.997 **(Figure 1).** Bland Altman Difference Plot showed a mean difference of 1.23 mmol/mol **(Figure 2).** Samples (n=12) with common haemoglobin variants were included in the method comparison. HbA_{1C} results from Menarini HA-8160V and CAPI3 were similar for all variants. HbS and HbC were visually well separated from the HbA_0 peak on the CAPI3. The number and type of variants detected has significantly increased during the 6 months since implementation. A total of 215 variants have been detected including HbS, HbE, HbD and HbC in addition to more rare variants such as HbH Barts, Hb Créteil and HbHofu.

HbA_{1C} was stable at RT and 4°C, for up to 15 days with a maximum difference of 1 mmol/mol at a HbA_{1C} level of 36 mmol/mol and 2 mmol/mol at a HbA_{1C} level of 73 mmol/mol.

Table 1. CAPI3: Between-run Laboratory Precision

HbA_{1C}	Control Level 1 (n=300) mmol/mol	Control Level 2 (n=300) mmol/mol
Mean	36.2	70.4
SD	0.5	0.6
Between-run CV_A%	1.34	0.87

Table 2. CAPI3: Trueness Experiment

HbA_{1C}	IFCC Target Value mmol/mol	Measured Value mmol/mol	Deviation from the target value mmol/mol	BIAS %
Sample 1	32	31	1	3.1
Sample 2	43	45	-2	-4.7
Sample 3	69	69	0	0.0
Sample 4	36	35	1	2.8
Sample 5	53	53	0	0.0
Mean			0	0.24

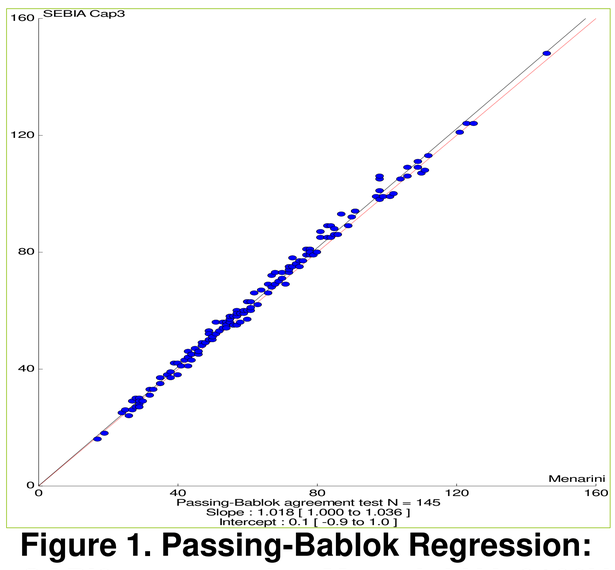

Figure 1. Passing-Bablok Regression: CAPI3 compared to Menarini HA-8160V

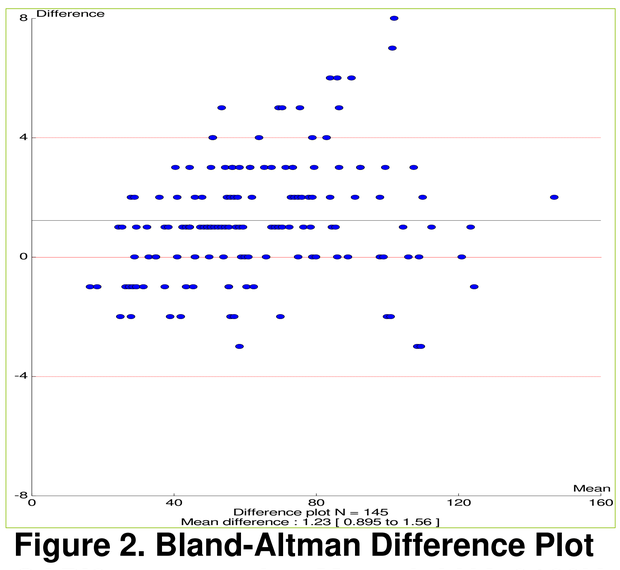

Figure 2. Bland-Altman Difference Plot CAPI3 compared to Menarini HA-8160V

CONCLUSION

This study found that the analytical performance specifications of the HbA_{1c} assay using the CAPI3 instrument meet the most stringent analytical quality criteria required for clinical use. Using these objective criteria the method is judged fit for purpose and recommended for implementation into routine clinical practice.

REFERENCE

1. CLSI. Clinical and Laboratory Standards Institute.EP15A3: User Verification of Precision and Estimation of Bias; Approved Guidelines. 2014;34.