Evaluation of a new enzymatic HbA1c test for the diagnosis of diabetes

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BACKGROUND

Glycated Hemoglobin A1c (HbA1c) is a well-established parameter for long-term monitoring and diagnosis of diabetes. Here, we present a novel enzymatic HbA1c test (HbA1c net FS) for the highly specific detection of HbA1c, that eliminates interferences of common hemoglobin (Hb) variants of existing test systems. HbA1c net shows excellent precision resulting from the innovative application type (twin-test), linking 2 calibrations and 2 detections for Hb and HbA1c in only one determination. The test principle is based on Hb determination after sample hemolysis at 570 nm and subsequent H_2O_2 release after oxidative cleavage of fructosylated HbA1c-dipeptides in the same cuvette. H_2O_2 concentration is determined colorimetrically at 660 nm, where the delta absorbance is proportional to the HbA1c concentration.

METHODS

Assay adaption and performance verification have been carried out on a BioMajesty®JCA-BM 6010/C (BM) fully automated clinical chemistry analyzer system. All reagents, calibrators and controls were obtained from DiaSys Diagnostic Systems GmbH. Method comparisons were performed on BM and HPLC (ADAMS A1c HA 8160, Arkray) as reference system. Data have been evaluated by using regression analysis according to Passing/Bablok. Intra-assay imprecision was performed according to CLSI (EP5-A2). Determination of hemoglobin subtypes was carried out by recovery measurements of IFCC Hb-subtype evaluation samples.

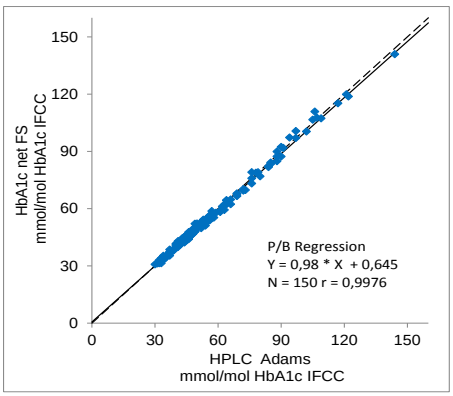

Figure 1: Method comparison HPLC vs HbA1c net

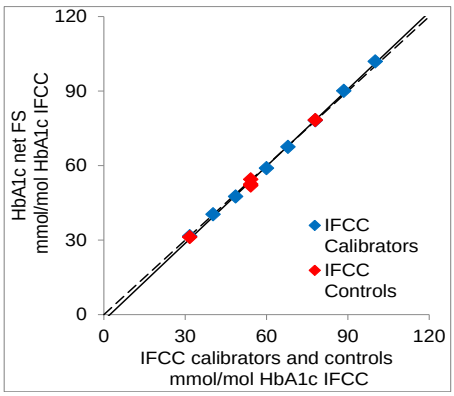

Figure 2: Recovery of IFCC calibrators and controls

Hb subtype	Abnormal variant in %	±10% DCCT
HbAS	40% S	yes
HbCC	90% C/0.6% F	yes
HbEE	86% E/12% F	yes
HbSC	49% S/ 48% C	yes
HbSS	65% C/20% F	yes
HbF	23% F	yes
β-thal	5.6% A2/3%F	yes

Table1: Interferences by Hb variants

RESULTS

A method comparison of HbA1c net FS against HPLC (figure 1) with 90 native samples demonstrated excellent correlation [r=0.9976; Passing/Bablok: y=0.980x – 0.645 mmol/mol HbA1c IFCC]. HbA1c net FS is highly precise with an intra-assay precision of CV<0.9% (for HbA1c values from 37 to 113 mmol/mol), (table 2). High accuracy of HbA1c net FS was demonstrated by recovery of IFCC controls (with varying Hb and HbA1c levels) within ± 3% of the target value (figure 2). Common Hb variants as e.g. HbAS, HbCC, HbEE, HbSC, HbSS, elevated HbF and ß-Thalassemia showed no significant interference (table 1).

CONCLUSIONS

DiaSys HbA1c net FS assay reveals outstanding specificity and precision. This test correlates very well to HPLC (NGSP/DCCT) and IFCC reference material. The application of HbA1c net on the fully automated system BioMajesty®JCA-BM 6010/C allows on-board hemolysis of samples with optimized workflow and eliminates additional error-prone and time-consuming manual sample preparation steps.

Intra-assay precision (n=20)	Mean [mmol/mol]	SD [%]	CV [%]
Sample 1	85.6	0.458	0.54
Sample 2	32.2	0.269	0.84

Intra-assay precision (n=20)	Mean [%]	SD [%]	CV [%]
Sample 1	10.0	0.042	0.42
Sample 2	5.10	0.025	0.48

Total precision (n=20)	Mean [mmol/mol]	SD [%]	CV [%]
Sample 1	86.3	1.696	1.97
Sample 2	30.8	0.681	2.21

Total precision (n=20)	Mean [%]	SD [%]	CV [%]
Sample 1	10.04	0.155	1.55
Sample 2	4.97	0.062	1.25

Table 2: Intra-assay precision

REFERENCES

1. Thomas L. Clinical Laboratory Diagnostics. 1st ed. Frankfurt:TH-Books Verlagsgesellschaft; 1998. p. 142-48.
2. Sacks DB. Carbohydrates. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B. Saunders Company; 1999. p. 790-6.
3. Sacks DB. Carbohydrates. In: Burtis CA, Ashwood ER, Bruns DE, editors. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 4th edition St. Louis Missouri: Elsevier Saunders; 2006; p. 878-884.
4. Jeppsson JO, Kobold U, Barr J, Finke A et al. Approved IFCC reference method for the measurement of HbA1c in human blood. Clin Chem Lab Med 2002; 40: 78-89.
5. Hoelzel W, Weykamp C et al. IFCC Reference System for Measurement of Hemoglobin A1c in Human Blood and the National Standardization Schemes in the United States, Japan, and Sweden: A Method-Comparison Study. Clin Chem 2004; 50 (1): 166-74.
6. The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes in the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med.1993; 329: 977-86.
7. Little RR, Rohlfing CL, Wiedmeyer HM, Myers GL et al. The National Glycohemoglobin Standardization Program: A Five-Years Progress Report. Clin Chem 2001; 47: 1985-92.