Evaluation of a novel enzymatic HbA1c test on the fully automated system respons®910

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Introduction

Glycated Hemoglobin A1c (HbA1c) is a well established parameter for long-term monitoring and the diagnosis of diabetes. Here, we present a novel enzymatic HbA1c test (HbA1c net FS) for highly specific detection of HbA1c, excluding putative interferences by common hemoglobin (Hb) variants. HbA1c net demonstrates excellent precision based on the application type (twin-test). This test links 2 calibrations and 2 detections for Hb and HbA1c in only one determination. The test principle is defined by Hb determination after sample hemolysis at 570 nm and H_2O_2 release after oxidative cleavage of fructosylated dipeptides in the same cuvette. H_2O_2 concentration is determined colorimetrically at 660 nm, whereas delta absorbance is proportional to the HbA1c concentration. The aim of this study was to establish a novel enzymatic HbA1c test, with superior performance, convenient handling and optimized workflow compared to other common methods in the market.

Methods and Procedures

Assay adaption and performance verification have been carried out on respons®910 clinical chemical analyzer. All reagents, calibrators and controls were provided by DiaSys Diagnostic Systems GmbH. Method comparisons were performed against HPLC as reference system. Data have been evaluated by using regression analysis according to Passing and Bablok. Inter- and intra-assay imprecision were performed according to the CLSI protocol (EP5-A2). Whole blood samples can be stored on-board in a plurality of primary tubes. To counteract sedimentation of erythrocytes and prolong the sample processing time the probe aspirates 10 mm below the surface and performs a mixing through aspiration and expulsion of whole blood. The on-board hemolysis is carried out in one reaction cuvette, the two stepped HbA1c determination is carried out in another reaction cuvette (figure 1).

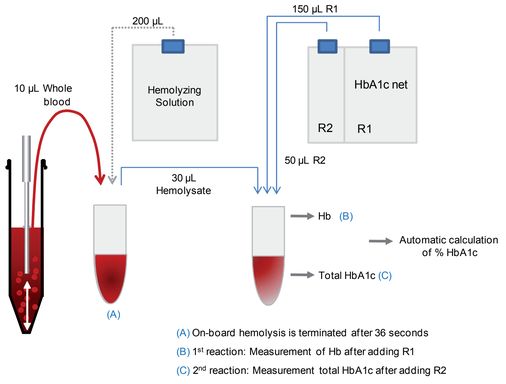

Figure 1: HbA1c net FS processing scheme on respons®910

Results

Due to the whole blood mixing ability of samples with a non-elevated erythrocyte sedimentation rate stored in common primary tubes, could be processed up to 90 min on-board and are recovered within ±10% limits (table 1).

A method comparison of HbA1c net FS against HPLC (figure 2) with 29 native samples demonstrated a good correlation [r=0.9924; Passing/Bablok: y=0.984x-0.974 mmol/mol HbA1c IFCC]. HbA1c net displays an intra-assay imprecision (table 2) of CV<1.4% for values from 39 to 112 mmol/mol and an inter-assay imprecision (table 3) of CV<3.5% for values from 36 to 111 mmol/mol. Good accuracy of HbA1c net FS was demonstrated by recovery of IFCC controls (with varying Hb and HbA1c levels) within ±9% of the target value (table 4).

Primary tube type	Whole blood on-board time until hemolysis	HbA1c [mmol/mol]	Recovery [%]
	Reference	23.6	100
Sarstedt 2 mL	30 min	22.1	93.4
Blood tube	90 min	21.9	92.8
EDTA preparation	180 min	21.0	88.7
Sarstedt 3 mL	30 min	23.9	101
Blood tube	90 min	24.1	102
EDTA preparation	180 min	26.8	113
Sarstedt 4 mL	30 min	25.7	108
Blood tube	90 min	23.5	99.1
EDTA preparation	180 min	25.1	106
BD 2 mL	30 min	23.1	97.5
Hemogard™ / Vacutainer®	90 min	23.1	97.3
EDTA preparation	180 min	22.7	95.7
BD 3 mL	30 min	23.5	99.1
Hemogard™ / Vacutainer®	90 min	23.4	98.6
EDTA preparation	180 min	22.5	95.0
BioGreinerOne 2 mL	30 min	23.0	96.9
Vacuette®	90 min	22.9	96.8
EDTA preparation	180 min	21.9	92.5
BioGreinerOne 4 mL	30 min	23.7	99.9
Vacuette®	90 min	23.5	99.0
EDTA preparation	180 min	-	-

Table 1: Whole blood on-board storage time until hemolyzation

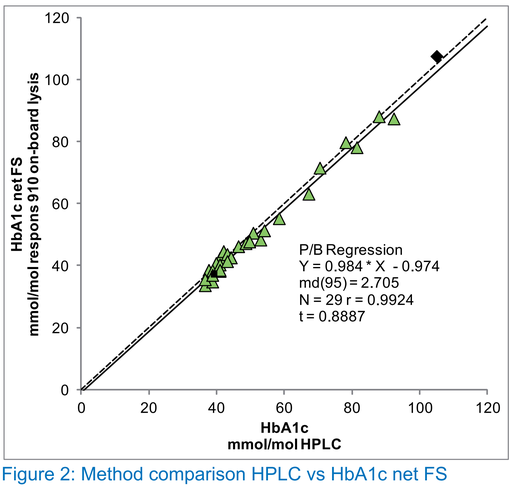

Figure 2: Method comparison HPLC vs HbA1c net FS

Intra-assay imprecision (n=20)	Mean [mmol/mol]	SD [mmol/mol]	CV [%]
Sample 1	38.6	0.53	1.38
Sample 2	49.3	0.33	0.66
Sample 3	84.2	0.39	0.46
Sample 4	112	0.29	0.26

Table 2: Intra-assay Imprecision

Inter-assay imprecision (n=20)	Mean [mmol/mol]	SD [mmol/mol]	CV [%]
Sample 1	36.3	1.26	3.47
Sample 2	61.3	1.44	2.34
Sample 3	75.7	2.29	3.02
Sample 4	111	3.02	2.72

Table 3: Inter-assay Imprecision

Hb	A1c	Mean HbA1c [mmol/mol]	Recovery [%]
Normal	Low	35.3	109
Normal	Medium	58.2	106
Normal	High	79.1	106
Low	Medium	57.7	105
High	Medium	55.6	101

Table 4: Recovery of IFCC controls

Conclusion

DiaSys HbA1c net FS assay on the fully automated respons®910 system reveals excellent specificity and precision. This test has a high correlation to HPLC (NGSP/DCCT) but also to IFCC reference material. The unique aspiration and expulsion feature of the system eases handling of whole blood samples. By application of HbA1c net to the DiaSys system respons®910, HbA1c workflow is optimized, due to the implemented on-board hemolysis eliminating error-prone and time-consuming manual preparations.

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