A FULLY AUTOMATED HbA1c ASSAY ON THE DxC 500 AU CLINICAL CHEMISTRY ANALYZERS

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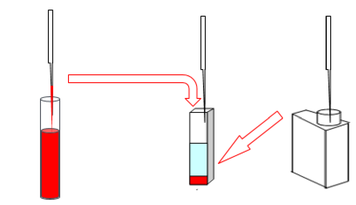
ABSTRACT

Background: Diabetes mellitus is a condition characterized by hyperglycemia resulting from the body's inability to use blood glucose for energy. In Type 1 diabetes, the pancreas no longer makes insulin and blood glucose cannot enter the cells to be used for energy. In Type 2 diabetes, the pancreas does not make enough insulin or the body is unable to use insulin correctly. According to the World Health Organization (WHO), 422 million adults were living with diabetes globally in 2014 with an estimated 1.6 million deaths directly associated with diabetes annually. This number of diabetes cases is expected to more than double in the next 25 years. HbA1c is the major species of glycohemoglobin in human blood. HbA1c formation occurs through a non-enzymatic reaction, called glycation, which occurs between glucose and the n-terminal valine of the hemoglobin β-chain of HbA. A Schiff base intermediate product is formed, which rearranges to form a stable ketoamine in an irreversible reaction. The rate of glycation is proportional to the glucose concentration in the bloodstream and is an accurate reflection of average blood glucose over a period of eight to twelve weeks. HbA1c testing is recommended for the diagnosis of diabetes by the International Expert Committee (IEC), the American Diabetes Association (ADA), and the WHO, who recommended a diagnostic threshold of ≥6.5% (≥48 mmol/mol) HbA1c and a range for pre-diabetes of 5.7-6.4% (39–46 mmol/mol) HbA1c. **Methods:** The HbA1c Advanced assay utilizes automatic sample pre-treatment, has batch and random access capability. No manual pre-treatment of the whole blood sample or additional washing steps are required. Firstly the red blood cells are hemolyzed automatically, total hemoglobin and glycated hemoglobin are then measured colorimetrically and immunoturbidimetrically respectively. **Results:** Precision studies were conducted according to CLSI EP05-A3. Commercial controls and four native K2 EDTA whole blood samples ranging from 5.1 to 12.0% HbA1c, were run twice daily, over twenty days using three lots of reagent on three DxC 500 AU Clinical Chemistry analyzers at a single site. Repeatability ranged from 0.86 to 1.44% CV and Total Precision ranged from 1.55 to 2.43% CV. Linearity studies were conducted according to CLSI EP06-A, and verified an analytical measuring range of 4.0 – 15.0% (NGSP) and 20 – 140 mmol/mol (IFCC). Method comparison and bias estimation was evaluated using CLSI EP09-A3. K2 EDTA patient samples (n=150) across the analytical range were run versus a Secondary Reference method and yielded a slope of 1.031, intercept -0.147% HbA1c, correlation coefficient R=0.997 for Weighted Deming regression, and slope 1.000 and intercept 0.06% HbA1c for Passing Bablok regression. Interference studies carried out demonstrated no significant interference from common endogenous interferences, a large panel of drugs, common Hb variants (HbC, HbD, HbE, HbA2 and HbS) and cross reactants (HbA0, HbA1a+b, acetylated hemoglobin, carbamylated hemoglobin, glycated hemoglobin, glycated albumin and labile HbA1c). **Conclusion:** The HbA1c Advanced assay on the DxC 500 AU* is a precise and accurate assay, requiring no manual pre-treatment and can be used for monitoring and diagnosing diabetes.

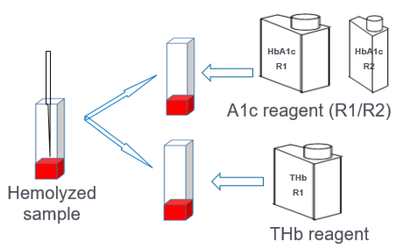
***Pending clearance by the United States Food and Drug Administration. Not currently available for in vitro diagnostic use.**

INTRODUCTION

A fully automated HbA1c assay was verified on the DxC 500 AU Clinical Chemistry analyzer, with automated whole blood sample pre-treatment capability. The DxC 500 AU aspirates and automatically performs the hemolysis of the whole blood sample on the instrument. The sample probe is designed to dive to 70% depth within the whole blood sample to minimize effects from erythrocyte settling. The DxC 500 AU contains a specialized wash well to clean the sample probe after each aspiration.



The hemolyzed whole blood is added to two separate cuvettes. HbA1c and Total Hemoglobin (THb) are then measured immunoturbidimetrically and colorimetrically respectively, and the overall %HbA1c result is calculated automatically by the instrument.



METHODS

Precision (Table 1)

- Precision studies were conducted according to CLSI EP05-A3: "Evaluation of Precision Performance of Quantitative Measurement Methods: Approved Guideline – Third Edition". Four native K2 EDTA whole blood samples, and two whole blood commercial controls ranging from 5.1 to 14.0% HbA1c, were run twice daily in random order, over twenty days using three lots of reagent on three DxC 500 AU Clinical Chemistry analyzers at a single site.

METHODS (CONTINUED)

Linearity (Figure 1)

- Linearity studies were conducted according to CLSI EP06-A: "Evaluation of the Linearity of Quantitiative Measurement Procedures: A Statistical Approach; Approved Guideline". High and low pools were prepared using human whole blood, which was spiked or diluted as required with human-based material. Samples were mixed together in varying ratios to obtain 9 linearity levels that were then run in quadruplicate. The measured values were compared to the theoretical values, based upon the dilution factor. Data was analyzed using weighted regression analysis.

Interferences (Tables 2 to 4)

- Interferences from endogenous substances, drugs, cross reactants and hemoglobin derivates were tested according to CLSI Guideline EP07 Third Edition: "Interference Testing in Clinical Chemistry; Approved Guideline" at two % HbA1c concentrations, approximately 6.5% HbA1c (low level human venous whole blood EDTA sample) and approximately 8.0% HbA1c (high level human venous whole blood EDTA sample). Testing of endogenous substances was completed using one lot of reagent on DxC 500 AU analyzer and drugs, cross reactants and hemoglobin derivates were tested on DxC 700 AU and are leveraged for the DxC 500 AU.
- To assess potential interferences from common hemoglobin variants, a minimum of 20 samples of each variant type were measured in singlicate and the results compared to that from a reference method, demonstrated to be free from hemoglobin interference.
- The criteria for no significant interference for all interference studies is recovery within 7% of the initial value (sample containing no interferent) or the reference value (value assigned in a Secondary Reference Laboratory), as applicable.

Method Comparison (Figure 2)

- A correlation study was performed using guidance document CLSI EP09-A3: "Measurement Procedure Comparison and Bias Estimation Using Patient Samples". Singlicate results from human K2 EDTA whole blood specimen results from the HbA1c Advanced (B93009) assay on the DxC 500 AU were compared with those from a Secondary Reference Laboratory method.

Total Error (Table 5)

- Using the results of the bias estimation (%bias) in the method comparison study and total precision estimates in the precision study (%CV), Total Error (TE) at four HbA1c concentrations was calculated as follows: %TE = |%Bias| +1.96*%CV*(1+%Bias/100).

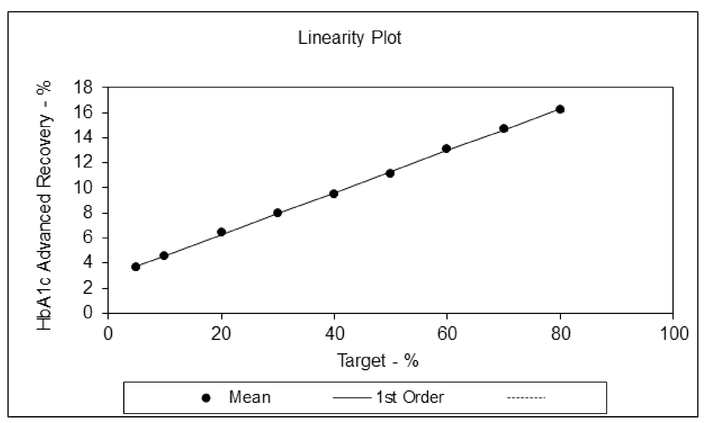
RESULTS

Table 1 Summary of 20 day precision results for 3 lots x 3 instruments (%NGSP units)

Sample Details	Mean %HbA1c	Repeatability (Within-Run)		Between-Run		Between-Day		Between-Lot		Between Instrument		Total Precision	
		%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD
Human Whole Blood 1	5.06	1.44	0.07	0.00	0.00	1.14	0.06	0.66	0.03	1.45	0.07	2.43	0.12
Human Whole Blood 2	6.28	0.86	0.05	0.00	0.00	0.79	0.05	0.53	0.03	0.88	0.06	1.55	0.10
Human Whole Blood 3	7.87	0.86	0.07	0.25	0.02	0.86	0.07	0.61	0.05	0.93	0.07	1.66	0.13
Human Whole Blood 4	11.97	0.97	0.12	0.00	0.00	0.96	0.11	0.65	0.08	1.10	0.13	1.87	0.22
Whole Blood Control 1	5.68	1.28	0.07	0.30	0.02	1.14	0.06	0.84	0.05	1.16	0.07	2.25	0.13
Whole Blood Control 2	10.28	0.89	0.09	0.27	0.03	0.75	0.08	0.72	0.07	0.87	0.09	1.64	0.17

Total precision results for all human whole blood samples and controls were ≤ 2% CV or ≤ 0.13 %HbA1c SD.

Figure 1 Linearity results



An analytical measuring range of 4.0 – 15.0% (NGSP) and 20 – 140 mmol/mol (IFCC) was verified. (IFCC results not shown.)

RESULTS (CONTINUED)

Table 2 Summary of cross reactant and hemoglobin derivative interference testing.

HbA0	12 mg/mL	Carbamylated hemoglobin	1.5 mg/mL
HbA1a+b	0.16 mg/mL	Glycated albumin	5 mg/mL
Acetylated hemoglobin	0.5 mg/mL	Labile Hemoglobin	2000 mg/dL

No significant interference is observed up to the concentrations stated in Table 2. Leveraged from the DxC 700 AU for the DxC 500 AU

Table 3 Summary of endogenous substances and drug interference testing.

Acarbose*	0.05 mg/dL	Levodopa*	20 mg/dL
Acetaminophen*	26 mg/dL	Lipemia (Intralipid)	500 mg/dL
Acetylcystein*	166 mg/dL	Metformin*	5 mg/dL
Acetylsalicylic Acid*	1000 mg/dL	Methyldopa*	20 mg/dL
Ampicillin-Na*	1000 mg/dL	Metronidazole*	200 mg/dL
Ascorbic Acid	300 mg/dL	Phenzylbutazone*	53.5 mg/dL
Calcium Dobsilate*	20 mg/dL	Rheumatoid Factor	1000 IU/mL
Cefoxitin*	2500 mg/dL	Rifampicin*	8 mg/dL
Conjugated Bilirubin	60 mg/dL	Rosiglitazone maleate*	0.33 mg/dL
Cyclosporine*	0.5 mg/dL	Salicyclic Acid*	4.76 mg/dL
Doxycyclin*	50 mg/dL	Sitagliptin*	0.2 mg/dL
Glucose	2000 mg/dL	Theophylline*	10 mg/dL
Glyburide*	0.12 mg/dL	Total Protein	21 g/dL
Heparin*	5500 IU/L	Triglyceride	1000 mg/dL
Ibuprofen*	50 mg/dL	Unconjugated Bilirubin	60 mg/dL

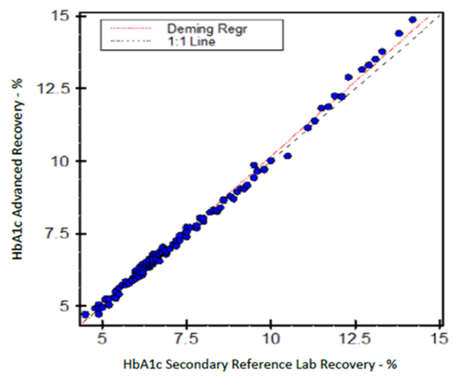
No significant interference is observed up to the concentrations stated in Table 3.
*Leveraged from the DxC 700 AU for the DxC 500 AU

Table 4 Summary of hemoglobin variant interference testing

Hemoglobin Variant	No. of Samples	% Variant	Range in %HbA1c Concentration	Relative %Bias 6.5% HbA1c	Relative %Bias 9.0% HbA1c
HbC	28	31 – 38	4.84 – 10.38	-0.73	-1.72
HbD	20	38 – 42	5.39 – 7.54	0.31	-1.56
HbE	22	23 – 27	5.20 – 9.44	3.91	4.66
HbS	24	35 – 41	4.95 – 12.84	1.45	0.71
HbA2	23	2.0 – 6.2	4.85 – 10.7	-0.99	0.14

No significant interference is observed up to 38% HbC, 42% HbD, 27% HbE, 6.2% HbA2, 41% HbS. Samples containing ≥ 7%HbF may result in lower than expected HbA1c results.

Figure 2 Method Comparison results (Deming Regression analysis)



Slope	Intercept =	r =	N =	Sample range =
= 1.031	-0.147 %HbA1c	0.997	150	4.5 -14.2% HbA1c

A comparison of the HbA1c Advanced assay versus the Secondary Reference Laboratory (SRL) HPLC method shows a high degree of correlation.

Table 5 Summary of Calculated Total Error

%HbA1c	% Bias	% CV	%TE
5.0	0.16	2.43	4.93
6.5	0.84	1.55	3.90
8.0	1.26	1.66	4.55
12.0	1.88	1.87	5.61

The Total Error results for all samples are within the 6% TE criterion.

CONCLUSION

The HbA1c Advanced assay on the DxC 500 AU is a precise and accurate assay, requiring no manual pre-treatment of the whole blood sample. The assay has no significant interference from common endogenous substances, drugs, cross reactants, hemoglobin derivatives, or common hemoglobin variants; can be used for monitoring and diagnosing diabetes.