



UPDATED: INTRODUCTION OF A NEW AND IMPROVED SOFTWARE v5.24F (G8 v5.24F) FOR HEMOGLOBIN A1C TESTING ON THE TOSOH HLC723-G8 CATION EXCHANGE HPLC ANALYZER

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ABSTRACT

Background:

The measurement of HbA1c is well established as an accurate index of the mean blood glucose concentration in monitoring glycemic control in patients with diabetes mellitus. A well accepted method for HbA1c measurement is the separation and quantitation of HbA1c in whole blood by Ion Exchange HPLC on the Tosoh G8 (Tosoh Bioscience, San Francisco, CA). The Tosoh G8 program is based on chromatographic separation of HbA1c on cation exchange column. The non-porous nature of the column makes it a very efficient method for separation of peaks of the chromatogram. The separation is optimized to reduce interferences from commonly occurring hemoglobin variants and Fetal Hb thus enhancing the performance of the G8.

Objectives:

The study was carried out to evaluate the Tosoh Automated Glycohemoglobin Analyzer HLC723-G8 with the v5.24F (G8 v5.24F) software modification to assess interference when measuring %HbA1c in the presence of hemoglobin (Hb) variants and HbF. The %A1c results obtained on the G8 v5.24F were compared to the results from the Trinity Biotech Ultra2 analyzer for the (HbAS, HbAC, HbAD, & HbAE) variants; and to results from the Bio-Rad Variant II Turbo 2.0 analyzer for HbF.

Methods:

A set of 25-30 specimens each with a pre-determined %A1c value ranging 4-10% with a variant concentration anywhere between 20-40% HbAC, HbAD, HbAS, & HbAE were run and the results obtained on the G8 v5.24F were compared with Trinity Biotech Premier Hb9210™ HbA1c Analyzer.

Similarly, for HbF, a set of 21 samples were run and results were compared with the Bio-Rad Variant II TURBO analyzer. The HbF samples were prepared by using a 10:0, 6:4, 5:5, 4:6 and 0:10 spiking protocol to achieve concentrations between 1-40%.

Conclusion:

The study concluded that the performance testing results met their pre-determined acceptance criteria. Accurate and reportable HbA1c% results in the presence of HbC (37.8%), HbD (39.5%) and HbS (38.9%), HbE (30.9%) and HbF of up to 25%. Clinically significant interference is defined as $\geq \pm 6\%$ relative difference in the results from a comparison method at 6% and 9% A1c. The results obtained were less than $\pm 6\%$ relative difference in the results from a comparison method at 6% and 9% A1c. All performance testing results met their pre-determined acceptance criteria. The modified software, version 5.24F, reduces interference from commonly occurring variants and Fetal Hb when measuring %HbA1c.

INTRODUCTION

Hemoglobin A1c is an important analyte for the detection and diagnosis of diabetes. Methods such as ion exchange HPLC separate the hemoglobin components into fractions enabling the visualization and the presumptive identification of hemoglobin variants. Not all A1c testing methods are the same. Charge based separation methods such as ion exchange HPLC are able to detect variant hemoglobins and structure based separation methods such as immunoassays and boronate affinity chromatography do not have the ability to detect variants. Charge based separation methods provide hemoglobin peak separation profile in the form of a chromatogram. The method is designed to quantitate A1c but also presumptively identifies the most commonly occurring variant hemoglobins which immunoassays are not able to achieve.

The most commonly occurring variant hemoglobins are HbS, C, D and E. In this poster the Tosoh new software v5.24F for G8 has been evaluated in the presence of variants.

The previous G8 software versions could not report HbA1c in the presence of heterozygous HbE. The version v5.24F has been evaluated to determine its ability to report A1c in the presence of HbE in addition to the variants HbS, HbC, HbD.

METHODS

A set of 25-30 specimens each with a pre-determined %A1c value ranging 4-10% with a variant concentration anywhere between 20-40% HbAC, HbAD, HbAS, & HbAE were run and the results obtained on the G8 v5.24F were compared with Trinity Biotech Premier Hb9210™ HbA1c Analyzer.

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STATISTICAL DATA

Hemoglobin Variant Interference Study:

Possible interference when measuring HbA1c in clinical specimens due to variant hemoglobins is well known and documented. Common hemoglobin variants have been shown to interfere with HbA1c results with some assay methods^{1,2}. The prevalence of hemoglobinopathies varies among populations³. The most common of the beta chain variants are hemoglobins S, C, D and E.

The study conducted at NGSP SRL site was designed in accordance with CLSI EP07 Interference Testing in Clinical Chemistry; Approved Guideline – Third Edition and CLSI EP09-A3 Measurement Procedure and Bias Estimation 2013. Venous whole blood specimens containing varying levels of the variant were tested in triplicate on both the Tosoh Automated Analyzer HLC-723G8 with software version 5.24F (G8 5.24F) and the comparator. The percent of the variant in question was ascertained from the Sebia Capillarys 2 instrument using the Hemoglobin method.

Interference studies were conducted on known concentrations of %HbA1c and the specified variant in venous whole blood. Clinically significant interference was defined as $\geq 6\%$ relative difference in the results from the comparator at 6% or 9% HbA1c. Based on the results, the G8 5.24F does not demonstrate any clinical interference on the HbA1c levels at the % levels of variant for each hemoglobin variant as listed in the Table below:

Table 1: Percent Relative Bias from Reference Method

Hemoglobin Variant/ Hemoglobinopathy	Percent Relative Bias from Reference Method at Low and High Concentrations of HbA1c Samples			
	~6.5 % HbA1c		~8.0 % HbA1c	
	Calibrated Relative % Difference	Range	Calibrated Relative % Difference	Range
HbAC	-1.9	0.03 to 0.17	-1.1	0.06 to 0.34
HbAD	-0.5	0.08 to 0.30	-1.7	-0.04 to 0.36
HbAE	-1.3	0.001 to 0.27	-1.2	-0.10 to 0.49
HbAS	-2.7	-0.04 to 0.13	-3.2	-0.14 to 0.21
HbA2	-4.2	-0.17 to 0.06	-5.1	-0.37 to 0.12
HbF	-0.7	0.10 to 0.25	-1.6	-0.01 to 0.34

Table 2: Variant Samples Used in Hemoglobin Variant Study

Hemoglobin Variant/Hemoglobinopathy	n	Range in % Abnormal Variant/Hemoglobinopathy	Range in % HbA1c Concentration
HbC	26	30.8 to 37.8	4.8 to 9.8
HbD	24	29.5 to 39.5	5.3 to 9.347
HbE	26	20.0 to 30.9	4.763 to 9.7
HbS	29	28.2 to 38.9	4.9 to 10.05
HbA2	20	2.7 to 5.5	5.85 to 10.1
HbF	21	15.4 to 43.5	4.36 to 8.9

Fetal Hemoglobin and interference:

Elevated levels of Fetal Hemoglobin (HbF) seen with Hereditary Persistence of Fetal Hemoglobin (HPFH) may interfere with the A1c result.

Samples spiked with various concentrations of umbilical cord blood were measured by the G8 v5.24F and a cleared diagnostic HbA1c device (Bio-Rad Variant II Turbo 2.0) to compare results at 6% and 8% HbA1c levels. At up to 25% Fetal Hb the G8 v5.24F device did not interfere at clinically relevant levels of HbA1c.

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FIGURES

Fig 1: Method Comparison Deming Regression Analysis of G8 v5.24 vs NGSP SRL (Tosoh G8 vs Trinity Premier)

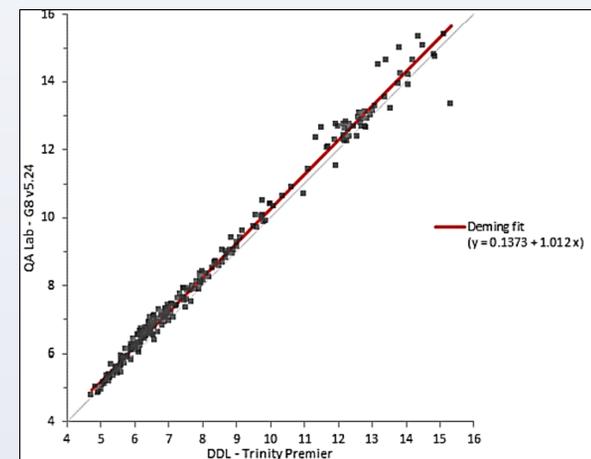
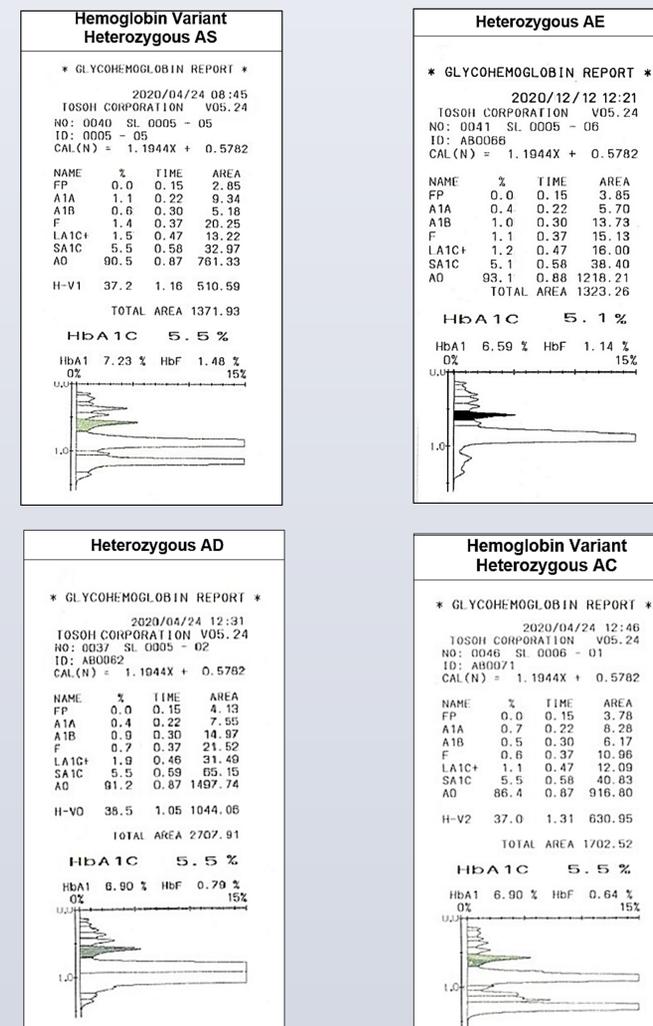


Fig 2: Sample chromatograms for variant hemoglobins



CONCLUSIONS

The study concluded that the performance testing results met their pre-determined acceptance criteria. Accurate and reportable HbA1c% results in the presence of HbC (37.8%), HbD (39.5%) and HbS (38.9%), HbE (30.9%) and HbF of up to 25%. Clinically significant interference is defined as $\geq \pm 6\%$ relative difference in the results from a comparison method at 6% and 9% A1c. The results obtained were less than $\pm 6\%$ relative difference in the results from a comparison method at 6% and 9% A1c. All performance testing results met their pre-determined acceptance criteria. The modified software, version 5.24F, reduces interference from commonly occurring variants and Fetal Hb when measuring %HbA1c. With version v5.24F HbA1c is reportable in the presence of HbE, HbS, HbC and HbD.