510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k131580

B. Purpose for Submission:

Addition of a diagnostic claim to a previously cleared device

C. Measurand:

Whole Blood Glycosylated Hemoglobin (HbA1c)

D. Type of Test:

Ion-exchange High Performance Liquid Chromatography (HPLC)

E. Applicant:

Tosoh Bioscience, Inc.

F. Proprietary and Established Names:

Automated Glycohemoglobin Analyzer HLC-723G8

G. Regulatory Information**:**

Product Code	Classification	Regulation Section	Panel
PDJ	Class II	21 CFR 862.1373	Chemistry 75

H. Intended Use:

1. Intended use(s):

   See indications for use below

2. Indication(s) for use:

The Tosoh Automated Glycohemoglobin Analyzer HLC-723G8 is intended for *in vitro* diagnostic use for the measurement of % hemoglobin A1c (HbA1c) (DCCT/NGSP) and mmol/mol hemoglobin A1c (IFCC) in whole blood specimens. This test is to be used as an aid in diagnosis of diabetes and identifying patients who may be at risk for developing diabetes.

3. Special conditions for use statement(s):

This device has known Hemoglobin E (HbE) interference. When a sample is suspected to contain HbE a flag will be displayed. The HbA1c result will not be reported from the analyzer.

Hemoglobin A1c should not be used to diagnose diabetes mellitus in patients with a hemoglobinopathy but normal red cell turnover (e.g. sickle cell trait).

Hemoglobin A1c should not be used to diagnose patients with abnormal red cell turnover (e.g. anemias from hemolysis and iron deficiency).

Hemoglobin A1c testing should not replace glucose testing for type 1 diabetes, in pediatric patients and in pregnant women.

This test should not be used to diagnose patients with iron deficiency and hemolytic anemia, various hemoglobinopathies, thalassemias, hereditary spherocystosis, malignancies and severe chronic hepatic and renal disease.

Hemoglobin A1c should not be used in pregnant patients, patients with homozygous sickle cell trait, hemolytic anemia, or other hemolytic diseases, and recent significant or chronic blood loss.

Hemoglobin A1c should not be used to diagnose diabetes mellitus in patients with hereditary spherocytosis, malignancies or severe chronic hepatic and renal disease.

Hemoglobin A1c should not be used in the diagnosis of gestational diabetes.

In cases of rapidly evolving type 1 diabetes the increase of HbA1c values might be delayed compared to the acute increase in glucose concentrations. In these conditions diabetes mellitus must be diagnosed based on plasma glucose concentration and/or the typical clinical symptoms.

For prescription use only.

4. Special instrument requirements:

All performance data was conducted using the Tosoh Automated Glycohemoglobin Analyzer HLC-723G8

I. Device Description:

The Tosoh Automated Glycohemoglobin Analyzer HLC-723G8 is an automated High Performance Liquid Chromatography (HPLC) system that separates and reports stable A1c (sA1c) percentage in whole blood. The operational portion of the G8 is composed of a sampling unit, liquid pump, degasser, column, detector, microprocessors, sample loader, floppy disk drive unit, operation panel and a printer.

The Tosoh Automated Glycohemoglobin Analyzer HLC-723G8 uses non-porous ion-exchange HPLC for rapid, separation of the stable form of HbA1c from other hemoglobin fractions. The G8 uses a cation exchange column and separated the hemoglobin components in the blood into six fractions, A1a, A1b, F, La1c, sA1c and A0. The separation is done by eluting the hemoglobins from the column with a stepwise elution of three elution buffers containing different salt concentrations. The result report includes a sample ID, date, percentage and retention time of each fraction, sA1c percentage and total A1 percentage (A1a+A1b+sA1c), along with a chromatogram of the elution pattern of the hemoglobin fractions. If a sample contains a hemoglobin variant, the column eluted the material depending upon the charge.

Calibrators (Tosoh A1c Calibrator Set) and controls (Canterbury Scientific Hemoglobin A1c) are recommended for use with this device. The calibrators and controls were previously cleared under 510(k) numbers k071132 and k021484, respectively.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Roche COBAS INTEGRA 800 Tina-quant HbA1cDx Gen.2 assay

2. Predicate 510(k) number(s):

k121291

3. Comparison with predicate:

Similarities and Differences		
Item	Candidate Device Tosoh Glycohemoglobin Analyzer HLC-723G8	Predicate Roche COBAS INTEGRA 800 Tina-quant HbA1c Dx. Gen.2 assay (k121291)
Indication for Use/Intended Use	The Tosoh Automated Glycohemoglobin Analyzer HLC-723G8 is intended for *in vitro* diagnostic use for the measurement of % hemoglobin A1c (HbA1c) (DCCT/NGSP) and mmol/mol	This test is to be used as an aid in diagnosis of diabetes and as an aid in identifying patients who may be at risk for developing diabetes The COBAS INTEGRA

Similarities and Differences		
Item	Candidate Device Tosoh Glycohemoglobin Analyzer HLC-723G8	Predicate Roche COBAS INTEGRA 800 Tina-quant HbA1c Dx. Gen.2 assay (k121291)
	hemoglobin A1c (IFCC) in whole blood specimens. This test is to be used as an aid in diagnosis of diabetes and identifying patients who may be at risk for developing diabetes.	800 Tina-quant HbA1cDx Gen.2 assay is an in vitro diagnostics reagent system intended for quantitative determination of mmol/mol hemoglobin A1c (IFCC) and % hemoglobin A1c (DCCT/NGSP) in hemolysate or whole blood on the Roche COBAS INTEGRA 800 clinical chemistry analyzer.
Specimen Type	Human Whole Blood	Same
Matrix	K3-EDTA Whole Blood	EDTA-Li-Heparin, Na Heparin, NaF/K-Oxalate Whole Blood
Assay Principle	Ion-exchange HPLC	Quantitative turbidimetric inhibition immunoassay
Detection Method	Visible wavelength detector	Absorption spectrum and measured biochromatically

K. Standard/Guidance Document Referenced (if applicable):

CLSI EP7-A2, Interference Testing in Clinical Chemistry; Approved Guideline

CLSI EP5-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline

CLSI-EP9-A2-IR, Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline

L. Test Principle:

The analyzer dilutes the whole blood specimen with Hemolysis & Wash Solution, and then injects a small volume of this specimen onto the TSKgel G8 Variant HSi column. Separation is achieved by utilizing differences in ionic interactions between the cation exchange group

in the column resin surface and the hemoglobin components. The hemoglobin fractions (designated as A1a, A1b, F, LA1c+, SA1c, A0, and H-V0, H-V1, H-V2) are subsequently removed from the column by performing a step-wise elution using the varied salt concentrations in the Variant Elution Buffers His 1, 2 and 3. The separated hemoglobin components pass through the LED photometer flow cell where the analyzer measures changes in absorbance at 415 nm. The analyzer integrates and reduces the raw data, and then calculates the relative percentages of each hemoglobin fraction. The Total Area of the sA1c is divided by the sum of the total areas of all peaks, excluding the HbF peak and any variant peak(s) to obtain a raw sA1c percentage. This uncorrected result is substituted as the "x" value in the linear regression formula determined during calculation. The analyzer prints the final numerical results and plots a chromatogram showing changes in absorbance versus retention time for each peak fraction.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

A precision study was performed testing 4 concentrations of unaltered EDTA whole blood samples at the following targeted HbA1c values: ~5%, ~6.5%, ~8% and ~12%. Precision was evaluated using three Tosoh Automated Glycohemoglobin HLC-723G8 analyzers and 3 lots of reagent on each analyzer. Measurements of 2 replicates in a single run, 2 times a day for 20 non-consecutive says for each specimen/ reagent combination were analyzed for a total of 40 runs and 80 determinants for each data set.

A summary of the results is shown below:

Analyzer 1: HLC-723G8 SN 12521504

	Repeatability		Between Run		Between Day		Between Lot		Total	
HbA1c	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Sample1 4.98%	0.04	0.86	0.01	0.22	0.09	1.72	0.07	1.32	0.09	1.83
Sample2 6.50%	0.02	0.29	0.01	0.18	0.04	0.63	0.04	0.56	0.04	0.68
Sample3 7.89%	0.02	0.26	0.01	0.16	0.08	0.95	0.09	1.19	0.08	0.98
Sample4 11.90%	0.02	0.18	0.01	0.12	0.15	1.30	0.04	0.30	0.16	1.31

Analyzer 2: HLC-723G8 SN 11078610R

	Repeatability		Between Run		Between Day		Between Lot		Total	
HbA1c	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Sample1 5.00%	0.05	1.00	0.02	0.37	0.08	1.62	0.04	0.84	0.09	1.79
Sample2 6.51%	0.02	0.35	0.01	0.20	0.07	1.11	0.01	0.10	0.07	1.14
Sample3 7.90%	0.03	0.32	0.02	0.20	0.07	0.85	0.03	0.36	0.07	0.89
Sample4 11.89%	0.03	0.23	0.02	0.17	0.19	1.57	0.05	0.45	0.19	1.58

Analyzer 3: HLC-723G8 SN 10316511

	Repeatability		Between Run		Between Day		Between Lot		Total	
HbA1c	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Sample1 4.93%	0.03	0.68	0.03	0.62	0.08	1.56	0.03	0.62	0.08	1.69
Sample2 6.44%	0.03	0.44	0.03	0.53	0.06	0.99	0.00	0.07	0.07	1.10
Sample3 7.81%	0.03	0.34	0.03	0.42	0.06	0.77	0.03	0.37	0.07	0.86
Sample4 11.79%	0.03	0.27	0.03	0.24	0.13	1.11	0.02	0.21	0.13	1.14

HLC-723G8 analyzers (combined)

Mean	Repeatability		Between Run		Between Day		Between Lot		Between - Instrument		Total	
HbA1c	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Sample1 4.95%	0.04	0.80	0.03	0.55	0.08	1.71	0.05	0.92	0.04	0.86	0.09	1.84
Sample2 6.48%	0.02	0.36	0.02	0.34	0.07	1.05	0.04	0.60	0.04	0.62	0.07	1.11
Sample3 7.87%	0.02	0.31	0.02	0.28	0.08	0.99	0.06	0.79	0.05	0.63	0.08	1.04
Sample4 11.86%	0.03	0.23	0.02	0.18	0.16	1.39	0.06	0.52	0.06	0.52	0.17	1.40

b. Linearity/assay reportable range:

Linearity was previously evaluated for this assay under k071132. The reportable range for this device is 4.0-16.9% HbA1c

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

The assigned HbA1c values of the Tosoh Automated Glycohemoglobin Analyzer are certified by The National Glycohemoglobin Standardization Program (NGSP). See the NGSP website for current certification at http://www.ngsp.org.

The final reportable result is traceable to both the International Federation of Clinical Chemistry (IFCC) and the Diabetes Control and Complications Trial (DCCT). The International Federation of Clinical Chemistry (IFCC) units of mmol/mol are calculated using the Master Equation NGSP (%) =0.09148 x IFCC (mmol/mol) + 2.52. HbA1c results are provided to the customers using two different units: NGSP equivalent units (%) and IFCC equivalent units (mmol/mol).

Calibrator and Control materials:

Value assignment procedures for calibrators (Tosoh Hemoglobin Alc Calibrator Set) that are recommended for use with this device were previously reviewed under submission k071132. Controls (Canterbury Hemoglobin A1c) were previously cleared under k021484.

Stability

Stability for calibrators (Tosoh A1c Calibrator Set) and controls (Canterbury Hemoglobin A1c) that are recommended for use with this device were previously reviewed under 510(k) numbers k071132 and k021484, respectively.

d. *Detection limit:*

The reportable range was previously established in k071132. The reportable range of this device is 4.0-16.9% HbA1c

e. *Analytical specificity:*

i.) Endogenous Interference

Studies were performed to assess common or known substances that could interfere with the Tosoh Automated Glycohemoglobin Analyzer. Whole blood samples with HbA1c values of 6.5% HbA1c and ~8% HbA1c were analyzed by spiking the interfering substance into each of the two whole blood samples and then preparing serial dilutions to achieve 10 concentrations. Ten replicates of each of the ten varying concentrations were analyzed and compared to the reference sample (sample containing no interferent). Significant interference was defined as % recovery ≥ +/- 5% of the expected 100% recovery.

The following substances showed that no significant interference at the concentrations described below:

Potential Interferent	Range tested
Bilirubin-Fractionated	2.0-18mg/dL
Conjugated Bilirubin	2.0-21 mg/dL
Lipemia	1-1000 mg/dL
Albumin	500-5000 mg/dL
Ascorbic Acid	3.0-25 mg/dL
Rheumatoid Factor	110-550 IU/mL

ii.) Cross Reactivity

Potential interferences from Acetylated hemoglobin (Hb), Carbamylated Hb, Aldehyde and Labile HbA1c were evaluated using whole blood samples with HbA1c values of ~ 6.5% and ~8% HbA1c. The results were concluded as follows:

- Acetylated Hb (up to 50 mg/dL) does not interfere with this assay.
- Carbamylated Hb (up to 25 mg/dL) does not interfere with this assay.
- Labile HbA1c (up to 1000 mg/dL) does not interfere with this assay.
- Aldehyde (up to 25 mg/dL) does not interfere with this assay.

Sponsor states The HPLC chromatogram shows the separation of HbA0, HbA1a, HbA1b, and HbA1c. The fractions elute in different "windows" and are visible on the chromatogram. There is no cross reactivity with these substances.

iii.) Hemoglobin Variant Interference:

A hemoglobin variant interference study was performed using a total of 120 samples known to contain Hemoglobin variants S,C,E,D, A2 and F. Two whole blood patient samples containing an HbA1c of ~6.5% and ~ 8% and the appropriate Hemoglobin variant were tested. 10 samples were tested at each of the two HbA1c concentrations of ~6.5 % and ~8%. Testing of the samples containing the Hemoglobin variants S,C,E,D and F was performed in singlicate. Testing was performed on the Tosoh Automated Glycohemoglobin HLC-723G8 Analyzer and compared to results obtained by a reference method that has been demonstrated to be free from the hemoglobin interference being tested. The following is a table of the samples that were measured.

Hemoglobin Variant	Number of Samples	% Content of Variant in sample	mMol/mol HbA1c
HbS	20	30-36% S	44-78
HbC	20	35-37% C	47-59
HbE*	20	HbE interferes	HbE interferes
HbD	20	36% D	43
HbF	20	7-24.4 %F	57-74
HbA2	20	12.7-13.9% A2	47.1-62.9

Hemoglobin Variant Results Summary

	Percent Relative Bias from Reference Method at Low and High Concentrations of HbA1c Samples	
Hb Variant	~6.5 % HbA1c	~ 8% HbA1c
S	0	-5
C	5	-5
E	HbE interferes	HbE interferes
D	1	-3
A2	-2	-2
F	5	-2

The results show there is no significant interference for HbS (≤36%), HbC (≤37%), HbD (≤36%), HbA2 (≤13.9%), HbF (≤24.4%)

*The labeling contains a boxed warning which states "The Tosoh Automated Glycohemoglobin Analyzer HLC-732G8 has known Hemoglobin E (HbE) Interference. When a sample is suspected to contain HbE a flag will be displayed. The HbA1c result will not be reported from the analyzer."

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

A method comparison study was performed using a 120 variant-free samples ranging from 4.5% to 16.5% HbA1c evaluated using the candidate Tosoh Automated Glycohemoglobin HLC-723G8 Analyzer. Samples were tested in singlicate over several days. The results were compared to testing performed at a secondary NGSP reference laboratory using a previously cleared HPLC method (Primus Ultra) HbA1c assay. The distribution of samples spanned the measuring interval (with a concentration of samples around the clinical decision points) as follows:

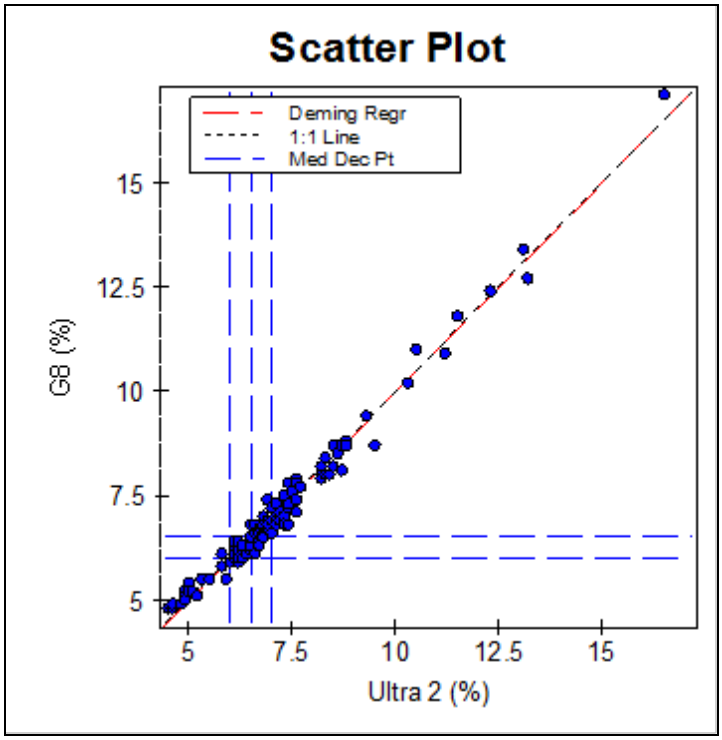
Hemoglobin A1c level	Number of samples	%samples tested
≤ 5%	5	4.2%
5 – 6%	15	12.5%
6 – 6.5%	30	25.0%
6.5 – 7%	30	25.0%
7 – 8%	20	16.7%
8 – 9%	10	8.3
> 9%	10	8.3%
Total samples	120	100%

Bias between Candidate and NGSP methods

Deming (weighted) and Passing-Bablok regression analyses were performed for the Tosoh Automated Glycohemoglobin HLC-723G8 Analyzer versus the reference method.

Summary of results are as follows:

	y-intercept	Slope
Deming	0.073 95%CI: (-0.110 to 0.255)	0.996 95% CI:(0.971 to 1.021)
Passing Bablok	0.05 95%CI: (-0.05 to 0.41)	0.99 95%CI:(0.93 to 1.00)



The following biases between the Tosoh Automated Glycohemoglobin HLC-723G8 Analyzer versus the reference method were observed:

Decision Level	Bias	%Bias
5.0	0.054	1.08
6.5	0.049	0.75
8.0	0.043	0.54
12.0	0.029	0.24

Total Near Cutoff

Using the results of bias estimation (%Bias) in the method comparison study and precision estimated in the reproducibility study, Total Error (TE) four concentrations: (5.0%,6.5%,8.0%, and 12.0%) was calculated as follows: %TE =|%Bias| + 1.96 *%CV*(1+%Bias). The results are presented in the tables below:

Decision Level	%Bias	%CV	%TE
5.0	1.08	1.84	5.8
6.5	0.75	0.68	2.8
8.0	0.54	0.98	3.0
12	0.24	1.31	3.1

b. Matrix comparison:

K3-EDTA whole blood specimens are to be used with this assay.

3. Clinical studies:

a. Clinical Sensitivity:

Not applicable

b. Clinical specificity:

Not applicable

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

   Reference Ranges (non-diabetic): HbA1c 4.0-6.0% (mean 5.0%, SD 0.5%)[1]

   The labeling states "The diagnosis of diabetes and identification of persons at increased risk of developing diabetes follows the ADA Guideline of 6.5% for the cut-off and values between 5.7% and 6.4% as being at increased risk".

   The labeling also states "Each laboratory should determine a reference interval that corresponds to the characteristics of the population being tested"

   [1] American Diabetes Association, Standards of Medical Care in Diabetes -2013; 36(Suppl.1).

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.