



**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY  
ASSAY AND INSTRUMENT**

**I Background Information:**

**A 510(k) Number**

K250073

**B Applicant**

Tosoh Bioscience, Inc.

**C Proprietary and Established Names**

Tosoh Automated Glycohemoglobin Analyzer HLC-723GR01

**D Regulatory Information**

Product Code(s)	Classification	Regulation Section	Panel
PDJ	Class II	21 CFR 862.1373 - Hemoglobin A1c Test System	CH - Clinical Chemistry
LCP	Class II	21 CFR 864.7470 - Glycosylated hemoglobin assay	HE - Hematology

**II Submission/Device Overview:**

**A Purpose for Submission:**

New Device.

**B Measurand:**

Glycosylated Hemoglobin (HbA1c)

**C Type of Test:**

### **III Intended Use/Indications for Use:**

#### **A Intended Use(s):**

See Indications for Use below

#### **B Indication(s) for Use:**

The Tosoh Automated Glycohemoglobin Analyzer HLC-723GR01 is intended for in vitro diagnostic use for the quantitative measurement of % hemoglobin A1c (HbA1c) (DCCT/NGSP) and mmol/mol hemoglobin A1c (IFCC) in human venous whole blood and hemolysate specimens using ion-exchange high performance liquid chromatography (HPLC). This test is to be used as an aid in diagnosis of diabetes and identifying patients who may be at risk for developing diabetes, and for monitoring of long-term blood glucose control in individuals with diabetes mellitus.

#### **C Special Conditions for Use Statement(s):**

- Rx - For Prescription Use Only
- This product is for IN VITRO DIAGNOSTIC use only
- For diagnosis purposes, results should be interpreted in conjunction with the patient's medical history and clinical findings.
- The HbA1c test is not suitable for use in:
  - Analyzing samples collected from newborns.
  - Diagnosing gestational diabetes.
  - Diagnosing diabetes in patients with any condition where the lifespan of red blood cells is compromised, including recent significant or chronic blood loss, transfusions, significant iron deficiency, hemolytic diseases, hereditary spherocytosis, patients with hemoglobinopathies, with heterozygous sickle cell trait, or thalassemias.
  - Diagnosing diabetes in patients with malignancies or severe chronic liver or kidney disease.
  - 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.
- Hemoglobin A1c testing should not replace glucose testing for type 1 diabetes, in pediatric patients and pregnant women.

#### **D Special Instrument Requirements:**

The Tosoh Automated Glycohemoglobin Analyzer HLC-723GR01

### **IV Device/System Characteristics:**

#### **A Device Description:**

The Tosoh Automated Glycohemoglobin Analyzer HLC-723GR01 (HLC-723GR01) is an automated high performance liquid chromatography (HPLC) that separates and reports stable hemoglobin A1c (HbA1c) in whole blood and hemolysate samples in both DCCT/NGSP % and IFCC mmol/mol units. The Tosoh Automated Glycohemoglobin Analyzer HLC-723GR01 (or HLC-723GR01) analyzer is used with the GR01 cation exchange column (TSKgel GR01 HbA1c Column), GR01 HbA1c elution buffers (Buffer No. 1, 2 and 3) with varying salt concentrations, Hemolysis and Wash Solution, Hemoglobin A1c Calibrator Set and Hemoglobin A1c Control Set (levels 1 and 2).

## **B Principle of Operation:**

The HLC-723GR01 is based on the principle of ion exchange high-performance liquid chromatography (HPLC). The analyzer has two settings to allow HbA1c be measured using either venous whole blood samples in tubes (K2 EDTA or K3 EDTA) or using manually diluted venous whole blood samples (hemolysate) in a sample cup. The whole blood sample setting incorporates an automatic dilution that hemolyzes the sample. Separation of hemoglobin fractions is achieved by using differences in ionic interactions between the cation exchange group on the column resin surface and the hemoglobin components by a step gradient elution with three elution buffers of increasing ionic strengths. Inside the column, the net charges of the hemoglobin proteins interact with the negative charges on the surface of non-porous resin. The separated hemoglobin fractions pass through a detector that measures absorbance at 415 nm. The analyzer plots a chromatogram showing the changes in absorbance versus retention time, showing as a peak for each fraction on the resulting chromatogram. The % HbA1c is calculated and is displayed along with the chromatogram.

## **C Instrument Description Information:**

### **1. Instrument Name:**

The Tosoh Automated Glycohemoglobin Analyzer HLC-723GR01

### **2. Specimen Identification:**

The built-in barcode reader reads the barcode label on the primary tube or vial adapter and the analyzer prints the barcode on the report in the ID field.

### **3. Specimen Sampling and Handling:**

Venous whole blood samples collected in K2EDTA or K3EDTA can be stored at 25°C for 24 hours or at 4°C for 14 days. Samples frozen at -80°C are stable for 9 months.

### **4. Calibration:**

The analyzer uses two levels of calibrators with different values of HbA1c (%). Calibration should be performed every 30 days, when a new column has been installed, when the analysis value of the QC control is out of the reference range, or the flow factor setting has been changed.

5. Quality Control:

It is recommended that at least two levels of controls be run on the GR01 analyzer at least once per day.

**V Substantial Equivalence Information:**

**A Predicate Device Name(s):**

Tosoh Automated Glycohemoglobin Analyzer HLC-723G8

**B Predicate 510(k) Number(s):**

K200904

**C Comparison with Predicate(s):**

<b>Device &amp; Predicate Device(s):</b>	<u>K250073</u>	<u>K200904</u>
Device Trade Name	Tosoh Automated Glycohemoglobin Analyzer HLC-723GR01	Tosoh Automated Glycohemoglobin Analyzer HLC-723G8, v5.24F
<b>General Device Characteristic Similarities</b>		
Intended Use/Indications For Use	Intended for in vitro diagnostic use for the measurement of % hemoglobin A1c (HbA1c) (DCCT/NGSP) and mol/mol hemoglobin A1c (IFCC) using ion-exchange high performance liquid chromatography (HPLC). This test is to be used as an aid in diagnosis of diabetes and identifying patients who may be at risk for developing diabetes, and for monitoring of long-term blood glucose control in individuals with diabetes mellitus.	Same
Test principle	Ion-exchange HPLC	Same
<b>General Device Characteristic Differences</b>		
Sample Type	Human venous whole blood and hemolysate	Human venous whole blood

## VI Standards/Guidance Documents Referenced:

21 CFR 862.1373 – Special controls for Hemoglobin A1c test system

Clinical and Laboratory Standards Institute (CLSI) EP05 – A3: Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition

CLSI EP06: Evaluation of Linearity of Quantitative Measurement Procedures – Second Edition

CLSI EP07: Interference Testing in Clinical Chemistry; Approved Guideline – Third Edition

CLSI EP09c – A3: Measurement Procedure Comparison and Bias Estimation Using Patient Samples – Third Edition

IEC 60601-1-2 Edition 4.1 2020-09 CONSOLIDATED VERSION: Medical electrical equipment - Part 1-2: General requirements for basic safety and essential performance - Collateral Standard: Electromagnetic disturbances - Requirements and tests

## VII Performance Characteristics (if/when applicable):

### A Analytical Performance:

#### 1. Precision/Reproducibility:

Precision studies were conducted using four K2EDTA venous whole blood samples containing HbA1c concentrations of approximately 5.0%, 6.5%, 8.0% and 12.0% from different donors and their corresponding hemolysates (manually diluted from the whole blood samples). Samples were tested at three sites on three analyzers (HLC-723GR01) with three lots of reagents, each sample was analyzed in duplicate per run, two runs per day, for 20 days. The results are shown below.

#### **All analyzers combined (n=720) with NGSP (%) unit.**

Whole Blood												
Mean % HbA1c	Repeatability		Between-Run		Between-Day		Between-Lot		Between Instruments		Total	
	SD	CV(%)	SD	CV(%)	SD	CV(%)	SD	CV(%)	SD	CV(%)	SD	CV(%)
4.91%	0.024	0.48	0.000	0.00	0.024	0.48	0.028	0.57	0.025	0.52	0.051	1.03
6.88%	0.026	0.38	0.015	0.22	0.043	0.62	0.050	0.73	0.011	0.17	0.074	1.07
8.24%	0.025	0.31	0.016	0.20	0.049	0.59	0.058	0.71	0.017	0.21	0.084	1.01
11.79%	0.031	0.26	0.023	0.19	0.063	0.53	0.078	0.66	0.032	0.27	0.112	0.95

Hemolysate												
Mean % HbA1c	Repeatability		Between-Run		Between-Day		Between-Lot		Between Instruments		Total	
	SD	CV(%)	SD	CV(%)	SD	CV(%)	SD	CV(%)	SD	CV(%)	SD	CV(%)
4.90%	0.017	0.35	0.012	0.25	0.024	0.50	0.029	0.60	0.027	0.56	0.052	1.05

6.87%	0.028	0.40	0.011	0.15	0.042	0.61	0.057	0.83	0.005	0.07	0.077	1.12
8.25%	0.025	0.30	0.012	0.14	0.050	0.61	0.057	0.69	0.016	0.20	0.082	1.00
11.82%	0.032	0.27	0.021	0.18	0.065	0.55	0.073	0.62	0.043	0.37	0.114	0.96

**All analyzers combined (n=720) with IFCC (mmol/mol) unit.**

Whole Blood												
Mean HbA1c (mmol/mol)	Repeatability		Between-Run		Between-Day		Between-Lot		Between Instruments		Total	
	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
30	0.188	0.62%	0.000	0.00%	0.280	0.93%	0.365	1.21%	0.252	0.84%	0.557	1.85%
52	0.155	0.30%	0.070	0.14%	0.402	0.78%	0.551	1.07%	0.169	0.33%	0.723	1.40%
67	0.153	0.23%	0.127	0.19%	0.514	0.77%	0.542	0.81%	0.138	0.21%	0.785	1.18%
105	0.166	0.16%	0.186	0.18%	0.696	0.66%	0.862	0.82%	0.387	0.37%	1.200	1.14%

Hemolysate												
Mean HbA1c (mmol/mol)	Repeatability		Between-Run		Between-Day		Between-Lot		Between Instruments		Total	
	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
30	0.125	0.42%	0.140	0.47%	0.279	0.93%	0.378	1.26%	0.246	0.82%	0.562	1.87%
52	0.141	0.27%	0.076	0.15%	0.401	0.78%	0.598	1.16%	0.145	0.28%	0.752	1.46%
67	0.137	0.20%	0.131	0.20%	0.520	0.78%	0.559	0.84%	0.154	0.23%	0.801	1.20%
106	0.191	0.18%	0.194	0.18%	0.693	0.66%	0.860	0.81%	0.504	0.48%	1.244	1.18%

2. Linearity:

A linearity study was conducted using Tosoh Automated Analyzer HLC 723GR01 according to the CLSI guideline EP06. A total of eleven 11 venous whole blood specimens collected in K2EDTA anticoagulant tubes were prepared by mixing samples containing low (3.92%) and high (20.41%) levels of HbA1c. Each sample was tested in replicates of four. The mean measured HbA1c values were compared to the expected values. The results of regression analysis:

$$\text{NGSP: } y = 1.001x + 0.06199, R^2 = 1.000$$

$$\text{IFCC: } y = 1.001x + 0.7028, R^2 = 1.000$$

The linearity study supports the claimed measuring range of 3.9% to 16.9% HbA1c (NGSP), corresponding to a measuring range of 19 - 161 mmol/mol HbA1c (IFCC).

3. Analytical Specificity/Interference:

**Endogenous and Exogenous Substances:**

Interference Studies were performed to assess the impact of endogenous and exogenous substances on the performance of the Tosoh Automated Glycohemoglobin Analyzer HLC-723GR01. Three K2EDTA venous whole blood samples containing approximately 5%, 6.5% and 10% HbA1c were separated into a control sample with no potential interferent added and a test sample containing the potentially interfering substances. Significant interference was

defined by the sponsor as a % difference greater than  $\pm 5\%$  between the mean of the test sample replicates and control sample replicates. The following substances demonstrated no significant interference at the concentrations described below:

<b>Endogenous Substance</b>	<b>Highest concentration tested with no significant interference</b>
Albumin	20 g/dL
Ascorbic Acid	300 mg/dL
Bilirubin - Conjugated	100 mg/dL
Bilirubin - Unconjugated	100 mg/dL
Rheumatoid Factor	750 IU/mL
Triglycerides	6,000 mg/dL

<b>Exogenous Substance</b>	<b>Highest concentration tested with no significant interference</b>
Acetaminophen	20 mg/dL
Acetylsalicylic acid	330 mg/dL
Ampicillin	1000 mg/dL
Cefoxitin	2500 mg/dL
Cyclosporin	0.7 mg/dL
Doxycycline	50 mg/dL
Heparin	5000 U/L
Ibuprofen	50 mg/dL
Levodopa	20 mg/dL
Metformin	5 mg/dL
Methyldopa	30 mg/dL
Metronidazole	200 mg/dL
Phenylbutazone	400 mg/dL
Rifampicin	6.4 mg/dL
Salicylic Acid	60 mg/dL
Theophylline	10 mg/dL

#### **Cross reactivity:**

Three venous whole blood samples collected in K2EDTA anticoagulant tubes, with HbA1c levels at approximately 5%, 6.5% and 10% HbA1c were used to assess potential cross-reacting substances that could interfere with the Tosoh Automated Glycohemoglobin Analyzer HLC-723GR01 performance. Samples were separated into a control sample with no treatment and a test sample containing the potentially interfering cross reactant. No significant interference was defined by the sponsor as within  $\pm 5\%$  change between the mean of the test sample replicates and the mean of the control sample replicates. Results are shown below:

Hemoglobin Derivatives	Highest Concentration Level Tested with No Interference
Labile Hb (glucose)	2000 mg/dL
Carbamylated Hb (Sodium Cyanate)	25 mg/dL
Aldehyde Hb (Acetaldehyde)	25 mg/dL
Acetylated Hb (Acetylsalicylic Acid)	50 mg/dL

#### Hemoglobin Variant Interference:

Hemoglobin variant studies were conducted using K2EDTA venous whole blood samples containing hemoglobin variants HbS, HbC, HbD, HbE, HbA2, and HbF. Results obtained using the Tosoh Automated Analyzer HLC-723GR01 were compared to results obtained using a valid comparator method. No significant interference was defined by the sponsor as a relative bias of  $\leq \pm 7\%$  at around medical decision level of 6.5% and 8.0% HbA1c ( $\leq \pm 6\%$  for HbF), when compared to the comparator method.

Variant	n	HbA1c Range	Variant Range	Relative % Bias (Range of %Bias) Relative to the Comparator	
				~6.5 % HbA1c	~8.0 % HbA1c
HbC	25	4.6% to 14.3%	30.3% to 38.8%	2.79 (0.74 to 4.65)	1.80 (-0.61 to 3.85)
HbD	22	5.7% to 10.9%	24.3% to 42.7%	0.50 (-3.08 to 5.26)	-1.16 (-4.71 to 1.27)
HbE	34	5.0% to 12.8%	19.5% to 31.4%	1.54 (-3.57 to 4.92)	2.08 (0.00 to 7.19)
HbS	29	4.7% to 14.4%	26.6% to 42.7%	2.49 (1.57 to 3.91)	2.71 (1.99 to 4.43)
HbA2	33	4.7% to 14.3%	3.5% to 6.7%	2.51 (0.77 to 4.65)	1.26 (-0.61 to 2.99)
HbF	33	5.5% to 11.7%	3.8% to 32.2%	-1.2 (-3.0 to 1.5)	-2.6 (-4.2 to 0.8)

#### 4. Assay Reportable Range:

The reportable range for this device is 3.9% to 16.9% HbA1c.

#### 5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

The assigned HbA1c values of the Tosoh Automated Analyzer HLC-723GR01 are certified with the National Glycohemoglobin Standardization Program (NGSP). See 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) + 2.152. HbA1c results are provided to the customers using two different units: NGSP equivalent units (%) and IFCC equivalent units (mmol/mol).



6. Detection Limit:

Not applicable.

7. Assay Cut-Off:

Not applicable.

8. Accuracy (Instrument):

See method comparison study below.

9. Carry-Over:

A carryover study was conducted and found to be acceptable.

**B Comparison Studies:**

1. Method Comparison with Predicate Device:

A method comparison study was performed using 135 venous K2 EDTA samples and the respective manual hemolysates. obtained on the candidate device HLC-723GR01 were compared to results obtained on the comparator method at a NGSP secondary reference laboratory. The HbA1c concentrations of the whole blood samples ranged from 4.3% to 16.5% and the corresponding hemolysate samples ranged from 4.2% to 16.6%, according to the comparator. Each sample was tested in singlicate on one of the three investigational analyzers across three different sites and in triplicate on the comparator method. Passing-Bablok Regression analyses were performed for the candidate device versus the comparator method.

The distribution of samples used in the study is summarized below:

%HbA1c	# whole blood samples tested (% of total)	# hemolysate tested (% of total)
≤5%	5 (3.7%)	5 (3.7%)
>5.0 – 6.0%	16 (11.9%)	15 (11.1%)
>6.0 – 6.5%	31 (23%)	32(23.7%)
>6.5 – 7.0%	31 (23%)	32 (23.7%)
>7.0 – 8.0%	21 (15.6%)	20 (14.8%)
>8.0 – 9.0%	9 (6.7%)	10 (7.4%)
>9.0%	22 (16.3%)	21 (15.6%)
Total	135	135

Summary of Passing-Bablok regression results in NSGP units are as follows:

Sample type (n=135)	Slope	Slope 95% CI	Intercept	Intercept 95% CI	R
Whole Blood	1.0045	1.000 to 1.0135	-0.0222	-0.0743 to 0.000	1.000
Hemolysate	1.0125	1.0039 to 1.0189	-0.0788	-0.1226 to -0.0241	1.000

**Total Error:**

The bias estimates determined from the method comparison study results using Passing-Bablok regression and the precision determined in the precision study were used to determine the total error (TE) of the device at each of the HbA1c levels was calculated as follows:

$$\%TE = |\%Bias| + (1.96 \times \%CV) \times (1 + \%Bias/100)$$

	%HbA1c	%Bias	%CV	%TE
Whole Blood	5.0%	0.006	1.03	2.02
	6.5%	0.108	1.07	2.21
	8.0%	0.172	1.01	2.16
	12.0%	0.265	0.95	2.13
Hemolysate	5.0%	0.326	1.05	2.38
	6.5%	0.038	1.12	2.23
	8.0%	0.265	1.00	2.23
	12.0%	0.593	0.96	2.49

2. Matrix Comparison:

A matrix Comparison study was conducted using a total of 51 paired venous K2EDTA and K3EDTA venous whole blood samples spanning the HbA1c measuring range and the results support the use of the HbA1c assay with sample collected in K2EDTA and K3EDTA.

Method of Analysis	n	Slope	Intercept	r
Ordinary Deming	51	0.9957	0.0211	1.000
Passing-Bablok	51	1.0000	0.0000	1.000

**C Clinical Studies:**1. Clinical Sensitivity:

Not applicable.

2. Clinical Specificity:

Not applicable.

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

Not applicable.

**D Clinical Cut-Off:**

Not applicable.

**E Expected Values/Reference Range:**

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

	In NGSP units	In IFCC units	Comment
HbA1c	≥ 6.5 %	≥ 48 mmol/mol	Cutoff point to diagnose diabetes
HbA1c	5.7 - 6.4%	39 – 47 mmol/mol	Increased risk for diabetes (prediabetes)

Reference:

American Diabetes Association. Classification and diagnosis of diabetes: Standards of Medical Care in Diabetes—2021. Diabetes Care 2021; 44 (Suppl. 1), S15-33.

#### **F Other Supportive Instrument Performance Characteristics Data:**

Additional studies were conducted to support the STAT function of the analyzer and the automatic dilution feature of the analyzer.

#### **VIII Proposed Labeling:**

The labeling supports the finding of substantial equivalence for this device.

#### **IX Conclusion:**

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