P0503902US

Tosoh Automated Glycohemoglobin Analyzer HLC-723®GR01

For users in the United States: Please refer to the Web IFU posted on www.tosohbioscience.us

Caution: Federal law restricts this device to sale by or on the order of a healthcare practitioner.

Instructions For Use

GR01 HbA1c Elution Buffer No. 1

No. 2

No. 3

TOSOH CORPORATION

Safety Precautions

To help protect you and/or your property from potential damage and ensure personal safety, please read this IFU thoroughly before using the product.

[Notational Convention]

Notation		Explanation
⚠	**CAUTION**	Indicates a hazard with a low level of risk which, if not avoided, could result in minor or moderate injury.

⚠ CAUTION

■ **Use only in well-ventilated areas**

In case of insufficient ventilation, spilled solvents can cause harm.

■ **First Aid**

Skin exposure

Wash exposed area with plenty of soap and water.

Eye exposure

Open eyes as wide as possible and wash with clean water for at least 15 minutes.

Immediately call for medical attention.

Ingestion

Please wash mouth with excess water and immediately call for medical attention.

■ **Do not spill solvents**

Spillage and leakage can cause fire, electric shock, poisoning, injury and corrosion. Wear appropriate protective gear when cleaning up a spill.

■ **Wear protective eye gear and gloves**

Organic acids are harmful and should not come in direct contact with the skin.

■ **Handle the package with care**

Inappropriate handling may cause rupturing and/or splattering of the product.

■ **Only use this product as intended**

This product is intended for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of HbA1c in venous whole blood and diluted blood (hemolysate) specimens by healthcare professionals.

■ **Proper disposal**

Dispose in accordance with local, state and federal laws and regulations.

NOTE

Keep this IFU with the product for future reference.

If any serious incident has occurred in relation to this device, report it to the manufacturer or the supplier.

Symbols on the product labels

Manufacturer

Supplied by

In vitro diagnostic medical device

Consult instructions for use

Catalogue number / Part number

Batch code / Lot number

Temperature limitation

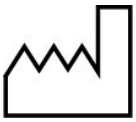
Use-by date

Serial number / Column number

Net volume
(after reconstitution for
lyophilized material)

For specified column lot only

Actual manufacturing site



Date of manufacture

A carrier containing unique
device identifier information

Contents

Published in December, 2024

1. Intended 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.

2. Summary and Explanation of the Test

Diabetes mellitus is a serious, chronic disease that occurs either when the pancreas do not produce enough insulin (Type 1 diabetes), or when the body cannot effectively or properly use the insulin it produces (Type 2 diabetes). The condition is characterized by hyperglycemia. The number of cases for both types of Diabetes have been steadily increasing over the past few decades (Ref. 1). Diabetes of all types can lead to complications in many parts of the body and can increase the overall risk of dying prematurely. Possible complications include heart attack, stroke, kidney failure, leg amputation, vision loss and nerve damage. In pregnancy, poorly controlled diabetes increases the risk of fetal death and other complications (Ref. 1).

The use of HbA1c testing has been recommended by various national and international bodies such as the American Diabetes Association (ADA) and the World Health Organization (WHO) to name a few. Ideally an A1c test must be carried out using a method certified by the National Glycohemoglobin Standardization Program (NGSP) and standardized or traceable to the Diabetes Control and Complications Trial (DCCT) reference assay. These organizations recommend a threshold of >/= 6.5% in NGSP units or >/= 48 mmol/mol in the International Federation of Clinical Chemistry (IFCC) units to diagnose diabetes. (Ref. 2 - 5).

HbA1c, a stable glycated hemoglobin component, is formed slowly and non-enzymatically from hemoglobin and whole blood glucose in two steps, first by binding of glucose to the N-terminal valine of the β-chain of hemoglobin to form the Schiff base (labile HbA1c or “LA1c+” as shown on the HLC-723GR01 chromatogram report), and second by a reaction known as the Amadori rearrangement to form a stable ketoamine (stable HbA1c or “SA1c” as shown on the HLC-723GR01) from the labile HbA1c. The labile HbA1c changes rapidly in response

to changes in whole blood glucose concentrations, whereas stable HbA1c usually reflects the average blood glucose level over the past couple of months.

3. Principle of the Procedure

The HLC-723GR01 (hereafter referred to as HLC-723GR01 or GR01 interchangeably throughout the document) is based on the principle of ion exchange high-performance liquid chromatography (HPLC). The system comprises of a GR01 HbA1c cation exchange column, named as TSKgel GR01 HbA1c Column, packed with TSKgel resin, and the GR01 HbA1c Elution Buffers No. 1 – 3 of varying salt concentrations.

After placing the samples to be analyzed on the system, the GR01 automatically aspirates, dilutes and injects samples onto the column. No manual pretreatment of samples is needed prior to placing on the system.

The GR01 introduces the buffers of increasing ionic strengths onto the column where the hemoglobins are separated into a total of six fractions including HbA1c based on their ionic interactions with the resin packed in the column. The separated hemoglobin fractions pass through a detector that measures absorbance at 415 nm.

The A1c results are obtained at 50 seconds/sample. The resulting chromatogram provides HbA1c (%) or (mmol/mol).

HbA1c (%) is determined as a relative percentage of the integrated area of the HbA1c fraction against the sum of the other hemoglobin fractions after being calibrated with the HbA1c Calibrator Set (S). The GR01 analyzer also calculates and can report HbA1c levels in IFCC units (mmol/mol) converted from HbA1c (%) in NGSP units using the master equation (Ref. 8).

In addition to providing an HbA1c result, the GR01 detects the presence of the most common heterozygous hemoglobin variants, HbAE, HbAS, HbAC or HbAD in samples. The A1c value is reportable in the presence of these commonly occurring variant hemoglobins.

4. Material Provided

Catalogue No.	Description	Content (per one pack)
0024760	GR01 HbA1c Elution Buffer No. 1	800 mL
0024761	GR01 HbA1c Elution Buffer No. 2	800 mL
0024762	GR01 HbA1c Elution Buffer No. 3	800 mL

The GR01 HbA1c Elution Buffers are organic acid buffers, each of which contains less than 0.05 % sodium azide as a preservative.

5. Materials Required but Not Provided

The following materials are required to perform HbA1c assays using the HLC-723GR01 analyzer with the GR01 column and buffers. They are available separately from Tosoh Corporation.

	Catalogue No.
HbA1c Calibrator Set (S)	0023502
HbA1c Diluting Solution	0023503
TSKgel GR01 HbA1c Column	0024759
HSi Hemolysis & Wash Solution	018431US

Only materials obtained from Tosoh Corporation or Tosoh Bioscience, Inc. should be used. Materials obtained elsewhere should not be substituted since assay performance is characterized based strictly on Tosoh Corporation materials.

6. Materials Optionally Used in Combination with the Device

The following material is an optional product to perform quality control of the HLC-723GR01 using the TSKgel GR01 HbA1c Column. This material is available separately from the Tosoh Corporation or Tosoh Bioscience, Inc.

	Catalogue No.
Hemoglobin A1c Control Set	0021974

7. Warnings and Precautions

1) The GR01 HbA1c Elution Buffer No. 1, No. 2 and No. 3 are for *IN VITRO* DIAGNOSTIC USE only.
2) The GR01 HbA1c Elution Buffer No. 1, No. 2 and No. 3 are intended for use on the Tosoh Automated Glycohemoglobin Analyzer HLC-723GR01 only.
3) Inspect the packaging and the exterior of the box for any sign of damage before use. If any damage is visible, contact your local Tosoh sales representative.
4) Use the GR01 HbA1c Elution Buffer No.1 and No. 2 in combination with the TSKgel GR01 HbA1c Column of the same lot number. The lot number of TSKgel GR01 HbA1c Column is indicated by a single uppercase alphabetical character (A, B, etc.) on the label of box. The lot number of the GR01 HbA1c Elution Buffer No. 1 and No. 2 are indicated by a single alphabetic character on its pack or box label as shown below. Note that the GR01 HbA1c Elution Buffer No. 3 is not lot-specific.

5) Always wear proper personal protective clothing (gloves, glasses, etc.) while handling all reagents, samples and waste fluids.
6) Do not use the GR01 HbA1c Elution Buffer No. 1, No. 2 and No. 3 beyond the expiration date.
7) Do not use the GR01 HbA1c Elution Buffer No. 1, No. 2 and No. 3 with an incorrect connection with the eluent tube. Check the colors of the caps and tube No. match with the colors and No. of the GR01 HbA1c Elution Buffers.
8) Some reagents contain sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.
9) Patient samples (clinical specimens) must always be considered as potentially infectious and should be handled using established laboratory procedures and/or institutional guidelines.
10) Any serious incident that has occurred in relation to the device should be reported to the manufacturer or the supplier and the regulatory authorities.

8. Storage and Stability

The GR01 HbA1c Elution Buffers are stable until the expiration date when stored at 4 to 30 °C. The expiration date is indicated on the package box and aluminum pack labels.
When stored at 4 to 30 °C, each buffer is stable for 90 days after opening. After opening, even when stored as stated above, they cannot be used beyond their expiration date.
The TSKgel GR01 HbA1c Column injection lifetime is 5,000 injections (column count).

9. Specimen Collection and Handling

A venous whole blood sample collected in a primary tube containing K2EDTA or K3EDTA is required for the analysis.
A venous blood sample should be collected aseptically. No special preparation is necessary. Blood samples collected in primary tubes stated above may be stored at 25 °C for 24 hours or at 4 °C for 14 days prior to analysis. If using a hemolysate sample, dilute the whole blood 1:150 with the Hemolysis & Wash Solution. Hemolysate should be freshly prepared at room temperature and run within 2 hours after preparation. Refer to the HLC-723GR01 Operator's Manual Sections 4.5 and 5.8.1 for further instructions on testing diluted/hemolysate samples using the STAT function.”

Venous whole blood samples frozen at – 80 °C are stable for 9 months (Ref. 9 and 10).

10. Procedures

Be sure to read the IFU included with the TSKgel GR01 HbA1c Column, HbA1c Calibrator Set (S) and HSi Hemolysis & Wash Solution, as well as the HLC-723GR01 Operator's Manual for detailed instructions for column replacement procedures.

I. Column and Reagent Installation

Place the following column and reagent appropriately on the analyzer. The column and reagents are provided ready for use.

- TSKgel GR01 HbA1c Column
- HSi Hemolysis & Wash Solution

II. Buffers installation

1) When the analyzer is busy with assays, wait for the ongoing assay to end and a “Standby” to be displayed, or tap the [Analysis stop] button on the top part of the screen to stop the ongoing assay and to display the “Standby” state (a “Standby” on the Main screen).
2) Tap the [Remaining reagent volume information/ Reagent replace] button on the Main screen or [Reagent Replacement] button on the Maintenance screen to show the “Reagent replacement” screen.
3) Uncap the Elution Buffer packs. Keep the caps in case the Elution Buffers are separately stored after being de-installed from the analyzer.
4) Gently squeeze the Elution Buffer pack by hand to remove all excess air inside, and tighten the cap firmly into place to create a vacuum that will prevent air from entering during operation.
5) Tap the buttons of the reagents to be replaced.
6) Tap the [Register] button.

III. Calibration

Calibration frequency on the GR01 is every 30 days. Laboratories may choose to calibrate more frequently based upon QC best practices protocol of each individual laboratory. For recalibration details, please refer to the HLC-723GR01 Operator's Manual for use of HbA1c Calibrator Set (S).

IV. Quality Control Procedure

In order to monitor and evaluate the accuracy and precision of the analytical performance, it is recommended that at least two levels of controls be run at least once per day.

The Hemoglobin A1c Control Set is provided lyophilized. Refer to the IFU of the Hemoglobin A1c Control Set for detailed instructions.

Assay quality control materials are described in the HLC-723GR01 Operator's Manual. If one or more control samples are out of the established ranges, it is necessary to investigate the cause including the validity of the calibration curve before reporting patient results. Follow the recommendations of local, state, and federal regulatory agencies as well as laboratory policies.

11. Procedural Notes

Refer to the HLC-723GR01 Operator's Manual for detailed instructions.

1) The minimum volume of a whole blood specimen collected in a primary tube is 1 mL for an assay. A whole blood specimen as small as 100 µL may be used by (1) manually diluting it with the HSi Hemolysis & Wash Solution and putting it in a sample cup and (2) assaying as a diluted sample after the appropriate software option is set. For more detailed procedures, refer to the HLC-723GR01 Operator's Manual.
2) Measurement values (%) on the chromatogram indicate the percentage of each peak in relation to the Total Area (excluding the front peak (FP) and HbF peak, “F”).
3) The minimum unit of HbA1c displayed is 0.1 % in NGSP units or 1 mmol/mol in IFCC units.
4) Bring the Elution Buffer to room temperature before inserting it on the analyzer.
5) Never use expired reagents. The expiration dates are printed on their labels. Assay results obtained with expired reagents will not be reliable. Also note that reagents must be used within 90 days after opening (provided that the pack is correctly maintained in vacuum state).
6) When there is leftover reagent in an aluminum pack, remove the pack from the analyzer and again gently squeeze the pack by hand to remove all excess air inside, and tighten the cap firmly to create a vacuum that will prevent air from entering during storage and store at 4 to 30 °C.
7) Always replace the aluminum pack with a full aluminum pack before the pack is empty. Avoid refilling leftover elution buffer into the aluminum pack as it can produce unreliable assay results.
8) For safe waste disposal, it is recommended that each laboratory comply with established laboratory procedures and local, state and federal regulations.
9) If the analyzer is installed with the Buffer Switching Unit, the buffer packs already used

are able to be replaced with new buffer packs even during “Analysis” states. Check the buffer packs during use on the “Reagent replacement” screen for their remaining volumes.

12. Results

The GR01 must pass calibration and the controls values must be in range. Results will not be reported if outside the Total Area range or the reportable range.

An HbA1c value (%) which is reported from the analyzer represents the percentage of integrated area of HbA1c fraction (shown as “SA1c” peak in the assay report) in relation to the Total Area (a total of the integrated peaks of all hemoglobin fractions excluding the front peak, “FP” and HbF peak, “F”) after calibration.

The GR01 presumptively identifies peaks associated with common hemoglobin variants, HbAD, HbAS, HbAC and HbAE, in a sample based on the defined elution time windows, “H-V0”, “H-V1”, “H-V2” and “H-V3”, respectively in the chromatogram., in a sample based on the defined elution time windows, “H-V0”, “H-V1”, “H-V2” and “H-V3”, respectively in the chromatogram.

HbA1c values are reported in % when the calibration is done in NGSP units, or in mmol/mol when the calibration is done in IFCC units.

The GR01 analyzer can report HbA1c results in IFCC units (mmol/mol) by applying the master equation (Ref. 8) to convert the measured NGSP units (%HbA1c) into IFCC units.

The GR01 software is equipped with a set of Flags that determine if an HbA1c result is reportable. The reportability depends on a combination of flag priority settings, flag levels and reference ranges. The parameters for these flags are set by Tosoh personnel when installing the analyzer and cannot be modified by users. Details about Flag settings and how they are used are provided in the GR01 Operator’s Manual Section 7.2 *Flag settings*.

13. Limitation of the Procedure

Rx - For Prescription Use Only

The HbA1c test is not suitable for use in the following (Ref. 2, 6):

- Samples collected from newborns should not be used.
- 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.

Note: Only the HbA1c values measured by this device should be reported to the health care provider. The test report displays %HbF and other detected hemoglobin variants; however, the performance characteristics of the hemoglobin variants detected by this device have not been reviewed or cleared by the FDA and therefore should not be reported to the healthcare provider. The hemoglobin variants detected by this device are only to ensure the validity of the HbA1c results because increased levels of hemoglobin variants may interfere with the percent HbA1c measurement. If a hemoglobinopathy is suspected, an FDA cleared test system for their measurement should be used.

General Considerations for Hemoglobin A1c testing:

1) When the Total Area of the chromatogram is < 300 or > 1800, the HbA1c value is not reported. In such a case, re-assay the sample using STAT function. Refer to the HLC-723GR01 Operator's Manual for further instructions.
2) Measuring range: The measuring range of HbA1c is from 3.9 % to 16.9 % in NGSP units which corresponds with 19 mmol/mol to 161 mmol/mol in IFCC units.
3) For diagnosis purposes, results should be interpreted in conjunction with the patient's medical history and clinical findings.
4) In homozygous and double heterozygous forms of variant hemoglobins (example: SS, CC, SC), there is no HbA present, therefore no HbA1c can be determined.
5) Only the most commonly occurring variant hemoglobins: HbAS, HbAC, HbAD and HbAE, have been evaluated for potential interference with the GR01. For the identification of any particular hemoglobin variant, alternative methods should be used.

14. Expected Values

The “Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes - 2021”, American Diabetes Association (Ref. 6) gives the following criteria for the diagnosis of diabetes and categories of increased risk for diabetes.

	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)

The HbA1c reference range (non-diabetic) is reported as below (Ref. 7).

HbA1c: 4.0 - 6.0 % (mean 5.0 %, SD 0.5 %)

This range can be converted into IFCC units as:

HbA1c: 20 - 42 mmol/mol,

using the following Master Equation (Ref. 8):

IFCC (mmol/mol) = 10.93 x NGSP(%) – 23.50

15. Performance Characteristics

a) Reproducibility/Repeatability

Repeatability (within-run precision) and reproducibility (inter-run precision) of the GR01 analyzer was determined measuring %HbA1c in both venous whole blood samples and corresponding hemolysates. HbA1c concentrations of approximately 5.0%, 6.5%, 8.0%, and 12.0% were tested over two (2) runs per day, with two (2) replicates per run, at three (3) sites/instruments, with three (3) reagent lots, for 20 days per reagent lot, for a total of 60 days. The study results for whole blood and hemolysate are summarized for NGSP units in Tables 1A and 1B, and for IFCC units in Tables 1C and 1D, respectively.

Table 1A. Summary of Precision Analysis – Whole Blood (NGSP Units)

%HbA1c Target [Actual Mean]	Repeatability		Between Run		Between Day		Between Lot		Between Site/Instrument		Total Variation / Reproducibility	
	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
5.0% [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%
6.5% [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%
8.0% [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%
12.0% [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%
Abbreviations: 'SD': Standard Deviation | 'CV': Coefficient of Variation												

Table 1B. Summary of Precision Analysis – Hemolysate (NGSP Units)

%HbA1c Target [Actual Mean]	Repeatability		Between Run		Between Day		Between Lot		Between Site/Instrument		Total Variation / Reproducibility	
	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
5.0% [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%
6.5% [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%
8.0% [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%
12.0% [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%
Abbreviations: 'SD': Standard Deviation | 'CV': Coefficient of Variation												

Table 1C. Summary of Precision Analysis – Whole Blood (IFCC Units)

HbA1c Target (mmol/mol) [Actual Mean]	Repeatability		Between Run		Between Day		Between Lot		Between Site/Instrument		Total Variation / Reproducibility	
	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
31 [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%
48 [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%
64 [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%
108 [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%
Abbreviations: 'SD': Standard Deviation | 'CV': Coefficient of Variation												

Table 1D. Summary of Precision Analysis – Hemolysate (IFCC Units)

HbA1c Target (mmol/mol) [Actual Mean]	Repeatability		Between Run		Between Day		Between Lot		Between Site/Instrument		Total Variation / Reproducibility	
	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
31 [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%
48 [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%
64 [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%
108 [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%
Abbreviations: 'SD': Standard Deviation | 'CV': Coefficient of Variation												

b) Method Comparison

The method comparison study was designed in accordance with CLSI EP 21: Evaluation of Total Analytical Error for Quantitative Medical Laboratory Measurement Procedures - Second Edition, and parts of CLSI EP09c: Measurement Procedure Comparison and Bias Estimation Using Patient Samples - Third Edition. Hemoglobin A1c (HbA1c) test results of the Tosoh Automated Glycohemoglobin Analyzer HLC 723GR01 (GR01 analyzer) in an Intended Use setting were compared with results obtained with comparator testing run on the Tosoh Automated Glycohemoglobin Analyzer HLC-723G8 (G8 analyzer); comparator testing was performed in a National Glycohemoglobin Standardization Program (NGSP) Secondary Reference Laboratory (SRL). One Hundred and thirty-five (135) K2EDTA venous whole blood with HbA1c concentrations that spanned the GR01 measuring range (3.9% HbA1c to 16.9% NGSP units; 19 to 161 mmol/mol IFCC units) were evaluated. Each sample was measured in triplicate with the G8 comparator device, and the mean value was defined as the "true" HbA1c value. Each sample was also measured in singlicate on one of three investigational GR01 analyzers placed at three (3) separate Moderate to High Complexity Clinical Laboratories. The data supports a correlation between the candidate and comparator devices, with no clinical or statistical difference between the two analyzers as the relative difference for all data points met the acceptance criteria of ≤±6%. The results are summarized in Table 2A (NGSP units) and Table 2B (IFCC units).

Table 2A. Summary of Method Comparison – Passing-Bablok Parameter Estimates (NGSP Units)

Specimen Type	n	Slope (95% CI)	Intercept (95% CI)
Whole Blood	135	1.0045 (1.0000 to 1.0135)	–0.0222 (–0.0743 to 0.0000)
Hemolysate	135	1.0125 (1.0039 to 1.0189)	–0.0788 (–0.1226 to –0.0241)

Table 2B. Summary of Method Comparison – Passing-Bablok Parameter Estimates (IFCC Units)

Specimen Type	n	Slope (95% CI)	Intercept (95% CI)
Whole Blood	135	1.0129 (1.0083 to 1.0180)	–0.4438 (–0.7260 to –0.1459)
Hemolysate	135	1.0141 (1.0097 to 1.0186)	–0.5471 (–0.8133 to –0.3166)

The total analytical error (%TE) was calculated using the estimated %bias at each decision point from the Method Comparison study and the total coefficient of variation (%CV) estimated in the Precision study. Results for the TE calculations are shown in Table 3 for NGSP units.

Table 3. Total Error Estimation (NGSP Units)

	HbA1c Level	%Bias [1]	%CV Total Variation [2]	Total Error% [3]
Whole Blood	Sample 1 [5.0%]	0.006	1.03	2.02
	Sample 2 [6.5%]	0.108	1.07	2.21
	Sample 3 [8.0%]	0.172	1.01	2.16
	Sample 4 [12%]	0.265	0.95	2.13
Hemolysate	Sample 1 [5.0%]	-0.326	1.05	2.38
	Sample 2 [6.5%]	0.038	1.12	2.23
	Sample 3 [8.0%]	0.265	1.00	2.23
	Sample 4 [12%]	0.593	0.96	2.49

[1] Estimated in the Method Comparison study
[2] Obtained from the Precision study
[3] %TE = |%Bias| + 1.96 * %CV * (1 + (%Bias/100))

c) Matrix Comparison

Matched sets (N=51) of venous whole human blood were collected in two anticoagulant tubes containing K2EDTA (primary specimen) and K3EDTA (candidate specimen). The paired specimens were tested with the GR01 analyzer using two replicates for each anticoagulant type. Data obtained for HbA1c concentrations were evaluated to compare precision and systematic differences between the candidate (K3EDTA) and primary (K2EDTA) anticoagulant collection tubes. The CV was 0.6% for both K2EDTA and K3EDTA, showing the same precision for both anticoagulants. Passing-Bablok fit parameters are shown in Table 4. Based on the test results, K2EDTA and K3EDTA performed equivalently.

Table 4. Summary of Matrix Comparison Result - K2 EDTA vs K3 EDTA

Method of Analysis	n	Slope (95% CI)	Intercept (95% CI)
Ordinary Deming	51	0.9957 (0.9885 to 1.0030)	0.0211 (-0.0271 to 0.0693)
Passing-Bablok	51	1.0000 (1.0000 to 1.0000)	0.0000 (0.0000 to 0.0000)

d) Traceability and Expected Values (calibrators)

The assigned HbA1c values of the Tosoh Automated Glycohemoglobin Analyzer are certified with the National Glycohemoglobin Standardization Program (NGSP). See NGSP website for current certification at https://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). (Ref. 8)

e) Linearity

The linearity of the Tosoh Automated Glycohemoglobin Analyzer HLC723-GR01 was evaluated based on CLSI EP06 - Evaluation of the Linearity Quantitative Measurement Procedures: Second Edition. Linearity across the expanded range (3.9% to 20.4% HbA1c) was performed using low (3.9% HbA1c) to high (20.4% HbA1c) whole blood de-identified remnant specimens collected in K2EDTA anticoagulant tubes. These samples were mixed in varying ratios and were run in four (4) replicates each on the HLC-723GR01. The means of the measured values were compared to the expected values based upon their dilution factor. Theoretical values were calculated using the following formula:
Theoretical value = (Mix ratio (A) × HbA1c (A) + Mix ratio (B) × HbA1c (B)) / 10

The recovery ratios were calculated by:
The Observed Recovery = Measured Value / Theoretical Value × 100

All samples recovered within the acceptable range of 100 ± 5%. The results are included in Table 5 below with Table 5A showing the results in NGSP units (%) and Table 5B showing the results in IFCC units (mmol/mol).

Table 5A. Linearity Study Results – NGSP Units (%)

High HbA1c (ratio)	Low HbA1c (ratio)	Measured Value HbA1c (%)	Theoretical Value HbA1c (%)	Observed Recovery
0	10	3.92	3.92	100.0
1	9	5.58	5.57	100.1
2	8	7.32	7.22	101.3
3	7	9.10	8.87	102.6
4	6	10.52	10.52	100.0
5	5	12.27	12.17	100.8
6	4	14.02	13.82	101.4
7	3	15.48	15.47	100.1
8	2	17.26	17.11	100.8
9	1	18.85	18.76	100.5
10	0	20.41	20.41	100.0

The study supports the claimed measuring range of 3.9% – 16.9 % HbA1c.

Table 5B. Linearity Study Results – IFCC Units (mmol/mol)

High HbA1c (ratio)	Low HbA1c (ratio)	Measured Value HbA1c (mmol/mol)	Theoretical Value HbA1c (mmol/mol)	Observed Recovery
0	10	19.4	19.4	100
1	9	37.5	37.4	100.3
2	8	56.5	55.4	102.0
3	7	76.0	73.5	103.4
4	6	91.4	91.5	99.9
5	5	110.6	109.5	101.0
6	4	129.7	127.6	101.6
7	3	145.7	145.6	100.1
8	2	165.1	163.6	100.9
9	1	182.6	181.6	100.6
10	0	199.6	199.6	100

The study supports the claimed measuring range of 19 – 161 mmol/mol.

f) Analytical Specificity

i. Endogenous Interfering Substances

The endogenous interference study was performed in compliance to CLSI EP07-A3, *Interference Testing in Clinical Chemistry; Third Edition*. Interference studies were conducted at known concentrations of HbA1c samples from both diabetic and non-diabetic donors. Specimens were spiked with increasing amounts of the interfering substance and then assayed ten (10) times. The mean result of each sample was then compared to the mean result of the untreated, reference sample. Significant interference was defined as an observed difference in HbA1c concentration greater than ± 5% from the untreated, reference sample.

Table 6. Endogenous Interfering Substances Tested

Potential Interfering Substance	Concentration with No Interference
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

Albumin, ascorbic acid, conjugated and unconjugated bilirubin, triglyceride and rheumatoid factor do not interfere with the Tosoh Automated Glycohemoglobin Analyzer HLC-723GR01 assay up to the stated concentrations.

ii. Drug Interference

The exogenous interference study was performed in compliance to CLSI EP07-A3, *Interference Testing in Clinical Chemistry -Third Edition*. Interference studies were conducted at known concentrations of HbA1c samples from both diabetic and non-diabetic donors. Specimens were spiked with increasing amounts of the interfering substance and then assayed ten (10) times. The mean result of each sample was then compared to the mean result of the untreated, reference sample. Significant interference was defined as an observed difference in HbA1c concentration greater than ± 5% from the untreated, reference sample.

Table 7. Exogenous Interfering Substances Tested

Potential Interfering Substance	Concentration with No Interference
Acetaminophen	20 mg/dL
Acetylcysteine	330 mg/dL
Ampicillin	1,000 mg/dL
Cefoxitin	2,500 mg/dL
Cyclosporine	0.7 mg/dL
Doxycycline	50 mg/dL
Heparin	5.000 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

The substances listed in Table 7 - Exogenous Interfering Substances Tested do not interfere with the Tosoh Automated Glycohemoglobin Analyzer HLC-723GR01 assay at therapeutic doses.

iii. Cross Reactivity with Hemoglobin Derivatives

A cross-reactivity study was performed to assess potential interferences from Acetylated hemoglobin (Hb), Carbamylated Hb, Aldehyde Hb, and Labile HbA1c with samples from both diabetic and non-diabetic donors. Specimens were spiked with increasing amounts of the interfering substance and then assayed ten (10) times. The mean result of each sample was

then compared to the mean result of the untreated, reference sample. Significant interference was defined as an observed difference in HbA1c concentration greater than ± 5% from the untreated reference sample. The following results were concluded as not interfering with the assay.

- Acetylated Hb up to 50 mg/dL
- Carbamylated Hb up to 25 mg/dL
- Aldehyde Hb up to 25 mg/dL
- Labile HbA1c up to 2000 mg/dL

In conclusion, Acetylated hemoglobin (Hb), Carbamylated Hb, Aldehyde Hb, and Labile HbA1c with HbA1c do not cross-react with the assay up to the concentrations shown above.

iv. Hemoglobin Variant Interference

The hemoglobin variant interference study was performed by comparing HbA1c results of samples with the presence of common Hb variants measured with both the GR01 and the reference method, Tosoh Automated Glycohemoglobin Analyzer HLC-723G8, Ver. 5.24F ("G8 v5.24F"), which is which has been demonstrated to be free from interference by these hemoglobin variants in compliance to CLSI EP07: *Interference Testing in Clinical Chemistry; Approved Guideline – Third Edition*.

Interference due to the presence of certain levels of common Hb variants (HbAC, HbAD, HbAE, and HbAS) and hemoglobinopathy, elevated HbA2 and elevated Fetal Hemoglobin (HbF) is well-known when measuring %HbA1c in clinical specimens.

Two interference studies were performed to identify the level of (1) Hb variants and HbA2, and (2) HbF likely to cause interference with HbA1c measurements.

(1) Common Hemoglobin Variants and Elevated HbA2

The hemoglobin variant study was performed using K2 EDTA venous whole blood samples known to contain hemoglobin variants HbS, HbC, HbE, HbD and elevated HbA2. The samples containing the hemoglobin variants were tested using the GR01 and using the comparator method (G8 v5.24F), which has been demonstrated to be free from interference by these hemoglobin variants. Non-clinically significant interference was defined as ≤±7% relative difference in the results from the comparative method at 6.5% or 8.0% HbA1c. The following table summarizes the test results.

Table 8. Hemoglobin Variant Sample Concentration Distribution

Hemoglobin Variant/ HbA2	N	Range in % Abnormal Variant/ HbA2	Range in % HbA1c Concentration
HbAC	25	30.0% to 38.8%	4.6% to 14.3%
HbAD	22	24.3% to 42.7%	5.7% to 10.9%
HbAE	34	19.5% to 31.4%	5.0% to 12.8%
HbAS	29	26.6% to 42.7%	4.7% to 14.4%
HbA2*	33	3.5% to 6.7%	4.7% to 14.3%
HbF*	33	3.8 % to 32.2 %	5.5 % to 11.7 %

*Hemoglobinopathy

Table 9. Hemoglobin Variant Interference Study – Relative Difference at Medical Decision Points

Variant	n	Mean (Range) of Relative Bias from Reference Method at Approximately 6.5% and 8.0% HbA1c	
		~6.5 % HbA1c	~8.0 % HbA1c
HbC	25	2.79 (0.74 to 4.65)	1.80 (-0.61 to 3.85)
HbD	22	0.50 (-3.08 to 5.26)	-1.16 (-4.71 to 1.27)
HbE	34	1.54 (-3.57 to 4.92)	2.08 (0.00 to 7.19)
HbS	29	2.49 (1.57 to 3.91)	2.71 (1.99 to 4.43)
HbA2	33	2.51 (0.77 to 4.65)	1.26 (-0.61 to 2.99)
HbF	33	-1.2 (-3.0 to 1.5)	-2.6 (-4.2 to 0.8)

(2) Fetal Hemoglobin (HbF)

No clinically significant interference with HbA1c results was observed up to 32.2% HbF

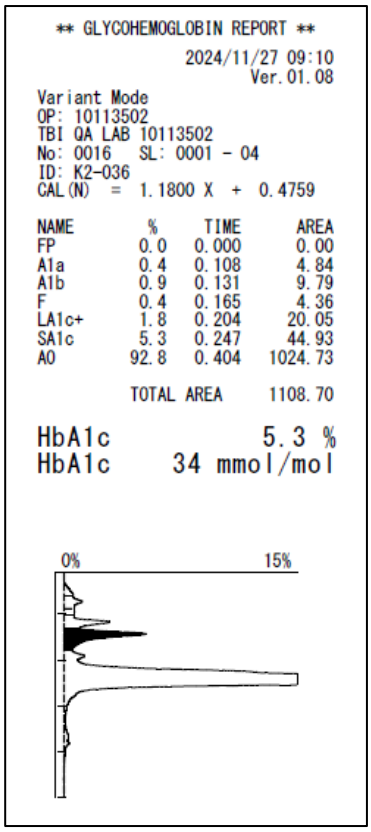
In conclusion, no clinically significant interference with HbA1c results was observed for the common hemoglobin variants HbAC, HbAD, HbAE or HbAS at the levels stated in the table above.

No clinically significant interference with HbA1c results was observed up to 6.7% HbA2.

No clinically significant interference with HbA1c results was observed up to 32.2% HbF.

16. Example chromatograms

Sample chromatograms for normal and diabetic specimens are given below.
The additional peak information provided in addition to %HbA1c on the chromatograms should only be used for example in troubleshooting and has not been validated. Only the HbA1c value should be reported to the healthcare provider.



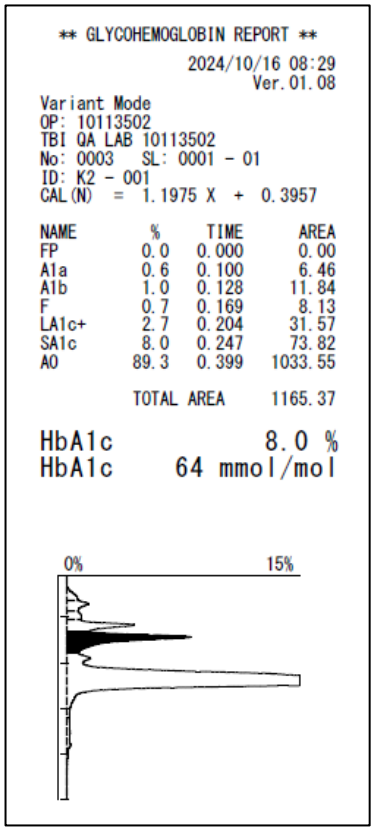
** GLYCOHEMOGLOBIN REPORT **

2024/11/27 09:10
Ver.01.08

Variant Mode
OP: 10113502
TBI QA LAB 10113502
No: 0016 SL: 0001 - 04
ID: K2-036
CAL(N) = 1.1800 X + 0.4759

NAME	%	TIME	AREA
FP	0.0	0.000	0.00
A1a	0.4	0.108	4.84
A1b	0.9	0.131	9.79
F	0.4	0.165	4.36
LA1c+	1.8	0.204	20.05
SA1c	5.3	0.247	44.93
A0	92.8	0.404	1024.73
	TOTAL AREA		1108.70

HbA1c 5.3 %
HbA1c 34 mmol/mol



** GLYCOHEMOGLOBIN REPORT **

2024/10/16 08:29
Ver.01.08

Variant Mode
OP: 10113502
TBI QA LAB 10113502
No: 0003 SL: 0001 - 01
ID: K2 - 001
CAL(N) = 1.1975 X + 0.3957

NAME	%	TIME	AREA
FP	0.0	0.000	0.00
A1a	0.6	0.100	6.46
A1b	1.0	0.128	11.84
F	0.7	0.169	8.13
LA1c+	2.7	0.204	31.57
SA1c	8.0	0.247	73.82
A0	89.3	0.399	1033.55
	TOTAL AREA		1165.37

HbA1c 8.0 %
HbA1c 64 mmol/mol

17. References

1) Global report on diabetes. World Health Organization; 2016.
2) The International Expert Committee (American Diabetes Association). International Expert Committee Report on the Role of the A1c Assay in the Diagnosis of Diabetes. Diabetes Care 2009; 32 (7): 1327–1334.
3) American Diabetes Association. Standards of Medical Care in Diabetes - 2010. Diabetes Care 2010;33 (Suppl. 1), S11-61.
4) IDF Clinical Practice Recommendations for managing Type 2 Diabetes in Primary Care. International Diabetes Federation; 2017.
5) Use of Glycated Haemoglobin (HbA1c) in the Diagnosis of Diabetes Mellitus, World Health Organization; 2011.
6) American Diabetes Association. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes – 2021. Diabetes Care 2021; 44 (Suppl. 1), S15-33.
7) American Diabetes Association. Standards of Medical Care for Patients with Diabetes Mellitus. Diabetes Care 2002; 25 (Suppl. 1): S33-49.
8) Geistanger A, Arends S, Berding C, Hoshino T, Jeppsson J-O, Little R, Siebelder C and Weykamp C, Statistical Methods for Monitoring the Relationship between the IFCC Reference Measurement Procedure for Hemoglobin A1c and the Designated Comparison Methods in the United States, Japan, and Sweden. Clin Chem 2008; 54(8) :1379-1385.
9) Bergmann et al, The influence of sample freezing at - 80 °C for 2-12 weeks on glycated haemoglobin (HbA1c) concentration assayed by HPLC method on Bio-Rad D-10® auto analyzer, Biochem Med (Zagreb). 2016 Oct 15;26(3):346-352.
10) Rohlfing et al, Effects of whole blood storage on hemoglobin a1c measurements with five current assay methods. Diabetes Technol Ther. 2012 Mar;14(3):271-5. doi: 10.1089/dia.2011.0136. Epub 2011 Oct 27.

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