Tosoh India Pvt. Ltd.,

Tosoh HLC-723GX

Automated Glycohemoglobin Analyzer

INSTALLATION QUALIFICATION

For

DOCTORS LABORATORY,

14-Q2 Nethaji By-Pass Road, Opp. Govt Hospital,
Dharmapuri, Tamil Nadu 636701

MARKETED BY:

Tosoh India Pvt. Ltd.

GEBI Industrial Park, Building No. “C”, Bhiwandi, Thane- 421302

TOSOH

Validation Protocol : Installation Qualification

System / Instrument : HLC-723GX

Protocol Written By : Tosoh India Pvt. Ltd.

Laboratory : DOCTORS LABORATORY

Engineering Approval By : GAVASKAR

Laboratory Approval By :DR.M.GANDHI

QA Approval By :DR.M.GANDHI

Objective

To ensure that the system / Instrument installed confirms to the purchase specifications and the manufacturers literature, and to document the information that the equipment meets the specifications.

Scope

To be performed at time of installation, modification or relocation.

Responsibility

Person overseeing the installation from Tosoh India Pvt. Ltd. will perform the qualification and record the information. He will verify the records and write the IQ Report.

Engineering Department at DOCTORS LABORATORY, DHARMAPURI site will review the IQ Results.

Quality Assurance Department at DOCTORS LABORATORY, DHARMAPURI site will approve the IQ Protocol and Report.

System / Equipment: HLC-723GX **Instrument ID:**

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a) Description of the System / Instrument being installed:

The HLC-723GX is intended to assay A1c (%) out of the total hemoglobin in blood for in vitro diagnostic use based on High Performance Liquid Chromatography principle with the cationic non-porous ion exchanger using the ionic difference. To use the analyzer, simply place the cap- pierced primary tube on the rack of the sample loader, and the analyzer will assay for A1c every 2.2 minutes with sampling and dilution.

Analyzer Characteristics:

1. Operation Panel

The operation panel is a monochrome LCD with touch keys. The operation is controlled with the touch keys on the screen. Various settings can be made on the screen. Individual basic function keys such as POWER, START, STOP, HOME and ERROR RESET are provided on the right side of the display. Routine operations are executed with these keys.

2. LED Panel

Three kinds of Light Emitting Diodes (LEDs) indicate the analyzer status: Power, Run, and ERROR

3. Printer

The printer paper roll is thermal-sensitive. It prints out assay results, error messages and parameter status. The assay results can be printed out in two different formats. A roll can handle about 350 sample results depending upon the format.

4. Storage Device

The analyzer is equipped with an internal USB socket. It is used to store assay results, update and backup program versions. A maximum of 12,000 sets of assay results (approximately 500 days) can be stored on one card (32 MB) formatted by the analyzer. The last 800 sets of assay results are also automatically saved in the analyzer's internal memory.

5. Line Filter

The line filter prevents impurities (such as dust from a broken valve seal) from entering the assay line. The filter element can easily be replaced by hand without any tools.

6. Column Oven

The column oven contains the column, a critical component in assaying. The column must be kept at a constant temperature always to prevent temperature fluctuations that can have an effect on the test results. The column oven maintains a constant temperature so that no wait time is required, unless the main power switch (left side) is turned off. The column can be manually connected and can be easily replaced without any special tools.

7. Drain Valve

If air enters the pump, open this valve and perform a drain flush in order to remove all air out of the instrument. Do not open this valve during assay.

8. Injection Valve

This valve is used to inject a sample into the assay line after it is diluted. The sample loop volume is 6 µL.

9. Rotary Valve

The rotary valve is used to switch flow paths during sampling and elution buffer priming.

10. Sampling Mechanism

By means of detectors the instrument can make a difference between sample cups and whole blood samples. In case of whole blood, the sample is automatically diluted and injected into the assay line. When the sample is injected into the column, the sample holder is rotated and will continue till last sample arranged on turn table.

11. Pump

The pump uses the plunger method to deliver the elution buffer required for the assay. The pump operates continuously to deliver the elution buffer during the assay and feeds three different concentration elution buffers in 2.2 minutes cycles by switching the solenoid valves. It also forms a gradient (concentration control), and the hemoglobin fractions are separated by the column.

12. Degassing unit

The degassing unit removes air bubbles in the elution buffer. The vacuum pump runs intermittently to keep a constant vacuum pressure in the chamber.

13. Turn Table

The Turn table has 10 sample holders for setting samples. Primary tubes and sample cups can be set in the sample holders. Detection of the presence of samples and identification of primary tubes and sample cups take place automatically and the samples are aspirated into the sampling mechanism. Whole blood samples are automatically diluted, and taken to the assay line.

b) List of the main components

Main Unit (HLC-723GX) 1

- Power Cord for the Main Unit 2 m 1
- Waste Eluent Bottle 5 L 1
- Waste Tank Container 1
- Screw Driver (+) 100 mm 1
- Sample Vial 50
- Flared Type Union 1
- Printer Paper (Thermal paper roll) 1
- System USB Stick (Smart Media) 1
- Holder for Reagent pack 1
- 5 L Bottle Cap 1
- Accessory box 1

c) Additional Accessories

*Accessory list included with the instrument

System/Instrument: HLC-723GX Laboratory: DOCTORS LABORATORY, DHARMAPURI

Checklist:

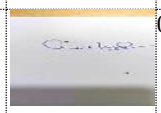
System / Equipment: HLC-723GX Instrument

	Required / Ordered	Actual	Deviations
Model	HLC-723GX	HLC-723GX	Nil
System Description	Glycohemoglobin Analyzer	Glycohemoglobin Analyzer	Nil
Dimensions of Analyzer Unit -			
Width	370mm	370mm	Nil
Depth	525mm	525mm	Nil
Height	482mm	482mm	Nil
Weight	25Kg	25Kg	Nil
Electrical Power Requirements -			
Line Voltage	100 – 240 VAC	100 – 240 VAC	Nil
Frequency	50/60HZ	50/60HZ	Nil
Power Consumption	180VA	180VA	Nil
Environmental Conditions -			
Temperature	15º C – 30º C	15º C – 30º C	Nil
Humidity	40% – 80%, (No condensation)	40% – 80%, (No condensation)	Nil
Dust	Typical office level	Typical office level	Nil

Water Requirements	Nil	Nil	Nil
Liquid Waste -			
Liquid Waste Container	5 Litres	5 Litres	Nil
Throughput Rate -			
Assay Measurement	2.2 Samples/min	2.2 Samples/min	Nil
Sampling System -			
Sample Pipette Principle	Cap Piercing	Cap Piercing	Nil
Sample Volume per Test	3ul Whole Blood, 120ul Diluted Sample	3ul Whole Blood, 120ul Diluted Sample	Nil
Tube / Sample Cup Detection	Possible	Possible	Nil
Sample Loading Capacity Continuous Loading	Maximum 10 Possible	Maximum 10 Possible	Nil
Reagent System -			
Reagents	Buffer 1, Buffer 2, Buffer 3 and Hemolysis/wash solution	Buffer 1, Buffer 2, Buffer 3 and Hemolysis/wash solution	Nil
No Extra Wash Solution	Yes	Yes	Nil

Validation Protocol: Installation Qualification

System/Instrument: HLC-723GX Laboratory: DOCTORS LABORATORY, DHARMAPURI

Installation Procedure	Protocol Location	Performed		Sign	Date
		Yes	No		
Installation of Hardware	See Chapter 2 – Installation of the Operators Manual	YES			01.01.2022
Installation Checks	See Chapter 2 – Installation of the Operators Manual	YES			01.01.2022

Performed by: **Signature / Date:**

Deviation: NIL

Reviewed By: Engineering Dept – Tosoh India Pvt. Ltd. **Signature / Date:** 01.012022

Tosoh India Pvt. Ltd.,

Tosoh HLC-723GX

Automated Glycohemoglobin Analyzer

OPERATIONAL QUALIFICATION

For

DOCTORS LABORATORY,

14-Q2 Nethaji By-Pass Road, Opp. Govt Hospital,
Dharmapuri, Tamil Nadu 636701

MARKETED BY:

Tosoh India Pvt. Ltd.

GEBI Industrial Park, Building No. “C”, Bhiwandi, Thane- 421302

TOSOH

Validation Protocol : Operational Qualification

System / Instrument : HLC-723GX

Protocol Written By : Tosoh India Pvt. Ltd.

Laboratory : DOCTORS LABORATORY

Engineering Approval By : GAVASKAR

Laboratory Approval By : DR.M.GANDHI

Q.A. Approval By :DR.M.GANDHI

Objective

To determine that the system/ instrument operates according to specifications and to record all relevant information and data to demonstrate it functions as expected.

Scope

To be performed after installation, modification, or relocation has been completed.

Responsibility

Person responsible for operating the system/ instrument from Tosoh India Pvt. Ltd will perform the qualification and record the information.

He will supervise the study, verify the completion of the records, and write the deviation report and the operational qualification report.

Customer quality assurance department will review and approve the OQ protocol and report.

Materials, SOP's, Documents:

Following are the topics course needed to perform the operation qualification -

- Daily operating procedures – Operator's Manual - Chapter 3.0
- Maintenance procedures – Operator's Manual - Chapter 5.0
- Special operation – Operator's Manual - Chapter Appendix

Procedure:

- Provide SOP's and data sheets for normal operation of the system
- Provide basic operation training and documenting the operators has been trained.
- Ensure adequate practice with general maintenance and some tips to trouble shooting.
- Test and record calibration data with QC report.
- Test and record outputs.
- Record any deviations to the procedures performed
- Prepare a deviation report including the justification of acceptance and impact on the operation.

Prepare an Operational Qualification Report:

- This should include data study initiated, data competed, observations mode, problems encountered, completeness of information collected, results of control/ alarm tests, sample data if appropriate, other information relevant to the study, and conclusions on the validity of the instrument/ system operations.

- Submit the reports to QA for review and approval.

Preparation: Document check

SOP Title and number	File Location	QA/QC approval date
Daily Operation		
Maintenance		
Special operations		

Training Records:

Name	Signature
MRS.YAMUNA	
MRS.BHARATHI	
MR.GOVINDHARAJ	
MR.TAMILSELVAN	
MR.UDHAYSHANKAR	
MR.SUBRAMANI	
MR.KARTHICK	

For training certificates contact local Support team members

Equipment make and model	**Manual available**
Tosoh HLC-723GX	Y { Y} N {Y }

Instrument SNo:

Results

Calibration and Control data:

Calibration data

Test	Date performed	Results	Acceptable Y/N
HbA1c	01.01.2022	VALID	**Y**
HbA1c	01.01.2022	VALID	**Y**
HbA1c	01.01.2022	VALID	**Y**
HbA1c	01.01.2022	VALID	**Y**
HbA1c	01.01.2022	VALID	**Y**

QC Data: Tosoh HbA1c Control

Test	Control	Results	Acceptable Y/N
HbA1c Level 1 Control			
HbA1c Level 2 Control			

Deviation Report

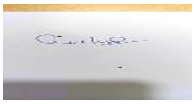
- **Deviation(s)** **:** NIL
- **Justification for Acceptance** **:** All operational requirements qualified.
- **Impact on Operation** **:** Instrument ready for its performance qualification & routine operation.

Operation Qualification Report

- **Date Study Initiated** **: 29.12.2021**
- **Date Study Completed** **:01.01.2022**
- **Observations Made** **:** Performance qualification complies as per manufacturer Recommendations
- **Problems encountered** **:** Nil
- **Completeness of Information Collected** **:** All information found to be complete
- **Results of the Tests** **:** Acceptable results.
  For Calibration and QC results refer to the attachment

Conclusions on the validity of the system operations:

- Study data has determined that the system described in this document meets/ does not meet all the criteria outlined in this operational qualification protocol.
- Operation qualification completed/ not completed successfully
- The system is ready for its performance qualification.

Performed By: Tosoh India Pvt. Ltd. **Signature /** 

Deviations: NIL

Verified By: DR.M.GANDHI **Signature / Date:**

01.01.2022

Tosoh India Pvt. Ltd.,

Tosoh HLC-723GX

Automated Glycohemoglobin Analyzer

PERFORMANCE QUALIFICATION

For

DOCTORS LABORATORY,

14-Q2 Nethaji By-Pass Road, Opp. Govt Hospital,
Dharmapuri, Tamil Nadu 636701

Tosoh India Pvt. Ltd.

GEBI Industrial Park, Building No. “C”, Bhiwandi, Thane- 421302

TOSOH

System/ Instrument: HLC- 723GX Automated HPLC Analyzer

Protocol Written by: Tosoh India Pvt.Ltd.

Doctors Laboratory, Dharmapuri

Laboratory Approval by:

QA Approval by:

Objective

To determine that the system/ instrument operates according to specifications and to record all relevant information and data to demonstrate it functions as expected.

Scope

To be performed after installation, modification, or relocation, after the installation qualification and Operational qualification has been completed.

Responsibility

Person responsible for operating the system/ instrument from Tosoh India Pvt. Ltd. will perform the Performance qualification report and record the information.

He will supervise the study, verify the completion of the records, and write the deviation report, assay validation and the Performance qualification report.
Customer quality assurance department will review and approve the PQ protocol and report.

a.) Description of the System/ Instrument being Installed:

HLC-723GX works on the principle of High-Performance Liquid Chromatography (HPLC), The analyzer uses the Cation exchange column to separate hemoglobin components by different ionic charge.

a. Variant Analysis Mode

Instrument interfaced either with 10 samples Turn Table (Sample loader).

Procedure:

Provide SOP's and data sheets for normal operation of the system

Provide basic operation training and documenting the operators have been trained.

Ensure adequate practice with general maintenance and some tips to trouble shooting.

Test and record calibration data with QC report.

Test and record outputs.

Record any deviations to the procedures performed

Prepare a deviation report including the justification of acceptance and impact on the operation.

Prepare an Performance qualification report:

This should include data study initiated; data competed; observations mode; problems encountered; completeness of information collected; results of control/ alarm tests; sample data if appropriate; other information relevant to the study; and conclusions on the validity of the instrument/ system operations.

Submit the reports to QA for review and approval.

Document the information requested below:

Instrument Manufacturer : TOSOH Corporation, Japan

Reagent Manufacturer: TOSOH Corporation, Japan

Reagents Used to Estimate the requested Test:

1. TSK gel HLC – 723 Variant HSi column
2. Variant Elution Buffer HSi No. 1
3. Variant Elution Buffer HSi No. 2
4. Variant Elution Buffer HSi No. 3
5. HSi Hemolysis & Wash Solution
6. Filter Element
7. Calibrator - 2 levels

Accessories:

1. Printer Paper
2. Control Level 1 & Level 2

Performed by: Tosoh India Pvt.Ltd., Sign/date

Deviations: NIL

Verified By:DR.M.GANDHI

Sign/date

01.01.2022

Results:

Calibration and Control Data

Calibration data:

Test	Date performed	Results (%)	Acceptable Y/N
Tosoh Calibrator 1	01.01.2022	5.9	Y
Tosoh Calibrator 2	01.01.2022	10.5	Y

QC Data

Test	Control Range	Results (%)	Acceptable Y/N
Tosoh HbA1c Control Level 1	5.2-5.8	5.5	Y
Tosoh HbA1c Control Level 2	8.2-9.5	8.9	Y

For Cal and QC results data refer to attachment

Performed by: Tosoh India Pvt. Ltd., Sign/date

Deviations: NIL

Verified By:DR.M.GANDHI Sign/date

01.01.2022

Purpose & Scope: Performance Validation Of Tosoh HLC-723G8

Specimen: Whole Blood

Experiments:

1. Accuracy check& Precision check
2. Method Comparison

***Refer to Attached Documents**

Performance Qualification Report

Date study initiated:

Date study completed:

Observations Mode:

Performance qualification complies as per manufacturer recommendations

Problems encountered:

Nil

Completeness of Information collected:

All information found to be complete

Results of the tests:

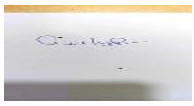
Acceptable results. For Calibration and QC results refer to the attachment

Conclusions on the validity of the system operations:

Study data has determined that the system described in this document meets/ does not meet all the criteria outlined in this operational qualification protocol.

Operation qualification completed/ not completed successfully

The system is ready after its performance qualification for routine operations.

Performed by: Tosoh India Pvt. Ltd., Sign/date 

Deviations: nil

Verified By:DR.M.GANDHI Sign/date

International Federation of Clinical Chemistry and Laboratory Medicine
Network on Standardization of HbA1c

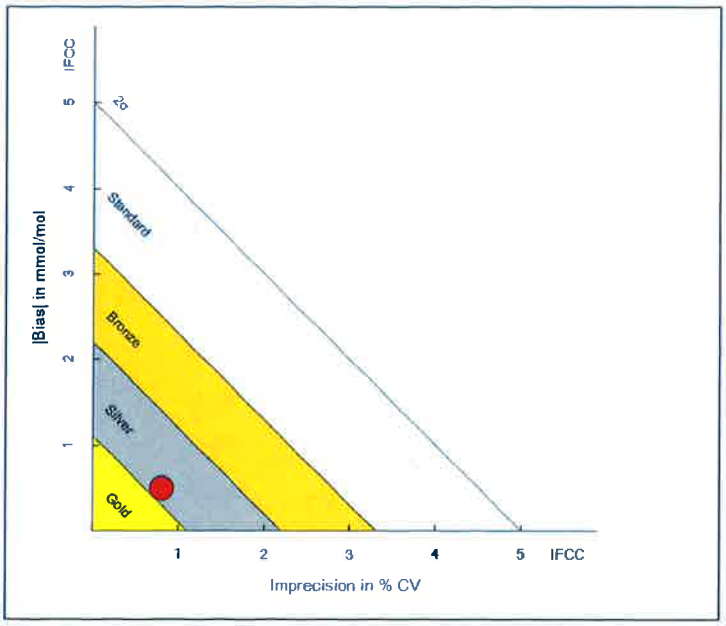
Certificate

Tosoh Corporation

using

HLC-723GX

participated in the IFCC HbA1c Certification Programme to demonstrate traceability to the IFCC Reference Measurement Procedure and performed as shown below.



Total Error =	1.3	mmol/mol
Bias =	+0.5	mmol/mol
Imprecision =	0.8	%
Grade =	Silver	

Criteria derived from the IFCC model for Quality Targets HbA1c (Clin Chem 2015;61 : 752-59)

Date of Certification : 01 January 2022

Date of Expiry : 01 January 2023

IFCC Network Coordinator
C. Siebelder

IFCC International Federation of Clinical Chemistry and Laboratory Medicine
Network on Standardization of HbA1c

Summary Analytical Performance

Organisation	**Tosoh Corporation**		
Instrument	**HLC-723GX**		
Date Of Certification	01 January 2022		
Bias	at 30 mmol/mol	0.7	mmol/mol
	at 50 mmol/mol	0.5	mmol/mol
	at 70 mmol/mol	0.3	mmol/mol
Imprecision	CV	0.8	%
Linearity	r	0.9997	
Total Error	TE	1.3	mmol/mol

Analytical Performance Individual Samples

Sample ID	Target Value	Your Result	Your Bias
01	45.2	46.3	1.1
02	42.6	43.5	0.9
03	40.3	41.6	1.3
04	96.2	96.5	0.3
05	53.5	54.1	0.6
06	66.3	66.5	0.2
07	48.2	48.6	0.4
08	77.6	76.9	-0.7
09	34.5	35.2	0.7
10	56.1	56.7	0.6
11	37.8	38.7	0.9
12	66.3	66.4	0.1
13	53.5	54.3	0.8
14	92.6	93.4	0.8
15	58.8	59.1	0.3
16	42.6	43.3	0.7
17	62.6	62.8	0.2
18	31.4	31.0	-0.4
19	83.1	83.2	0.1
20	77.6	77.3	-0.3
21	50.9	51.6	0.7
22	72.1	71.9	-0.2
23	58.8	59.2	0.4
24	88.7	89.3	0.6

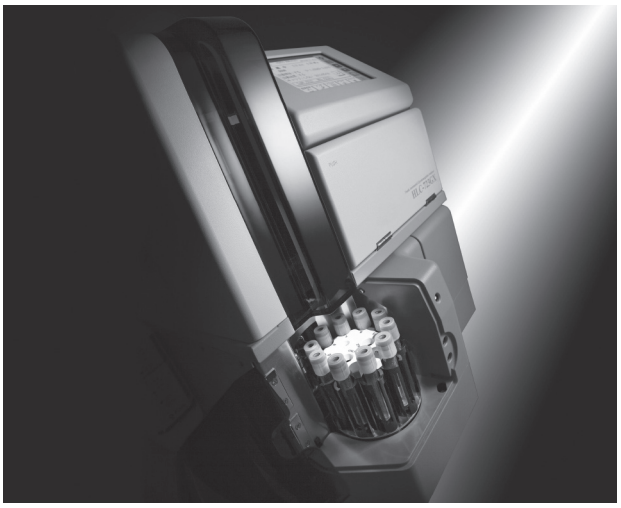
* Only if applicable: Blunder, excluded from the calculations

TOSOH

Tosoh Automated Glycohemoglobin Analyzer

HLC-723GX

Technical Report



Contents

1. Introduction

Diabetes mellitus is a disorder of metabolism featuring chronic hyperglycemia. It is caused by deficient secretion of insulin, which is secreted by the pancreas and is the only hormone that decreases the blood sugar level. Another cause of diabetes mellitus may be relative deficiency of insulin action due to insulin resistance.

In 2011, the International Diabetes Federation (IDF) reported that the population of diabetic patients was 366 million, and that the number of people dying due to diabetes mellitus was 4.6 million/year. Compared with the 2008 announcement, the diabetic population had increased 1.3 times, and the number of deaths due to diabetes had increased 1.2 times. Specifically, the numbers of diabetic patients are exploding in developing countries, and to solve this problem, upgrading the medical care system and the education system for diabetes is a major issue.

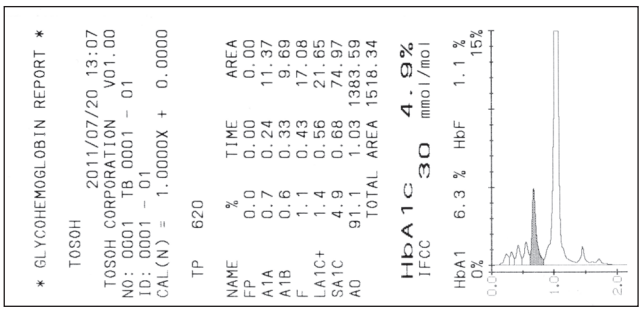
When diabetes is uncontrolled, microvascular damage that leads to complicating disorders such as retinopathy, nephropathy, and neuropathy can occur. Visual loss or kidney injury is increasing dramatically, and these are caused by progression of these disorders. In addition, recently, it has been reported that diabetes mellitus is related to macrovascular disease, such as myocardial infarctions and strokes [1)]. Thus, reduction in the number of diabetic patients is the worldwide goal.

Under such circumstances, in June 2009, an international committee of experts organized by the American Diabetes Association (ADA), IDF, and European Association for the Study of Diabetes (EASD) compiled a report that recommended the adoption of a diagnostic cut point of HbA1c over 6.5% (NGSP value)[2)]. This publication showed again the clinical usefulness of HbA1c for observing chronic glycemic levels. Then, in January 2010, the ADA proposed a new diagnostic cut point: that an HbA1c (NGSP value) of 6.5% or more should be treated as a diabetic disorder. Furthermore, the World Health Organization (WHO) also recommended using HbA1c for the diagnosis of diabetes mellitus[3)]. In this way, HbA1c now has a significant role to play in diabetes mellitus diagnosis internationally, and a measurement system with “high-accuracy and high speed” is increasingly requested.

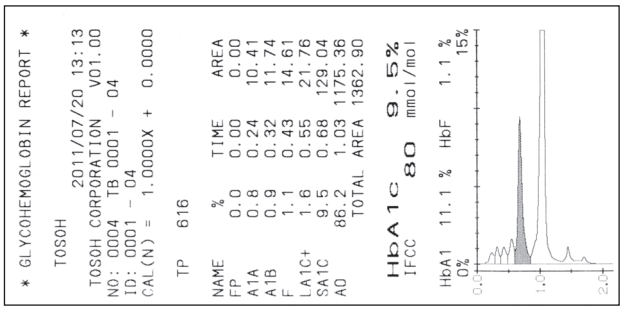
The HLC-723GX (GX), Tosoh’s latest model, has a downsized analyzer maintaining high precision measurement, and typical variant hemoglobins (HbD/S/C) do not interfere with the results. This highly precise measurement is helpful for diagnosis and for monitoring therapeutic progress in diabetic patients. The GX is particularly well suited for laboratories and clinics that deal with or monitor ambulatory care patients.
Not only is the GX expected to work well in Europe and the United States, it is also expected to work well in countries with variant hemoglobin carriers, such as Asia and Africa, identifying abnormal hemoglobins, as well as easily measuring the HbA1c values of the specimens.

Typical chromatogram

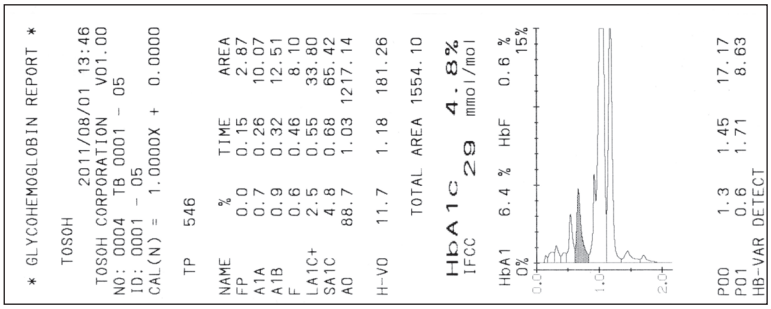
● Healthy individual



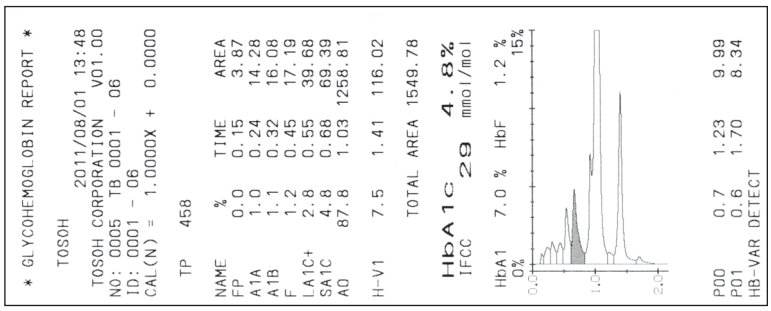
● Diabetic patient



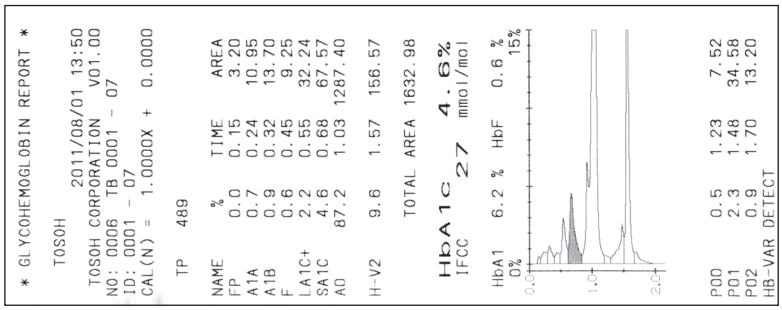
● HbD specimen



● HbS specimen

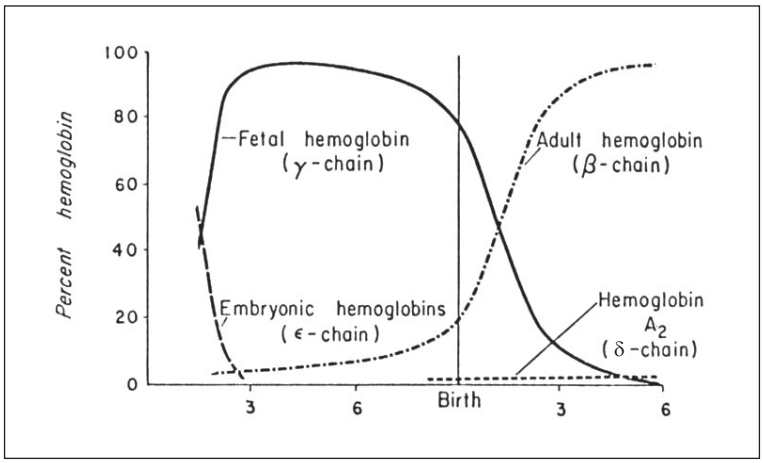


● HbC specimen



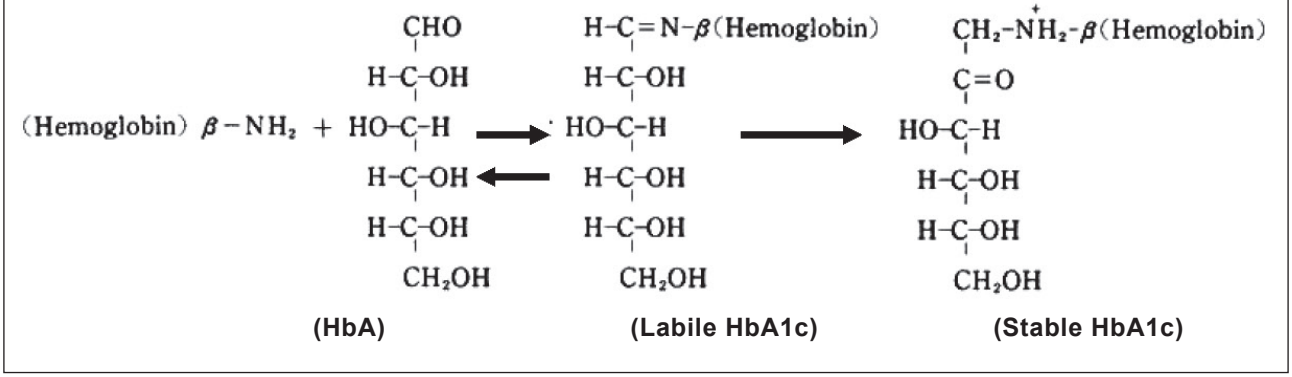
2. Clinical Significance of HbA1c

Human hemoglobin is a protein with a molecular weight of 64,500. It is a complex of 4 units of two different types of polypeptide chains. The hemoglobin of a normal adult primarily contains α- and β-chains, with γ- and δ-chains as minor components. The hemoglobin consisting of the α-chain and β-chain ($\alpha 2\beta 2$) is called HbA (HbA0), and the hemoglobin consisting of the α-chain and γ-chain ($\alpha 2\gamma 2$) is called HbF (fetal hemoglobin). HbF is dominant in the fetus and is then almost completely replaced by HbA0 in about the first 6 months of life[4].



Ontogeny and associated hemoglobin chain synthesis (Bunn et al., 1977)

Within HbA, sugar bound to the N-terminus of the β-chain is generally called HbA1. Of the different HbA1 components, HbA1c which is the glucose-bound β-chain, accounts for the highest percentage and is regarded to be the most clinically important indicator[5]. HbA1c is synthesized in two-step non-enzymatic reaction described in the picture below. Labile form A1c (L-A1c), the intermediate of the reaction, increases temporarily after a glucose tolerance test or eating food, while the stable form A1c (s-A1c) does not change with temporary increases in the blood glucose level. Today, HbA1c measurement based on the principle of HPLC is most widely performed, and modified hemoglobins (labile A1c, aldehyde Hb, or carbamylated Hb) have only a minimal effect through advances in column technology.



HbA1c is closely linked to the severity of diabetes mellitus in patients and is recognized as the best indicator of diabetes and a convenient marker of control. It was reported by the Diabetes Control and Complications Trial Research Group (DCCT) in 1993[6] and by the Kumamoto Study in 1998[7] that strict long-term control of blood glucose significantly decreased the occurrence of complications such as diabetic retinopathy and nephropathy. In the report, A1c (s-A1c) was used as a control marker, and the significance of s-A1c measurement was acknowledged.

3. Abnormal Hemoglobins

Hemoglobins with a point mutated amino-acid sequence of the polypeptide chain are generally termed variant hemoglobins, and they are commonly observed in patients with inherited hemoglobin disorders. About 800 forms of variant hemoglobins have been found thus far, and HbS, HbD, and HbC are typical hemoglobin variants[8]. Most hemoglobin variants are associated with no clinical symptoms, but some types of variant hemoglobins are associated with serious disorders such as sickle cell syndrome (HbS). Even now, many different varieties of hemoglobin variants are found by HPLC, which enables medical treatment. Therefore, when using HbA1c as the marker for diabetes mellitus diagnosis or blood sugar control, checking for variant hemoglobin is necessary.

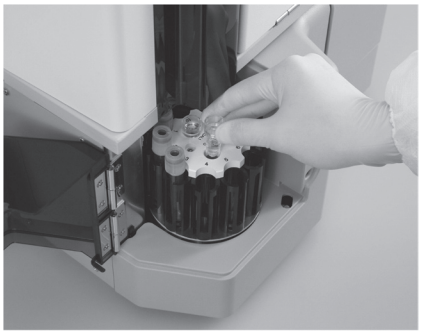
4. Worldwide Standardization of HbA1c Measurement

Diabetic patients are increasing significantly worldwide, and, thus, it is a serious problem that HbA1c values, indispensable for blood sugar control, are not internationally unified. In June 2007, 4 associations from all over the world (ADA, EASD, International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), and IDF) published a consensus statement on worldwide standardization of the HbA1c measurement[9] and proposed to go ahead with the standardization with a scientifically grounded IFCC value (mmol/mol).

5. Instrument Outline

The HLC-723GX is a dedicated system for HbA1c measurement based on the principle of high-performance liquid chromatography (HPLC). The system is a single bench-top type unit, including a turntable sampler, elution pump, degassing unit, sampling unit, detector, printer, operation panel, USB socket, control section, and a power supply in a compact body. The sampler with a turntable is available for 10 samples, with two exclusive holders for calibrators.

Appearance of the analyzer



Turntable

The sampler unit uses the cap piercing method. This allows vacuum blood collection tubes (12 to 15 ϕ, 75 mm, and 100 mm) to be set directly and measured with the caps on. In addition, when pre-diluted samples, calibrators, controls, and other samples are placed and set in sample cups, the instrument automatically recognizes the dilution and executes measurements accordingly.
Data management now includes a new built-in USB device. With the recommended setting, measurement results for approximately 250,000 samples can be stored in a USB memory storage device of 1 Gbyte.
A query function for selecting measurement samples through communication with an up-line host and visual monitoring of the Eluent Buffer and the Hemolysis & Wash Solution are available. In addition, the pop-up function, which prompts the confirmation of standard values during calibration, as well as the flag setting function, checking and supporting abnormal values to be reported, has been upgraded for greater safety.
The newly developed TSKgel® GX (4.6 mm I.D.×20 mm) is used as the column for the GX. Hemoglobin components can be divided into 6 fractions, including A1a, A1b, HbF, L-A1c+, s-A1c, and A0, through the use of a step-wise gradient eluting method that uses 3 buffers with different salt concentrations. If hemoglobin variants (i.e., HbS, HbD, HbC) are present, GX can separate them from normal hemoglobin components. Since the hemoglobin variants are eluted behind A0 as H-V0, H-V1, and H-V2, they cause no interference with the HbA1c value.
Separated components are measured at a wavelength of 415 nm, followed by a wave-forming process, and the measurement results are printed.
Furthermore, GX offer consumable supplies, such as a simplified “GX Assay Kit”, consisting of Buffers, Hemolysis & Wash Solution, Filter Elements, and roll papers.

6. Performance Data

● Intra-assay precision (n=10)

	Low	Medium	High
MEAN	5.30	7.86	10.46
SD	0.00	0.05	0.05
CV%	0.00	0.64	0.48

● Inter-assay precision (n=10)

	Low	Medium	High
MEAN	5.37	7.84	10.42
SD	0.05	0.05	0.04
CV%	0.93	0.64	0.38

● Stability of HbA1c measurement values at the beginning of the routine

Day	Low	Medium	High
1	5.3	7.8	10.4
2	5.4	7.9	10.5
3	5.3	7.9	10.5
4	5.4	7.9	10.4
5	5.4	7.9	10.4
6	5.3	7.8	10.4
7	5.4	7.8	10.4
8	5.4	7.8	10.4
9	5.4	7.8	10.4
10	5.4	7.8	10.4
MEAN	5.37	7.84	10.42
SD	0.05	0.05	0.04
CV%	0.93	0.64	0.38

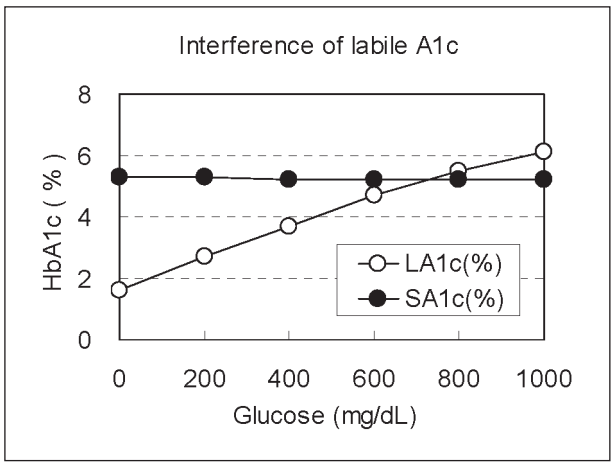
● Recovery study by mixing samples

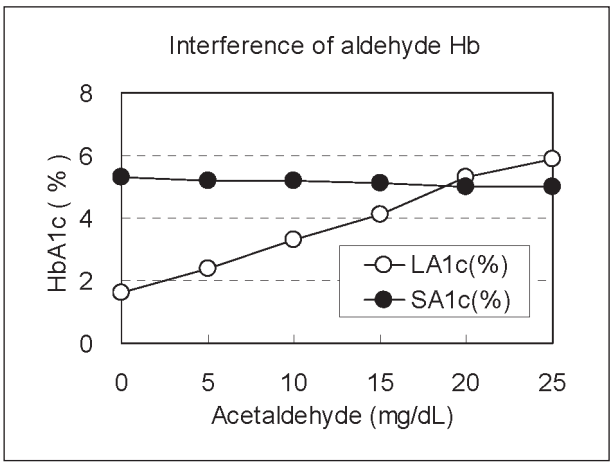
High HbA1c sample (ratio)	Low HbA1c sample (ratio)	Measured value s-A1c (%)	Theoretical value s-A1c (%)	Recovery (%)
0	10	2.7	2.7	-
1	9	4.5	4.5	100.3
2	8	6.3	6.3	100.4
3	7	8.0	8.1	99.7
4	6	9.7	9.8	98.5
5	5	11.6	11.6	99.9
6	4	13.2	13.4	98.9
7	3	15.0	15.1	99.3
8	2	16.8	16.9	99.5
9	1	18.6	18.7	99.9
10	0	20.4	20.4	-

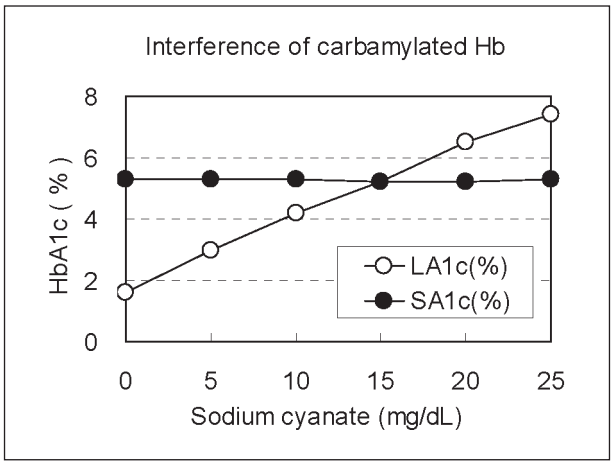
● Dilution linearity

Hb conc (g/dL)	s-A1c(%)	TOTAL AREA
4	5.1	309.43
9	5.4	732.21
10	5.4	867.50
16	5.4	1375.34
21	5.4	1768.12
26	5.5	2203.57
32	5.5	2754.26
40	5.5	3411.56
49	5.5	4192.41
72	5.9	6174.03

● **Interference**







● **Effects of coexisting substances**

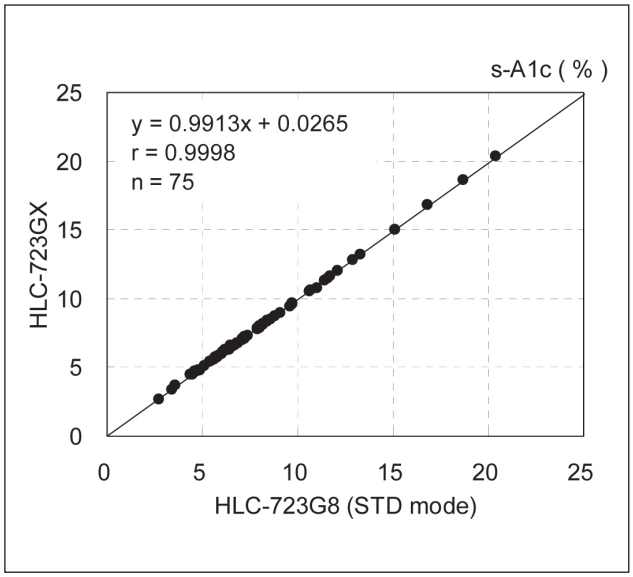
Free bilirubin	~	19.2	mg/dL
Conjugated bilirubin	~	20.1	mg/dL
Lipids	~	1,500	formazine turbidity
Acetylsalicylic acid	~	25	mg/dL
Ascorbic acid	~	200	mg/dL

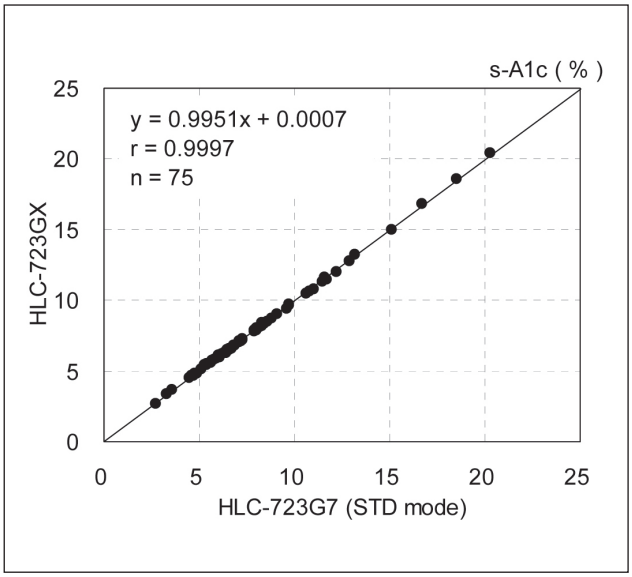
No Interference from coexisting substances up to above concentrations.

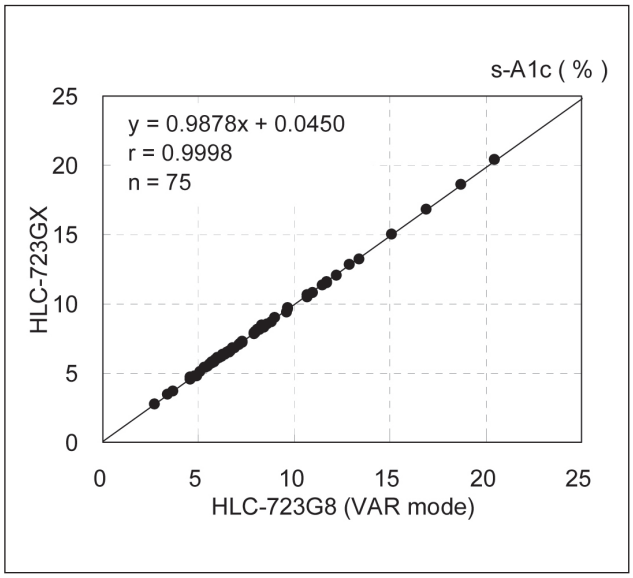
● **Correlation**

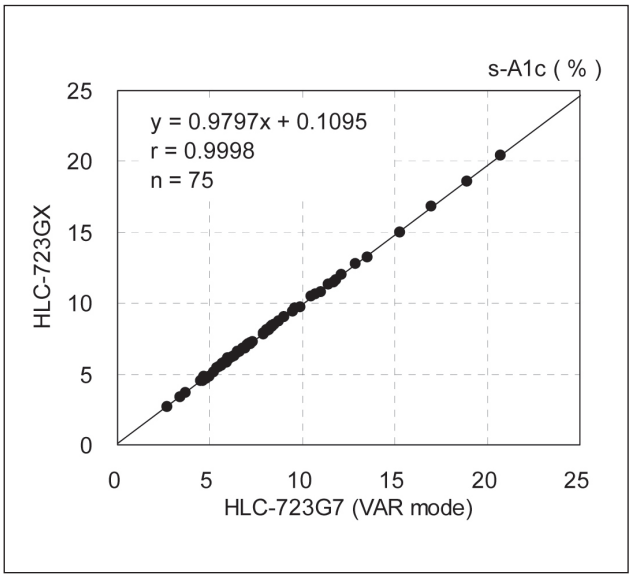
Correlation between GX and current systems

HbA1c

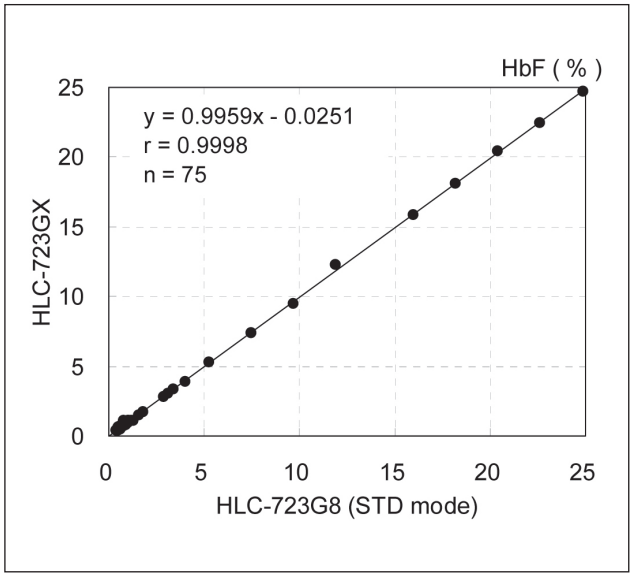


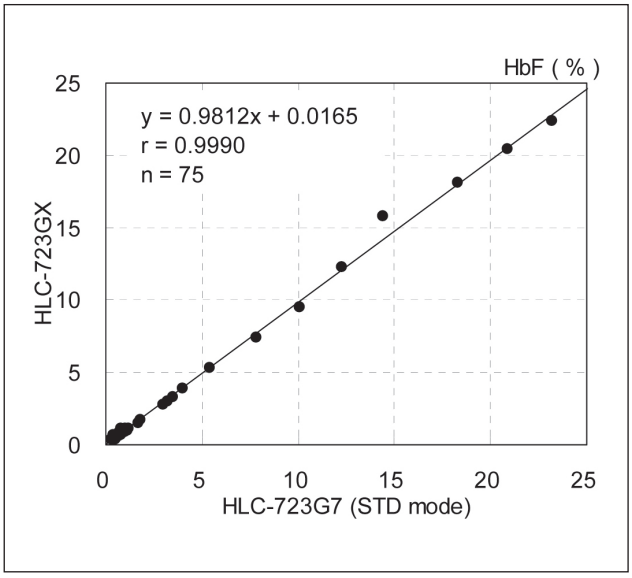


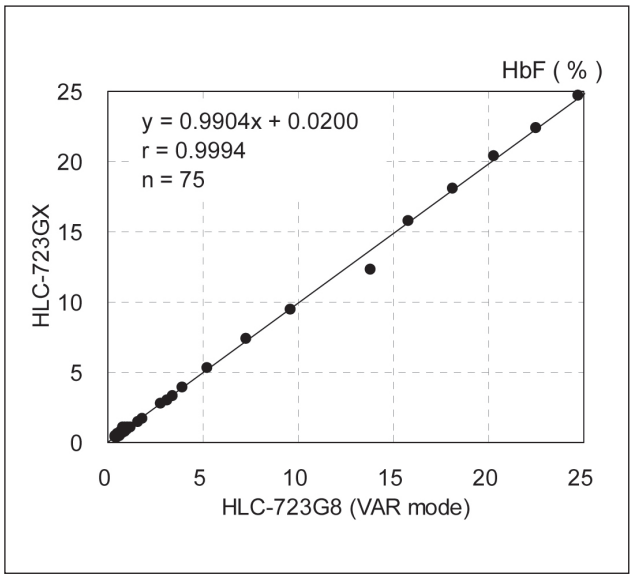


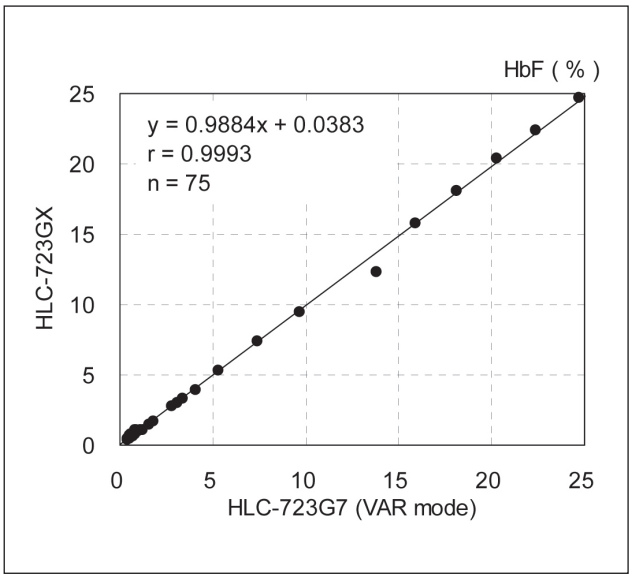


HbF

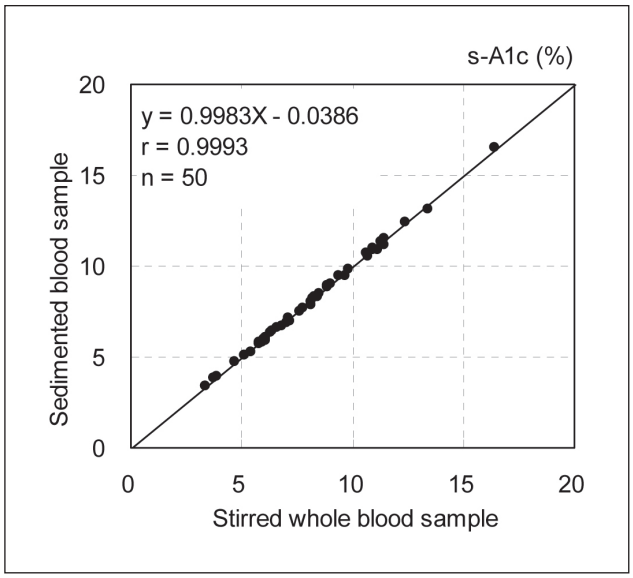

HbF (%)
y = 0.9959x - 0.0251
r = 0.9998
n = 75
HLC-723GX
HLC-723G8 (STD mode)
0
5
10
15
20
25


HbF (%)
y = 0.9812x + 0.0165
r = 0.9990
n = 75
HLC-723GX
HLC-723G7 (STD mode)
0
5
10
15
20
25

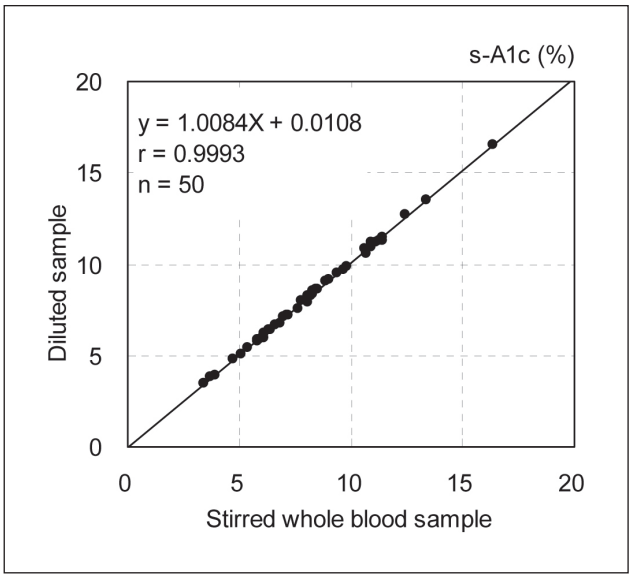

HbF (%)
y = 0.9904x + 0.0200
r = 0.9994
n = 75
HLC-723GX
HLC-723G8 (VAR mode)
0
5
10
15
20
25


HbF (%)
y = 0.9884x + 0.0383
r = 0.9993
n = 75
HLC-723GX
HLC-723G7 (VAR mode)
0
5
10
15
20
25

Difference between HbA1c values under several sample conditions
HbA1c



This shows the correlation of the measurement results with a whole blood sample stirred and not stirred.



This shows the correlation of the measurement results with a stirred whole blood sample and a diluted sample.

● **Stability study of stored samples**

s-A1c (%)

Storage period (days)	EDTA-2K	EDTA-3K	EDTA-2Na	3.8% Citrate	NaF+Citrate +EDTA-2Na	Heparin-Na	Heparin-Li	NaF+Heparin +EDTA-2Na
0	5.2	5.1	5.1	5.1	5.1	5.1	5.1	5.1
1	5.1	5.1	5.1	5.1	5.0	5.1	5.2	5.1
2	5.1	5.1	5.1	5.2	5.0	5.1	5.1	5.1
3	5.1	5.1	5.1	5.1	5.0	5.1	5.1	5.1
7	5.1	5.0	5.0	5.1	5.1	5.0	5.1	5.0
10	5.1	5.1	5.1	5.1	5.1	5.1	5.2	5.1
14	5.0	5.0	4.9	5.0	5.0	5.0	5.0	5.0

The data included in this Technical Report are those obtained from studies Tosoh Corporation performed unless specific notification is given.

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Printed in Japan 3204 FO− Cat.No.0992298 A