UMA CO., LTD.

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Matsudo, Chiba, Japan

MEASURE HbA1c II

Reagent for determination of Hemoglobin A1c

Latex Immunoturbidimetric Method

2 - 8°C	**IVD** *In vitro* Diagnostics	**QUALITY MANAGEMENT SYSTEM (BY TUV)**
DO NOT freeze	24 months/block from light	**ISO 13485:2016**

1. PURPOSE OF USE

Providing a quantitative in vitro assay for the Glycated Hemoglobin (HbA1c) concentration in whole blood or blood cells.

2. GENERAL INSTRUCTION

a. For in vitro diagnostics use only.

b. Diagnosis should be made in a comprehensive manner, in accordance with other related test results and clinical symptoms by the doctor in attendance.

c. For guaranteed results, usage of this product must comply with the instruction in this manual.

d. If you use automatic analyzers, follow their instructions carefully.

SUMMARY

Glycosylated hemoglobin (HbA1c) is the form of hemoglobin that is used widely to identify the average blood glucose levels of a person over the past three months and also can correlate to complications of high blood sugar (diabetes mellitus). It is recommended by international guidelines for evaluating the overall control of diabetes mellitus (DM). The World Health Organization (WHO) and the American Diabetes Association (ADA) also uses the value of HbA1c for the diagnosis of DM.

HbA1C is formed by non-enzymatic glycation of the beta chain of hemoglobin A by the plasma glucose. This glycation is irreversible and occurs continuously throughout the life span of red blood cells, which is 120 days (three months). The HbA1C or the fraction of glycated hemoglobin increases in a predictable manner according to the average level of plasma glucose. Therefore, it gives the blood sugar level estimate of the past three months, with the recent glucose levels having the greatest influence on its value. Various researchers in their studies have shown that the mean blood glucose of previous 1 month, 2 months and 3 months contributes 50%, 40% and 10% respectively to the final result and thus mathematically calculating, the half-life of HbA1c is estimated to be 35.2 days (indicating that half of the glycation of hemoglobin occurred in the previous 35.2 days from the time of its estimation).

3. MATERIALS REQUIRED BUT NOT INCLUDED

- Saline 0.9 % and high grade purified water

- Micropipet and other basic laboratory equipment.

- HbA1c Diluent and HbA1c Calibrator Set and HbA1c Control Set

4. REAGENT COMPOSITION & PREPARATION

- Reagent R-1: Mouse anti-human hemoglobin A1c monoclonal antibody-sensitized latex.

Reagent R-1 is ready for use

- Reagent R-2: Mouse anti-human hemoglobin A1c monoclonal antibody label-goat anti-mouse IgG polyclonal antibody

Reagent R-2 is ready for use

- Once open, Reagent stored on board the instrument is stable for 30 days with Hitachi 7180 Analyzers.

- Applicable to various automated analyzers.

- Calibrators & Controls (separately sold): Put 1 mL of purified water to each vial of calibrators (0, L, M1, M2, H) and controls (L, H), leave at room temperature for 60 minutes before use. After reconstituting, calibrators and control can be used without dilution can be used without dilution.

5. SAMPLE PREPARATION & STORAGE

- Whole blood: Dilute blood sample 51 times (or add 20 µL of whole blood into 1000 µL of HbA1c Diluent or purified water), mix well and confirm hemolysis (transparent solution) before use.

- Blood cell: Treat sample by anticoagulant (i.e EDTA), leave sample to stand for 3 hours or centrifuge at 2000 rpm for 2 minutes; take and dilute blood cell 101 times (or take 10 µL of blood cell layer and add to 1000 µL of HbA1c Diluent or purified water), mix well and confirm hemolysis (transparent solution) before use.

- Analyze sample soon after collection. In case, it could not be analyzed soon, store sample 2 - 8°C and analyze within 3 days.

- Stability:

- 8 hours at 15 - 25°C
- 3 days at 2 - 8°C
- 30 days at < -20°C

6. MEASUREMENT PRINCIPLE

In the first reaction, HbA1c interacts with anti-human hemoglobin A1c mouse monoclonal antibody- sensitized latex. In the second reaction, it further interacts with anti-human HbA1c mouse monoclonal antibody labeled-anti-mouse IgG goat polyclonal antibody. Measure absorbance of coagulated reaction solution and determine the ratio of HbA1c volume against total Hb amount from concentration of HbA1c and values of calibrator.

7. ASSAY PROCEDURE

This product is compatible with various types of clinical analyzer. An example of the assay procedure is indicated below.

Sample	+	Reagent 1	$\xrightarrow[280s]{37^\circ C}$	Reagent 2
4.0µL		150µL		50µL
$\xrightarrow[55s]{37^\circ C}$		Meas. Abs I	$\xrightarrow[265s]{37^\circ C}$	Meas. Abs II
		(660/800nm)		(660/800nm)

$\longrightarrow$ ΔAbs at 660/800nm $\longrightarrow$ Concentration HbA1c

Perform the assay according to the instructions for operating the automated analyzer Hitachi models. Refer to the **13. INFORMATION FOR AUTOANALYZERS** for the details of the assay method. Contact **HUMA MEDICAL CO., LTD.** for information about the parameters for other automated analyzers.

8. CALCULATION & UNIT CONVERSION

Calculation

- Calculate ΔAbs of specimen & standards vs blank

- Plot a calibration curve HbA1c = f(ΔAbs)

- Calculate HbA1c in specimen using the curve

(doing same procedure for Controls)

Unit conversion

NGSP [%] = IFCC [mmol/mol] x 0.09148 + 2.152

9. PERFORMANCE & CORRELATION TEST

a. Measuring range

- The assay is linear within an HbA1c concentration range in whole blood/blood cells of 3 - 15%.

The analytical measuring range of this assay extends from the concentration of calibrator level 0 to H. Samples exceeding the upper limit of the analytical measuring range for HbA1c should not be diluted, but instead should be reported as % HbA1c > 15%.

b. Detection Limit

Limit of Blank (LoB)	=	1%
Limit of Detection (LoD)	=	2%
Limit of Quantitation (LoQ)	=	3%

The LoB, LoD and LoQ were determined in accordance with CLSI EP17-A2 requirements.

The LoB is the highest apparent analyte concentration expected to be found when replicates of a blank sample containing no analyte are tested. The LoB corresponds to the concentration below which analyte-free samples are found with a probability of 95%.

The LoD is determined based on the LoB and standard deviation of low concentration samples. The LoD corresponds to the lowest analyte concentration which can be detected (value above the LoB with a probability of 95%).

The LoQ is the lowest analyte concentration that can be reproducibly measured with a total error of 20%. It has been determined using low concentration samples.

c. Performance

- Sensitivity: Absorbance was less than 0.7 at 660 nm when purified water was used as sample, and more than 1.0 when standard solution of 12% was used as sample.

- Accuracy: When measuring a control sample, the result is within ±10% of assigned value.

d. Precision (on Biolis 30i / SK300)

Representative performance data on the analyzers are given below.

Results obtained in individual laboratories may differ.

Precision was determined using human samples followed the CLSI Approved Guidline EP5-A2 with repeatability, reproducibility and total precison (1 aliquot per run, 2 run per day, 20 days).The following results were obtained.

Criterion: CV of Repeatability (aka. Within-run precision) is less than 1% and Total Precision is less than 3%.

Repeatability	*Mean* %	*SD* %	*CV* %
Human sample 1	5.67	0.05	0.79
Human sample 2	7.66	0.07	0.85
Human sample 3	11.36	0.10	0.91

Reproducibility	*Mean* %	*SD* %	*CV* %
Human sample 1	5.67	0.09	1.65
Human sample 2	7.66	0.13	1.71
Human sample 3	11.36	0.2	1.76

Total precision	*Mean* %	*SD* %	*CV* %
Human sample 1	5.67	0.10	1.74
Human sample 2	7.66	0.14	1.82
Human sample 3	11.36	0.21	1.87

e. Correlation Test

- Same Principle Method

Regression equation: $y = 0.992x + 0.06$ ($n = 65$)

Correlation coefficient: $r = 0.997$

- HPLC Method

Regression equation: $y = 1.01x - 0.14$ ($n = 65$)

Correlation coefficient: $r = 0.994$

(y: Value obtained from using UMA's reagent)

Reference Materials for Calibration

JCCRM 411

10. EXPECTED VALUES

Normal reference range

4.6 - 6.2% by NGSP value

4.3 - 5.8% by JDS value

Reference range should be established at each facility and judgement should be based on measurement results in a comprehensive manner together with clinical symptoms and other measurement results.

11. INTERFERENCES

- Icterus: No significant interference of conjugated bilirubin concentration up to 40 mg/dL and free bilirubin concentration up to 40 mg/dL

- Hemolysis: No significant interference of hemoglobin concentration up to 500 mg/dL

- Lipemia (Intralipid): No significant interference triglycerides concentration up to 3000 FTU

- Ascorbic Acid: No significant interference of ascorbic acid concentration up to 50 mg/dL

- For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings. Please use another methods if the result is affected by any factors

12. HANDLING, USAGE & DISPOSAL

Handling

1. Specimen can be potentially positive for infectious agents including hepatitis B virus and HIV. Wear glove and goggle when needed.

2. In case reagents got into skin, eye or mouth by mistake, wash it immediately with plenty of water and consult the doctor if needed.

3. If reagents are spilled, dilute with water and wipe it out. If specimen is spilled, spray 80% of alcohol over the specimen and wipe it out.

Usage

1. Store reagents under specified condition. Do not use after expiration date.

2. Do not use the container and auxiliaries included in this kit for other purposes.

3. Do not mix reagents of different lot for use.

4. Do not add to the reagent being used even if it is the same lot number.

Disposal

1. All specimens, as well as all instruments (e.g. test tubes) that come in contact with the specimens, must be treated by the following methods, or they must be treated according to the manual for infectious medical waste provided in each facility.

• Sterilize with an autoclave, subjecting them to high pressure saturated steam at 121 °C for more than 20 minutes. Do not process waste containing sodium hypochlorite solution with an autoclave.

• Immerse at least one hour in sodium hypochlorite solution (active chloride concentration of over 1000 ppm).

2. This reagent contains sodium azide. Sodium azide can react with lead pipe and/or steel pipe and can generate explosive metal azide. Make sure to use plenty of water at disposal. Concentration of sodium azide in R-2 is 0.05%.

13. INFORMATION FOR AUTOANALYZERS

❖ **For Hitachi Model**

Calculation Method		Two point
Temperature		37°C
Volume (µL)	Specimen	4.0
	R1	150
	R2	50
Wavelength (nm)	Main	660
	Sub	800
Measurement (cycle)	Point 1	10
	Point 2	19
	Point 3	34
Calibration type		Spline
Unit		%

14. OTHER INSTRUCTIONS AND CAUTION

- Results may differ depending on the sample/reagent ratio. Adjust parameters for different analyzer.

- Perform the QC procedure on the day of determination.

15. PACKING AND KIT CONFIGURATION

Code	Package	Test/Kit*	Test/Kit**
11H031B	1x30mL; 1x10mL	160	250
11H031A	1x60mL; 1x20mL	310	500
11H031A2	2x60mL; 2x20mL	620	1000
11H031A3	3x60mL; 3x20mL	930	1500
11H031A4	4x60mL; 4x20mL	1240	2000
11H011A	5x60mL; 5x20mL	1550	2500
11H031A6	6x60mL; 6x20mL	1860	3000
11H031	1x90mL; 1x30mL	510	750
11H031-2	2x90mL; 2x30mL	1020	1500
11H011	3x90mL; 3x30mL	1530	2250
11H031-4	4x90mL; 4x30mL	2040	3000
11H031-5	5x90mL; 5x30mL	2550	3750
11H701B	1x30mL; 1x10mL; HbA1c Diluent 4x60mL	160	250
11H701B2	1x30mL; 1x10mL; HbA1c Diluent 2x60mL	160	250
11H701B-2	2x30mL; 2x10mL; HbA1c Diluent 4x60mL	320	500
11H601A	1x60mL; 1x20mL; HbA1c Calibrator Set 5x1mL	310	500
11H701A	1x60mL; 1x20mL; HbA1c Diluent 4x60mL	310	500
11H701	1x90mL; 1x30mL; HbA1c Diluent 6x60mL	510	750
11H7601	1x90mL; 1x30mL; HbA1c Diluent 4x60mL; HbA1c Calibrator Set 5x1mL	510	750
11H111	R1 1x90mL	-	-
11H211	R2 1x30mL	-	-

* For middle-scale automatic analyzers such as: SK300; BS series; BA200; BA400. Chemwell Series; Dirui Series; Biolyzer series, HumanStar 300, Erba Series; Bioelab Series, BX 3010; Pictus P500;…

** For large-scale automatic analyzers such as: CA800; CA400; Randox Imola; Randox Modena+; BM 6010; Biolis50i; SK500; AU Series; Pictus P700; C series; Ci series; HumanStar 600; Kenolab series ...

The above-mentioned test's number are calculated base on technical specifications of each analyzer. The real number of test per kit may higher than the calculation's number.

The above-mentioned test's number cover the loss of the dead volume of reagent bottles but not cover the loss of Calibrator and Control.

Please feel free to contact authorized distributor for further confirmation.

16. REFERENCES

1. M.J. York, in A Comprehensive Guide to Toxicology in Nonclinical Drug Development (Second Edition), 2017
2. CLSI/NCCLS Evaluation of Precision Performance of Clinical Chemistry Devices, EP05-A2, 2004
3. CLSI EP17 · Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, 2nd Edition, 2017
4. In house data, UMA Diagnostics

17. MANUFACTURER

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