HbA1c II DIRECT

Diagnostic reagent for determination of HbA1c concentration.

Liquid. Dual Reagent./ Store at 2°C - 8°C. For in Vitro Diagnostic Use. Do not freeze.

Tube 1	Lyse	1000 uL
	Whole Blood	50 uL
	Mix and Incubate for 5 minutes (it can use for 4Hrs)	
Tube 2	**Reagent 1**	300 uL
	Sample (From Tube 1)	10 uL
	Mix and incubate for fixed 2 minutes	
	After incubation Add **Reagent 2**	100 uL
	Mix and Read within 5 seconds.	

Correlation between HbA1c and DAYTIME Glucose levels

HbA1c (%)	Gluc. (mg/dL)	Gluc. (mmol/L)
5	97	5,4
6	126	7
7	154	8,6
8	183	10.2
9	212	11.8
10	240	13.4
11	269	14.9
12	298	16.5

INTENDED USE

The HbA1c assay is used in clinical laboratories for the quantitative in vitro measurement of HbA1c (hemoglobin fraction) in human whole blood on auto analyzers and semi-auto chemistry analyzer.

TEST SUMMARY

The HbA1c assay is used as an aid in the monitoring of long-term blood glucose control and compliance in individuals with diabetes mellitus.

The HbA1c assay is used for the measurement of the concentration of HbA1 c relative to the concentration of the total hemoglobin (THb).

An increase in the percentage of HbA1 c has been found in individuals diagnosed with diabetes. Uncontrolled diabetes can lead to acute complications of hyperglycemia and ketosis. In addition, long-term complications such as cardiovascular disease, retinopathy, nephropathy, and neuropathy can occur. According to several studies, including the findings of diabetes Control and Complications Trial (DCCT), these complications can be prevented by long-term control of diabetes

Therefore, measurement of HbA1c percentage can be invaluable in the monitoring of long-term glycemic control of diabetic patients.

The HbA1c assay has no cross reactivity with labile HbA1c since the antibody used in this assay is specific for the ketoamine form of HbA1c. Stable HbA1c does not increase or decrease in response to rapid changes in physiological factors and therefore allows the measurement of individuals' average blood glucose levels over several months.

TEST PRINCIPLE

the HbA1c test is recommended for patients with diabetes every 2-3 months as part of the patient diabetes management program. Glycohemoglobin is produced by non-enzymatic addition of glucose to amino groups in hemoglobin. HbA1c refers to glucose modified hemoglobin A (HbA) specifically at N-terminal valine residues of hemoglobin beta chains. HbA1c test is used both as an index of mean glycemia and as a measure of risk for the development of diabetes complications. Therefore, the HbA1c test is a good indicator of glycemic control in the preceding 2-3.

This method utilizes the interaction of antigen and antibody to directly determine the HbA1c in whole blood. Total hemoglobin and HbA1c have the same unspecific absorption rate to latex particles. When mouse antihuman HbA1c monoclonal antibody is added (R2), latex-HbA1cmouse anti human HbA1c antibody complex is formed. Agglutination is formed when goat antimouse IgG polyclonal antibody interacts with the monoclonal antibody. The amount of agglutination is proportional to the amount of HbA1c absorbed on to the surface of latex particles. The amount of agglutination is measured as absorbance.

TEST PRINCIPLE

Method : Immunoturbidimetric

Wavelength : 660 nm (sub: Optional 800nm)

Linearity : 15%

REAGENT COMPONENTS

Lyse Reagent	Reagent R1	Reagent R2:
Stabilizers Buffers, Lysing agent, water	Latex: <0, 15% Buffer Stabilizers.	Mouse anti-human HbA1c monoclonal Antibody<0.06 mg/mL, goat anti-mouse IgG polyclonal antibody < 0.09 mg/dL, buffer, Stabilizers.

REAGENT PREPARATION

Reagent are ready for use

REAGENT STABILITY AND STORAGE

Reagents are stable at +2/+8°C till the expiration date stated on the label which is only for closed vials.

Once opened vials are stable for 30 days at +2/+8°C in optimum conditions. On board stability is strongly related to auto analyzers' cooling specification and carry-over values.

SAMPLE

The assay is formulated for use with human whole blood samples. Venous whole blood samples collected with EDTA anticoagulant can be used. It is recommended that samples be used within 7 days of collection when stored refrigerated. Prior to testing, whole blood samples should be mixed by gentle inversion to re-suspend settled erythrocytes.

Auto analyzer usage: Samples should be tested by stat mode (Emergency mode) to avoid precipitation.

TEST PROCEDURE

Preparation of Hemolysate,

1) Whole blood samples are taken to room temperature,
2) Blood samples are mixed in order to mix erythrocytes homogeneously,
3) Using a calibrated pipette, transfer 1000 mL Lyse solution to the sample cup,
4) 50 mL of homogenized blood sample is transferred to the sample cup with Lyse added,
5) Hemolysate is mixed thoroughly, incubated for 5 minutes at room temperature,
6) Hemolysate is ready for use for HbA1c.

ASSAY SCHEME FOR ANALYSERS

For analyzers capable of handling only 2-reagents, use the following scheme as a guideline for analyzer application. Note: HbA1c is an end-point assay and the first reading point A1 is right before the addition of reagent R2.

QUALITY CONTROL AND CALIBRATION

The Turbidimetric HbA1c assay requires monthly calibration. Place calibration series on the analyzer in the order of lowest to highest. Enter calibrator lot specific values provided on the specification Calibrator sheet.

The assay requires the use of an HbA1c Calibrator 4-Levels.

We recommend:
HbA1c Calibrator 4-Levels

All calibrator vials are stable until their expiration date when stored at +2/+8°C. HbA1c calibrator set is in lyophilized form. HbA1c calibrator set for the auto analyzers on-board Lysis Application includes four levels of calibrator material. Levels 1-4 are in lyophilized form. Reconstitute the lyophilized contents according to the directions on each label and mix gently. Let the vials equilibrate at room temperature for 30 minutes before use. Reconstituted calibrators are stable for 30 days when capped tightly and stored at +2/+8°C.

Commercially available control material with established values determined by this method may be used. We recommend:
HbA1c Control-Low/High Set

Turbidimetric HbA1c control set can be purchased separately. Users should follow the appropriate local guidelines concerning the running of external quality controls and handling of bio-hazardous material.

The International Federal of Clinical Chemistry (IFFC) values are calculated according to the formula given:

CONVERSION FORMULA:
NGSP%=[0.09148 X (IFCC)] + 2.152

REFERENCE INTERVAL (NORMAL VALUUES)

Expected Values : %4.5 – 6.5 (NGSP/DCCT)
Expected Values : 26-48 mmol/mol (IFCC)

Levels above 6.5% HbA1c are suitable for the diagnosis of diabetes mellitus according to the data provided by NGSP. Patients with levels between 39-46 mmol/mol (IFCC) or 5.7% - 6.4% HbA1c (NGSP) have a possibility of developing diabetes risk. [9,10]

It is recommended that each laboratory establish its own normal range**.**

Limitations:

- The linearity of the assay is up to 15% HbA1c. Samples with values above 15% should not be diluted and retested. Instead the values should be reported as higher than 16% (>16%).
- It has been observed that the patients who have alcoholism, high dose of acetyl salicylic acid, opiate and lead poisoning may lead to inconsistency.
- The assay is formulated for use with human whole blood samples in EDTA.
- Elevated levels of HbF may lead to insufficient evaluation of HbA1c and uremia does not interfere with HbA1c determination by immunoassay.

PERFORMANCE CHARACTERISTICS

The following HbA1c value data were obtained by comparing this Direct Turbidimetric HbA1c assay to a legally market HPLC method.

Whole blood application	
n	100
Slope	1.001
Intercept	0.027
Correlation Coefficient	0.990
Range of Values	5% - 14% HbA1c

Limit of Quantitation (LoQ) [LoQ values are based on Coefficient of Variation Percentage (CV) 20%]:" 4%.

High Linearity: The method is linear up to 15.0%. Linearity may considerably vary depending on the instrument used.

Precision Studies:

Repeatability (within run) (Intra-assay)

Mean concentration	CV%	n
Low 5.46	1.45	40
High 10.1	1.73	40

Reproducibility (Day to Day) (Inter-assay)

Mean concentration	SD	n
Low 5.46	2.81	40
High 10.1	2.72	80

Interference

No significant interactions were observed for Conjugated Bilirubin, Triglycerides, Ascorbic Acid, Acetlated Hb, Carbamylated Hb up to the interferent concentration given in the table,

Ascorbic acid	: 40 mg/dL
Total bilirubin	: 48 mg/dL
Acetylated Hb	: 4.8 mmol/L
Triglycerides	: 2000 mg/dL
Carbamylated Hb	: 7.3 mmol/L

Stable glycated hemoglobin serves as a substrate for the Turbidimetric reaction used in Direct Turbidimetric HbA1c assay.

The acceptable interference limit is set 10% below the highest interference concentration within ± 10% recovery of the target.

Interferences may affect the results due to medication or endogenous substances.

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