HbA1c

Immunoturbidimetric Method

DIATEK

CLINICAL SIGNIFICANCE :

HbA1c is a glycated product of hemoglobin A_0. In erythrocytes the relative amount of HbA converted to stable HbA1c increases with the average concentration of glucose in the blood. The conversion to stable HbA1c is limited by the erythrocyte's life span of approximately 100 to 120 days. As a result, HbA1c reflects the average blood glucose level during the preceding two to three months. HbA1c is thus suitable to monitor long-term blood glucose control in individuals with diabetes mellitus. More recent glucose levels have a greater influence on the HbA1c level.

TEST PRINCIPLE :

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 anti-human HbA1c monoclonal antibody is added (R2), latex- HbA1c-mouse anti-human HbA1c antibody complex is formed. Agglutination is formed when goat anti-mouse IgG polyclonal antibody interacts with the monoclonal antibody. The amount of agglutination is measured as absorbance. The HbA1c value is obtained from a calibration curve.

REAGENTS COMPOSITION :

Reagent 1 (R1) Latex Reagent : Latex 0.13%, Buffer, stabilizer Sodium azide (0.95 g/L)

Reagent 2 (R2) HbA1c Antibody : Buffer, Mouse anti-human HbA1c monoclonal antibody: 0.05 mg/mL, goat anti-mouse IgG polyclonal antibody 0.08 mg/dL., Stabilizers

Reagent 3 (R3) Hemolysing reagent : Water and stabilizers

All components of the kit are stable until the expiration date on the label when stored tightly closed at 2 - 8 ºC, and contaminations prevented during their use. Do not use reagents over the expiration date.

KIT CONTENTS

CODE No.	*HBA1C01*	*HBA1C02*
Pack size :	(20 ml)	(40 ml)
Reagent 1	1 x 15 ml	1 x 30 ml
Reagent 2	1 x 5 ml	1 x 10 ml
Reagent 3	1 x 40 ml	2 x 50 ml

REAGENT PREPARATION

All the Reagents are ready to use and unopened reagent is stable upto expiry. Once opened the onboard stability is 30 days in Fully Automated Analyser under on board cooling system.

SAMPLES : Use fresh EDTA blood.

Hemolysate Preparation :
Dispense 0.5 mL Hemolysis Reagent into a test tube. Place 10 µL of well mixed whole blood (Samples, Calibrators and Controls) into the test tube. Mix very well. Allow to stand for 5 minutes or until complete lysis is evident. Stability of the hemolysate is 10 days at 2-8 °C.

Materials required (But not provided) :

CALIBRATION :
Use HbA1c Calibrator set of four calibrators and normal saline as 0 (For Semi Auto input value as 0.01 for normal saline).

QUALITY CONTROL :
Use HbA1c Control Low and HbA1c Control High for test validation.

TEST PROCEDURE:

Before the assay begins, bring all the reagents to room temperature.

Calibrator/Sample/Control: Hemolysate
Reference curve : Calibrator series. Use saline as zero point.
Reagent Blank : Reagent 1 (375 µl) + Reagent 2 (125 µl)

1. Pipette into cuvette:
   Reagent 1 : 375 µl
   Add Hemolysate (Calibrators / Samples / Controls) : 10 µl
   Mix well and incubate for 5 min.
2. Then add Reagent 2 : 125 µl
   Mix well and incubate for 5 Min. at 37°C.
3. Read Absorbance at wavelength 600 nm (590 - 670 nm) against reagent blank
4. Plot a calibration curve
5. Calculate the concentrations of samples/controls based in the calibration curve.

TEST SPECIFICATIONS:

The performance datas were measured on Hitachi 917
Precision:

Intra-assay	Low	High
Mean	5.48	10.28
Std. Dev.	0.078	0.176
%CV	1.43	1.72

Inter-assay	Low	High
Mean	5.48	10.28
Std. Dev.	0.152	0.275
%CV	2.77	2.68

TEST COMPARISON

Comparison with HPLC (Tosoh) : y = 1.050x – 0.481 / r = 0.988

REFERENCE RANGE :

HbA1C value is approximately :
4 - 5.9% non diabetics
6 - 7% controlled diabetics
Over 7% uncontrolled diabetics.

The above reference range is guideline and all the laboratories must establish their own normal reference range. Final diagnosis should be made with correlation of clinical factors.

Measuring range :

2 - 16 %

ASSAY CONDITIONS:

Wavelength	600 nm
Cuvette	1 cm light path
Constant temperature	37ºC
Reaction (Mode)	End Point
Calibration	Multipoint Nonlinear
No. of Calibrator	5
Calibrator concentration	As specified
Linearity	16
Unit	%
Blanking	Reagent
Slope of Reaction	Increasing

PRECAUTIONS :

1. Storage conditions as mentioned on the kit to be adhered.

2. Use clean glassware and microtips while pipetting Reagents

3. Avoid contamination of the reagent during the assay process.

4. No interference for ascorbic acid (50 mg/dL), Triglyceride (2000 mg/dL), Bilirubin (50 mg/dL), carbamylated Hb (7.5mmol/L) and acetylated Hb (5 mmol/L)
- **HbA2, HbC and HbS do not interfere.**
- **Results could be inconsistent in patients with opiate additions,alcoholism, at large doses of aspirin, lead poisoning**
- **Elevated levels of HbF my lead to under-estimation of HbA1c**

5. Programmes for specific autoanalysers are available on request.

6. For accuracy of results, the assay procedure, reagent preparation and storage has to be meticulously followed.

7. As with all the diagnostic procedures, the physician should evaluate data obtained by the use of this kit in light of other clinical information.

BIBLIOGRAPHY

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