Hemoglobin A1c (HbA1c)

Turbidmetric Method

INTENDED USE:

This reagent kit is intended for ***"in vitro"*** quantitative determination of Glycohemoglobin **(HbA1C)** in human whole blood by Latex Turbidimetric method.

CLINICAL SIGNIPICANCE.

Glycosylated Hemoglobin (GHb) is formed continuously by the adduction of glucose by co-valent bonding to the amino-terminal valine of the hemoglobin beta chain progressively & irreversibly over a period of time & is stable till the life of the RBC. This process is slow, non- enzymatic and is dependent on the average blood glucose concentration over a period of time. HbA1c is a glycated product of hemoglobin A0 (HbA1c), the predominant form of hemoglobin in adults. Measurement of the percentage of HbA1c reflects the mean blood glucose concentration over the preceding one to two months, and is therefore considered to be an important diagnostic marker for monitoring blood glucose levels.

PRINCIPAL:

HbA1c-Turbilatex is a quantitative turbidimetric test for the measurement of Glycohemoglobin A1c percent in human whole blood. In first reaction HbA1c interacts with antihuman hemoglobin A1c mouse monoclonal antibody-sensitized latex and in the second reaction, it wil further interact with anti-human hemoglobin A1c mouse monoclonal antibody labeled-anti-mouse. IgG goat polyclonal antibody. Then, 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.

REAGENT COMPOSITIONS:

Reagent 1 : Anti human Hemoglobin A1c Mouse Monoclonal antibody-Sensitized latex.

Reagent 2 : Anti human Hemoglobin A1c Mouse Monoclonal antibody-labeled anti mouse IgG goat polyclonal antibody.

Reagent 3 : Lysing Reagent

Calibrator : 4 Lyophilized calibrator vials. Reconstitute with distilled water. Concentration printed on vial label.

MATERIALS REQUIRED BUT NOT PROVIDED

Clean & Dry Glassware.
- Micropipettes & Tips.
- Bio-Chemistry Analyzer

SAMPLES:

Whole blood samples (EDTA, NaF-EDTA, heparin) can be used.
HbA1c in samples is stable for 3 days when stored at 2-8°C.

WORKING REAGENT PREPARATION & STABILITY:

Reagent should be stored at 2-8°C.
Reagents are stable till expiry date mentioned on the labels when stored at 2-8°C.
All reagents are ready to use. Allow reagents to attain room temperature before performing the test.
Do not freeze the reagents. Frozen Latex or Diluent could change the functionality of the test.

GENERAL SYSTEM PARAMETERS:

Reaction type	Multipoint Fixed Time Kinetic
Wave length	630 nm (600-660nm)
Light Path	1 Cm
Reaction Temperature	37°C
Blank / Zero Setting	With Distilled Water
Reagent volume	300
Sample Volume	10µl
Lag / Delay Time	5 Sec.
Read Time	200 Sec.
Number of Calibrators	5 (DW, Cal 1, Cal 2,Cal 3, Cal 4)
Calibrator Concentration	DW = 0, Cal 1 to Cal 4 As mentioned on vial
Linearity	3.5 -16%

ASSAY PROCEDURE:

Lysing of Calibrator: Dispense 500 µl of Lysing Reagent in a micro centrifuge tube, add 15 µL of reconstituted calibrator, mix and wait for 10-15 mins.

Lysing of Whole Blood : Dispense 500 µl of Lysing Reagent in a micro centrifuge tube, add 15 µL of whole blood, mix and wait for 10-15 mins.

	Calibrator	Test
Reagent 1	**300 µl**	**300 µl**
Calibrator	**10 µl**	**----**
Sample	**----**	**10 µl**

Mix well and incubate for 2 minutes at 37°C

Reagent 2	**100 µl**	**100 µl**

Mix well and after 5 seconds, read initial absorbance A1. Exactly after 200 seconds interval, read absorbance A2 at 37°C.

Determine the △Aabsorbance.

△Abs. = A2-A1

CALCUTION:

At 630 nm with 1cm Light path,

$$\text{HbA1C conc. In \%} = \frac{\triangle \text{ Abs. of sample}}{\text{A Abs. of Calibrator}} \times \text{Calibrator conc.}$$

LINEARITY:

Reagent is Linear up to Glycohemoglobin HbA1c concentration of 3.5 -16%

REFERENCE NORMAL VALUE:

4-5.9%	Non Diabetes,
6-7%	Controlled Diabetes.
Over 7%	Uncontrolled Diabetes.

The reference values are only indicative in nature. Every laboratory should establish its own normal ranges to reflect the age, sex, diet and geographical location of the population.

QUALTY CONTROL:

For accuracy it is necessary to run known controls with every assay.

BIBLIOGRAPHY:

1. Trivell,L.A., Ranney, H.M. and Lai, H.T., (1971) New Eng. J. Med. 284, 353.
2. Nathan,D.M. et al, Clin. Chem. (1983), 29, 466-469.
3. Goldstein, D.E., et al, Clin. Chem. (1986) 32, B64-B70.
4. American Diabetes Association: Clinical Practice Recommendations. Diabetes Care 30

Gala No 217, 218, 219 Building No.3,
2nd Floor Jay Vijay Industrial Estate
Naigaon East, Mumbai.