GLYCOHEMOGLOBIN

(ION EXCHANGE RESIN METHOD)

DIATEK

Diagnostic reagent for quantitative in vitro determination of Glycohemoglobin in Blood on photometric systems.

Presentation

Pack Size	Ion Exchange Resin (R1)	Lysing Reagent (R2)	Resin separators (R3)
10 T	10 nos	5ml	10 nos

Summary

Glycosylated hemoglobin(GHb) reflects the average blood glucose concentration over the preceding several weeks & sudden fall from high to low glucose concentrations will not produce a correspondingly rapid fall in glycosylated hemoglobin. Thus GHb reflects the metabolic control of level over a period of time, unaffected by diet, insulin, other drugs or exercise on the day of testing. GHb is now widely recognized as an important test for the diagnosis of diabetes mellitus and is a good indicator of the efficacy of thereby.

Method

Ion exchange resin method.

Principle

Whole blood is mixed with a lysing reagent containing a detergent and a high concentration of borate ions. Thus the labile schiff's base is eliminated. The following mixture of the hemolysed preparation of whole blood with weak cation - exchanged resin, results in the binding of the HbA_o to the means of a separator, the resin is separated from the buffer solution containing unbound HbA1. The percent age of HbA1 (Glycohemoglobin) is determined by measuring the absorbance of HbA1 fraction and the total hemoglobin.

Storage Instructions and Reagent stability

The reagents are stable upto the end of the indicated month of expiry, if stored at 2-8°C and contamination is avoided. Do not freeze the reagents. The Resin separators can be removed on opening the kit & stored at R.T.

Waste Management

Please refer to local legal requirements.

Specimen

Venous blood is collected with EDTA/ heparin using aseptic .techniques. GHb in blood is stable for 7 days at 2-8°C.

Assay Procedure

Wavelength	:	415nm (405-420nm)
Optical path	:	1 cm
Temperature	:	R.T
Measurement	:	Against distilled water

Step 1: Hemolysate Preparation

1. Pipette 250 l of Lysing reagent (R2) into required number of labelled tubes for different samples.
2. Pipette 50u.l of well mixed whole blood sample into the appropriately labelled tube & mix well.
3. Incubate for 5 min at room temperature & allow complete lysis of R.B.C.

Step 2: Glycohemoglobin Separation

1. Take pre-pipetted ion exchange resin tubes (R1)& remove cap.
2. Add 100 l hemolysate reagent obtained in step 1 into the appropriately labeled ion exchange tubes.
3. Insert a resin separator (R3) into each tube so that the rubber sleeve is approximately 1 cm above the liquid level of the resin suspension.
4. Mix the tubes on a rocker, rotator or a vortex mixer continuously for 5 minutes.
5. Allow the resin to settle, then push the resin separator into the tubes until the resin is firmly packed.
6. Pour the supernatant into a cuvette, read the absorbance A_1 at 415nm against water.

Step 3: Total Hemoglobin

1. Dispense 5 ml distilled water into labelled tubes.
2. Add 20 l hemolysate obtained in step 1 into the appropriately labeled tubes. Mix well & read absorbance at 415 nm against distilled water.

Calculation

$$\%HbA,(\%GHb) = \frac{A\ GHB}{A\ THB} \times \text{Factor}$$

Factor = 4.61

Linearity

The Glycohemoglobin procedure is linear in the range of 4%-20%

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Reference Range

Precautions & Notes

The glycohemoglobin level is a good method for determination of average blood glucose concentrations during past 4 - 6 weeks.

Non Diabetic level	-	5 - 8%
Diabetic level	-	8 - 9%
Fair control	-	9-10%
Poor Control	-	above 10%

Quality Control

To ensure adequate quality control each run should include assayed normal & abnormal controls.

Precaution & Notes

1. If rocker or rotator is not available, the tubes may be swirled manually conlinuously for at least 5 minutes. Vigorous shaking is very much necessary as the non glyco hemoglobin binds effectively & completely with the resin leaving the Glyco fraction in the supernatant.
2. Ineffective and incomplete rocking or shaking may give erroneous results.
3. The lysing reagent contains sodium azide, Do not swallow, avoid contact with skin and mucous membrane. Also avoid pipetting by mouth.
4. There is no interference observed from bilirubin & triglycerides.

Literature

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DIATEK

P - 25 Kalindi Housing Scheme
Kolkata - 700 089
Tele : 033 - 2522 3075 / 3448 / 3646
Fax : 033 - 2522 2401
E - mail : diatek@rediffmail.com