GLYCOSYLATED HEMOGLOBIN

Ion Exchange Resin Method

Intended Use:
Glycosylated Hemoglobin or its variants test reagent/kits is a medical device intended for the estimation of Glycosylated Hemoglobin or its variants including A1a, A1b, and A1c in whole blood.

Clinical Significance
Glycohemoglobin (GHb) is formed continuously by the adduction of glucose by covalent bonding to the terminal valine of the Hemoglobin beta chain progressively and irreversibly over a period of time and is stable till the life of the RBC. This process is non enzymatic and is dependent on the average blood Glucose concentration over a period of time.
A single Glucose determination reflects the glucose level at that time. GHb on the other hand reflects the mean glucose level over an extended period of time. Thus GHb reflects the metabolic control of glucose 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 reliable indicator of the efficacy of therapy.

Principle
Glyco Hemoglobin (GHb) has been defined operationally as the fast fraction hemoglobins HbA1 (HbA1a, A1b, A1c) which elute first during column chromatography. The Non glycosylated hemoglobin, which consists of the bulk of Hemoglobin has been designated HbAO. A hemolysed preparation whole blood is mixed continuously for 5 minutes with a weakly binding cation-exchange resin. The labile fraction is eliminated during the hemolysate preparation and during the binding. During this mixing, HbAO binds to the ion exchange resin leaving GHb free in the supernatant. After the mixing period, a filter separator is used to remove the resin from the supernatant. The percent Glyco hemoglobin is determined by measuring absorbances of the ratio of the absorbances of the Glyco hemoglobin of the total hemoglobin fraction of the control and sample.

Working Reagent Preparation
Reagent is ready to use.

Stability and Storage
Reagent are stable at 2-8^{0}C till the expiry mentioned on the labels. **Do not freeze**.
The Resin separators can be removed on opening the kit and stored at R.T.

Materials required
- Photometer or spectrophotometer with a thermostat cell compartment set at 25/30/37ºC, capable of reading at 415 nm (405-420 nm).
- Stopwatch, strip-chart recorder or printer.
- Cuvettes with 1-cm path length
- Pipettes to measure reagent and samples.

Sample and Stability

Serum or plasma
Venous blood is collected with EDTA/Heparin using aseptic techniques. GHb in blood is found to be stable for one week at 2-8^{0}C.

Assay Procedure
STEP I - Hemolysate Preparation
1. Dispense 250µl Lysing Reagent into required number of labeled tubes for different samples.
2. Place 50 µl of the well-mixed whole blood sample into the appropriately labeled tube and mix well.
3. Incubate for 5 minutes at R.T. to allow complete lysis of R.B.C.

STEP II- Glycohemoglobin (GHb) Separation
1. Remove cap from the Ion-Exchange Resin tubes and label as Control & Test.
2. Add 100ul of the hemolysate form Step I into the approximately labeled Ion Exchange Resin tubes.
3. Insert a resin Separation 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 5-7 minutes.
5. Allow the resin to settle, and then push the resin separator into the tubes until the resin is firmly packed.
6. Pour or aspirate each supernatant directly into a curvette and measure each absorbance against distilled water.
7. Read the absorbance of the supernatant against distilled water at 415 nm (405-420 nm).

STEP III - Total Hemoglobin (THb) Fraction:
1. Dispense 5ml of distilled water into tubes labeled as Control & Test.
2. Add to it 20ul of hemolysate from Step I into the approximately labeled tube.
3. Mix well.
4. Read the absorbance against distilled water at 415 nm (405-420 nm).

Calculations
Results for the unknown samples are calculated as follows:

$$\text{GHb in \%} = \frac{\text{Abs. of Glyco hemoglobin (GHb)}}{\text{Abs. of Total Hemoglobin (THb)}} \times 4.61^*$$

* 4.61 = Assay Factor

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

Linearity
The Glycosylated hemoglobin procedure shows linearity for GHb levels in the range of 4.0% - 20.0%.

Reference Values

	GHB%	HBA1c
Non Diabetic:	5.0 - 8.0%	< 6.0%
Good Control:	8.0 – 9.0 %	6.0 – 7.0 %
Fair Control:	9.0 -10.0 %	7.0 – 8.0 %
Poor Control:	10.0 % & Above.	> 8.0 %

Note: Please refer conversion table of GHB A_1 and A_{1c} to M.B.G (Mean Blood Glucose)

Notes
1. Due to variation in inter-laboratory assay conditions, instruments and demography, it is recommended that each laboratory should establish its own normal range.
2. If rocker or rotator is not available, the tubes may be swirled manually continuously for at least 5 minutes. Vigorous shaking is very much necessary as the non-glycosylated hemoglobin binds effectively and completely with the resin leaving Glycosylated hemoglobin fraction in the supernatant.
3. Ineffective and incomplete rocking or shaking may give erroneous results.
4. Avoid mouth pipetting of lysing reagent.

Reference

1. Trivelli, L.A., Ranney, H.M. and Lai, H.T., New Eng.J.Med. 284, 353 (1971).
2. Nathan, D.M., et al., New eng.J.Med. 310, 314-346 (1984).
3. Bunn, H.F., Diabetes 130, 613 (1981).
4. Bates, H.M., Lab Manag., Vol 16 (Jan. 1978).

For *in vitro* Diagnostic use only.